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Different Modes of Transmissibility and Virus-Vector Relationship in the Occurrence of Leaf Curl Disease of Mesta (*Hibiscus subdariffa* L.)

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A B S T R A C T

Mesta (*Hibiscus sabdariffa*) is an important commercial fibre crop after cotton and jute, which belong to the family Malvaceae. The crop is mainly grown for a green leafy vegetable purpose in North Karnataka, India. Mesta crop is being affected by many diseases of which leaf curl disease caused by *Begomovirus* is a main constraint in its production. The disease is observed to be transmitted by different means mainly by whitefly (*B. tabaci*), dodder (*Cuscuta sp.*), grafting and sap. Similarly minimum 10 min of Acquisition Access Period (AAP) and Inoculation Access Period (IAP) is required for the disease transmission and exhibits the disease symptoms. Infected plants exhibited the characteristic symptoms of leaf curl disease as exhibits under natural conditions.

Introduction

Mesta is one of the most important commercial fibre crops after cotton and jute, which belong to the family Malvaceae with chromosome number 2n = 72. It is an herbaceous annual crop believed to be originated from Afro- Asian countries. It is more adaptive and drought tolerant than jute under diverse conditions of climate and soil. In Karnataka, the area under mesta cultivation is very less. However, in North Karnataka apart from fiber purpose it can be grown for leafy vegetable as mixed or inter crop in cotton, sugarcane, pigeon pea and vegetable crops. With the irrigation facility the crop can be grown throughout the year as pure crop for leafy vegetable purpose.

The crop is being affected by various diseases such as powdery mildew, leaf spot, root rot, yellow vein mosaic and leaf curl disease (Paul

et al., 2006). Among the different diseases, leaf curl disease caused by *begomovirus* is one of the important bottle neck for production and cultivation of mesta in Karnataka. The emergence of the *Bemisia tabaci-begomovirus* complex around the world depends on various factors, such as evolution of variants of the viruses, changes in the biology of vectors, movement of infected planting materials, introduction of new crops and host susceptibility genes through the exchange of germplasm, changes in cropping systems and climatic factors (Varma and Malathi, 2003).

Geminiviruses are classified into seven genera: *Mastrevirus, Curtovirus, Topacuvirus, Begomovirus, Becurtovirus, Erarovirus* and *Turncurtovirus*, based on their insect vector, host range and genomic characteristics (Varsani *et al.*, 2014). Among these viruses, whitefly [*Bemisia tabaci* (Gennadius)]-transmitted begomoviruses are considered to be one of the largest and most important group of plant viruses infecting a wide range of crops, particularly in tropical and subtropical regions of world. The leaf curl disease caused by *begomovirus* belongs to family *Geminiviridae* which affects both mesta and cotton belong to the malvaceae family. It is of great concern because cotton and mesta crop has covered larger area in North Karnataka. Since, there is no much research work was done on this, the work has been carried out for investigations on modes of transmission, virus –vector relationship of the virus in relation to disease spread.

Materials and Methods

Maintenance of virus pure culture under insect proof condition

Diseased mesta plants exhibiting typical symptoms of leaf curl were collected from different formers field and the pure culture of the virus was maintained under glasshouse (Fig. 1) and used for further transmission studies.

Source and maintenance of whitefly (*B. tabaci*) culture

Initially, Tobacco whiteflies (*B. tabaci*) were collected from brinjal plants at Entomology plot, New area and Agricultural Engineering research field, UAS Raichur, Karnataka, India and the colony was established on freshly grown cotton (*Gossypium hirsutum* cv. DCH-2) and brinjal (*Solanum melongeana* L.) plants kept in insect proof net house (Fig. 2). There after one generation, freshly emerged whiteflies were collected using an aspirator and were transferred onto freshly grown cotton plants kept in an insect proof net house. The colony developed, from the egg was referred to be pure (a-viruliferous) and further the colony of whiteflies were used for further studies.

Raising of healthy mesta seedlings

The seeds obtained from healthy mesta plants were sown and maintained in insect proof cages. Seedlings at two leaf stage were inoculated with 20 whiteflies for 24 h of inoculation access feeding period after 24 h acquisition access feeding on leaf curl infected mesta plants. The inoculated seedlings were kept in insect proof glasshouse for symptom development. Observations were made on time for initial and final symptoms expression and type of symptoms. Further the healthy mesta seedlings were also used for symptom characterization by transmitting leaf curl virus of mesta through different means of transmission.

Whitefly transmission

The healthy whiteflies maintained on cotton and brinjal plants were collected using an

aspirator and allowed to feed on leaf curl infected mesta plant for a different acquisition access period *viz.*, 10 min, 30 min, 1 h, 2 h, 3 h, 6 h, 12 h, 24 h. The viruliferous whiteflies were again transferred to healthy mesta seedlings to inoculate the virus for a period of 24 h. Later the whiteflies were killed using systemic insecticide triazophos at 1 ml/l. The inoculated seedlings were maintained in insect proof cages till the expression of symptoms.

Dodder transmission

The dodder seeds collected from the vein twined and established on mesta plants. The seeds collected have been sown in pot containing leaf curl infected mesta plants, the seeds have germinated and started twining to the infected plants after 12-15 days after sowing. After complete establishment of the dodder, the veins are twined to the series of healthy seedlings of mesta and kept in a insect proof cages for further symptom expression. The symptoms expressed were recorded.

Graft transmission

Healthy seedlings of mesta were grown in pots and maintained in insect proof cages. The healthy scions are collected and grafted on to the root stock of leaf curl virus infected mesta plants through wedge grafting. The scion and root stocks were tied tightly with thin plastic strips. Plants were maintained under insect proof cages and monitored for the development of disease symptoms over a period of time and recorded the symptoms.

Sap transmission

Mesta leaf samples showing typical symptoms of leaf curl along with the healthy samples were taken separately for mechanical sap transmission. The samples were washed in tap water to remove the dust particles adhering to them and dried between the folds of blotting paper. The leaves were then macerated in chilled mortar and pestle using potassium phosphate buffer (pH 7.0, 0.05M) at the rate of 1ml/g of leaf tissue. The leaf extract was filtered through Whatman no. 47 filter paper, then add ceilite (600 mesh) at the rate of 0.025 g/ml to the extract and 0.02 % of mercapta ethanol. The inoculum was applied gently on the upper surface of the leaves and rubbed unidirectionally, with a small piece of absorbent cotton wool. After 10-15 minutes, the inoculated leaves were washed with a fine jet of distilled water from a squeeze bottle to remove the excess of inoculum. The inoculated plants were maintained under insect proof cages for symptoms expression over a period of time.

Number of whiteflies (*B. tabaci*) required for transmission of disease

To determine the minimum number of whitefly required for successful transmission of virus onto a healthy mesta seedlings (Economic threshold level ETL), whiteflies were tested at different number varied from 1, 2, 4, 5, 10, 15 and 20/plant. Method of virus acquisition and inoculation was followed for each test number, 20 plants were inoculated. After 24 h of each acquisition access period and inoculation access period, seedlings were sprayed with triazophos at 1 ml/l and kept for symptoms expression in insect proof cages. Observations were made on number of seedlings exhibits leaf curl symptoms among the total number of plants inoculated per each test number of *B. tabaci*.

Viral acquisition and inoculation assay

The healthy whiteflies maintained on cotton and brinjal plants were collected using an aspirator (Fig. 3) and allowed to feed on leaf curl virus infected mesta leaf for different acquisition access periods *viz.*, 10 min, 30 min, 1 hr, 2 hr, 3 hr, 6 hr, 12 hr and 24 hr

(AAP). The viruliferous vectors of the respective AAP were again transferred to healthy mesta seedlings to inoculate the virus for a specific period of 24 hr. In order to standardise the inoculation access period required for the disease transmission healthy colonies of whiteflies were allowed to feed on leaf curl virus infected mesta leaf sample for a specific period of 24 hrs. After 24 hrs of AAP, such viruliferous whiteflies were allowed to feed on healthy mesta seedlings for different inoculation access periods *viz.*, 10 min, 30 min, 1 hr, 2 hr, 3 hr, 6 hr, 12 hr and 24 hr (IAP) to inoculate the virus (Fig. 4).

After, respective periods of inoculation and acquisition access periods, whiteflies were killed using systemic insecticide triazophos at 1 ml/l. Later inoculated seedlings were maintained in insect proof cages till the expression of symptoms. Observations were recorded on AAP and IAP required to achieve minimum and maximum per cent transmission based on number of seedlings exhibits diseases symptoms upon inoculation of virus.

Results and Discussion

The biological characterization of mesta leaf curl disease was studied to know the different symptoms exhibited over a period of time, through different modes of transmission *viz*., whiteflies, dodder, grafting and sap. The results obtained are presented here.

Whitefly transmission

Whiteflies are considered as an efficient vectors in transmission of *begomovirus* (leaf curl). A single viruliferous whitefly can transmit the disease to an extent of 40 per cent. Among 75 plants inoculated, 68 showed symptoms, with a mean incidence of 90.66 per cent (Table 1). Twenty viruliferous whiteflies were used per seedling with given a 24 h of AAP and IAP to achieve maximum transmission efficiency (100 %). The affected plants showed the characteristics symptoms such as, vein clearing, vein thickening, chlorosis, upward curling, leathery leaves, twisting of petioles, reduction in leaf size and stunted growth of the plant (Fig. 5) The initial symptoms were observed at 18 days after inoculation and the final symptoms were observed at 45-48 days after inoculation.

The results are in conformity with other whitefly transmitted geminiviruses in different crops such as hibiscus, (Rajeshwari *et al.*, 2005) and sunflower (Vindyashree *et al.*, 2016). In contrary Paul *et al.,* (2009) reported that, minimum of five and 30 whiteflies were required per plant for transmission of kenaf leaf curl disease to an extent of 10 and 70 per cent respectively.

Dodder (*Cuccuta subinclusa*) transmission

The transmission of leaf curl disease of mesta through dodder was achieved from mesta to mesta. The healthy mesta plants connected by strands of dodder established on infected plants. The transmission efficiency was ranged from 60 to 100 per cent with a mean incidence of 73.33 per cent (Table 1).

The parasitized plants showing the initial symptoms like, vein clearing and vein thickening at 27 days of after parasitisation. Further symptoms like upward curling of leaves, leathery leaves and twisting of petioles, small sized leaves and stunted growth of plant (Fig. 6) can be recorded at 45-48 days after inoculation.

Same kind of work has been carried out by Welliver (1992) in tomato for the tomato ring spot virus disease (TmRSV) through dodder (*Cuscuta gronovii)* in *Chenopodium quinoa* and Garnier and Bove (1983) in periwinkle from sweet orange plant infected with yellowing disease

Graft transmission

Wedge grafting method was employed for the transmission of leaf curl disease of mesta from infected mesta to healthy mesta. The grafting was carried out by using healthy scion to infected root stock. The successful grafting was recorded in 30 plants among 50 plants grafted. The transmission of the virus was ranged from 40-80 per cent with a mean incidence of 60 per cent (Table 1). The symptoms were observed at 33-48 days after grafting. The grafted plants exhibited the symptoms such as vein clearing, vein thickening, upward curling of leaves, leathery leaves, small sized leaves and stunted growth of the plants (Fig. 7).

Similar kind of results were obtained in Jatropa *Jatropa mosaic virus* (JMV) through grafting, (Aswathanarayana *et al.,* 2007). Beyene *et al.,* (2013) reported the transmission of cassava brown streak virus pathogen by chip grafting.

Sap transmission

The sap was obtained from the leaf curl infected mesta plants using sodium and pottssium phosphate buffer. The sap was inoculated to 30 days old mesta and *Nicotiana benthamiana* seedlings. Among the 45 mesta plants inoculated, 25 plants expressed the leaf curl symptoms after 30 days of inoculation with a mean incidence of 28.88 per cent. Similarly out of 30 *N. benthamiana* plants inoculated 12 plants showed the symptoms with mean incidence of 39.95 per cent with a average incidence of 34.41 per cent (Table 1). The sap inoculated plants showed only the vein clearing, vein thickening and leaf curling symptoms in both mesta and *N. benthamiana* plants (Fig. 8).

These results are in accordance with some of the works carried out in different crops. Jha and Rayachaudhuri (1956) demonstrated that, CMV was sap transmitted and produced systemic symptoms on *N. tabacum, N. glutinosa, Solanum nigrum, Cucumis melo,* safflower, *D. stramonium* and potato.

Cucumber Mosaic Virus (CMV) was mechanically sap transmissible (Iqbal *et al.,* 2011) and Sohrab *et al.,* (2014) reported the sap transmission of tomato leaf curl New Delhi virus to sponge guard. The virus can be transmitted by sap inoculation to sponge gourd, ridge gourd (*Lufa acutangula*), and *Nicotiana benthamiana* using standardized buffer. The sap transmission was confirmed by using the total DNA isolated from symptomatic leaves and PCR amplification of CP genes using specific primers

Virus vector relationship

Number whiteflies required, AAP, IAP required in relation to spread of leaf curl disease of mesta are presented here. To ascertain the minimum number of *B. tabaci* required for efficient transmission of mesta leaf curl disease, different number of whiteflies *viz.,* 1, 2, 4, 5, 10, 15, and 20 per plant were used for inoculation. Viruliferous whiteflies were enclosed on test plants with 24 h of acquisition access period (AAP) and inoculation access period (IAP). The results showed that, single whitefly was able to transmit mesta leaf curl disease with 40 per cent efficiency.

The transmission efficiency increased to more than 60 per cent when four viruliferous whiteflies were caged on healthy mesta seedlings. Transmission efficiency of 100 per cent was achieved with 20 viruliferous whiteflies used per plant. This indicates that the single whitefly is enough to transmit the disease. Further number of insects and the transmission efficiency was positively correlated.

Table.1 Different modes of tranmsission of leaf curl disease of mesta

Sl. No.	Modes of transmission	Transmission (%)
1.	Whiteflies	90.66
2.	Dodder	73.33
3.	Grafting	60.00
5.	Sap	34.41

Table.2 Virus-vector relationship in relation to disease spread

No. of whiteflies	Transmission %	AAP	Transmission %	IAP	Transmission %
1	40	10 min	45	10 min	30
2	55	30 min	60	30 min	60
4	65	1 h	60	1 h	60
5	90	2 h	65	2 h	70
10	95	3 h	80	3 h	80
15	95	6 h	85	6 h	85
20	100	12 h	100	12 h	95
-	-	24 h	100	24 h	100

Fig.1 Maintenance of mesta leaf curl disease samples in insect proof cages

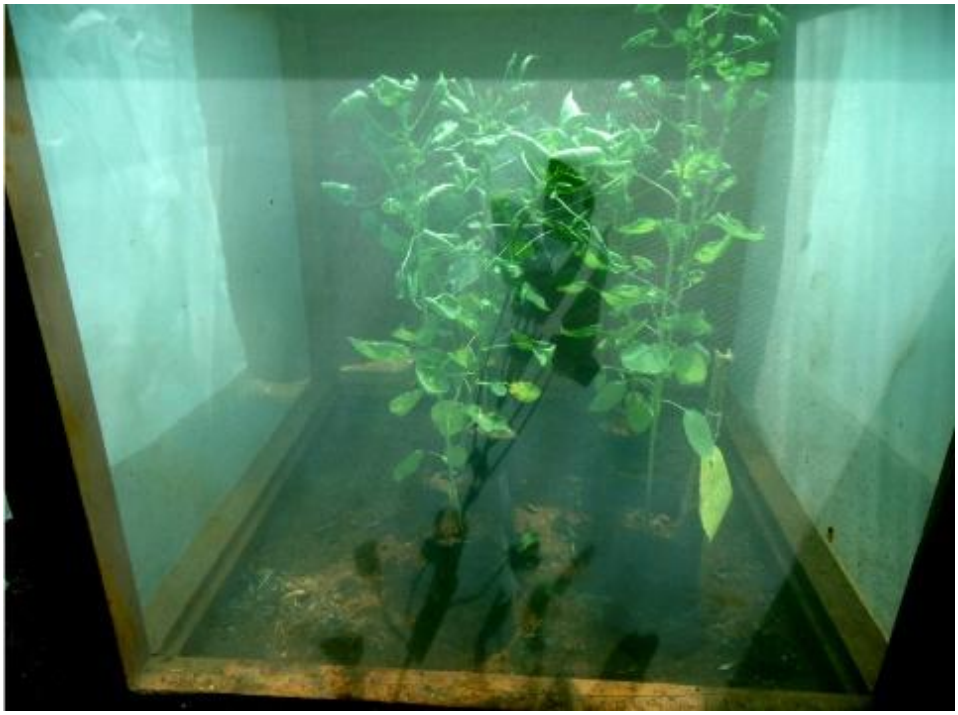

Fig.2 Maintenance of whitefly culture on cotton and brinjal plants

Fig.3 Materials used in transmission studies

a) Insect proof cage b) Inoculation tubes c) Acquisition tubes d) Aspirators

Fig.4 Acquisition and inoculation of leaf curl virus from whiteflies

Fig.5 Whitefly transmission **Fig.6** Dodder transmission **Fig.7** Graft transmission

Fig.8 Sap transmission

Similar findings were reported by Mathew (1988) in cassava mosaic virus. 100 per cent transmission of cassava mosaic virus was obtained with 40 or more whiteflies per seedling. Mandal and Muniyappa (1991) and Nateshan (1992) were observed that 15 viruliferous *B. tabaci* per plant were required for 100 per cent transmission of Croton yellow vein mosaic virus (CYVMV) and Cotton leaf curl virus (CLCuV) respectively.

Viral Acquisition and inoculation Assay for transmission of leaf curl disease of mesta

After standardizing the number of whiteflies required for the maximum disease transmission, a group of 20 non–viruliferous adult whiteflies was allowed to feed on leaf curl infected mesta plant for different AAP from 10 min to 24 h. The 20 viruliferous whiteflies were then inoculated to healthy plants enclosed for 24 h IAP in micro cages to estimate the comparative efficiency of AAP of *B. tabaci*. The studies indicated that a minimum 10 min of AAP was necessary for *B. tabaci* to acquire virus, which resulted in 45 per cent transmission. More than 50 per cent tansmission was observed with 30 min of AAP. An AAP of at least 12 hr was required for 100 per cent transmission of the disease from whiteflies. It has been shown that the per cent transmission of the disease increased with the increase in AAP. A group of 20 non–viruliferous adult whiteflies were allowed to

access leaf curl disease of mesta on infected seedlings for 24 h of AAP. The inoculation access period varied from 10 min to 24 h. Minimum IAP required by viruliferous whiteflies to transmit leaf curl virus of mesta was 10 min to achieve 30 per cent transmission efficiency. An IAP of 24 h was required to achieve 100 per cent transmission with 24 h AAP. The results indicated that per cent transmission increased with the increase of IAP (Table 2).

Similar findings were reported by Muniyappa *et al.,* (1991) in Horsegram yellow vein mosaic virus transmission. whiteflies required a minimum of 30 min AAP to transmit Horse gram yellow vein mosaic virus in Horsegram under greenhouse studies and Rajeshwari *et al.,* (2005) reported that a minimum of 25 *B. tabaci* adults were required for the transmission of CLCuMV-Hib[Ban] with 20 per cent efficiency. The transmission rate increased to 70 per cent when 50 adults per test plant were used. *B. tabaci* adults required a minimum feeding period of 12 h on diseased hibiscus plants for acquisition of CLCuMV-Hib[Ban] and achieved 30 per cent transmission rates. The transmission efficiency increased to only 60 per cent with 48-h AAP. *B. tabaci* adults required a minimum IAP of 24 h and achieved 30 per cent transmission rates, whereas transmission was 50 per cent following an IAP of 48 h.

Paul *et al.,* (2009) studied the transmission of kenaf leaf curl disease using different acquisition access period and inoculation access period, a minimum of 6 h of AAP and IAP is required for disease incidence (10 per cent) whereas maximum disease incidence of 70 per cent has been observed in 24 h of AAP and IAP.

The leaf curl disease of mesta can be successfully transmitted though different means of transmission *viz.,* whitefly, dodder, grafting and sap from mesta to mesta and exhibited the characteristics symptoms similar to the symptoms of natural field conditions.

Single viruliferous whitefly can transmit the mesta leaf curl disease. However 20 viruliferous whiteflies were required for 100 per cent transmission. A minimum period of 10 min was necessary for *B. tabaci* to acquire and to inoculate the mesta leaf curl virus and maximum transmission of the disease was recorded at 24 h of AAP and IAP (100 %).

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