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Survey and biological characterization of leaf curl disease of Mesta (*Hibiscus sabdariffa* L.)

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Abstract

Mesta is an important fibre crop and is affected by several biotic and abiotic factors. Among the different biotic factors, mesta leaf curl disease is new emerging threat for the production of mesta. The incidence of the disease varied from 20 to 80 per cent in southern India with highest disease incidence of 81.66 per cent observed in Raichur district of Karnataka state. The infected plants showed vein clearing, vein thickening, upward curling of leaves with twisting of petioles, leathery and small sized leaves and further the infected plants showed stunted growth. The samples were collected from the adjoining talukas of Raichur and subjected to amplification. All the samples failed to give amplification for DNA-B component. So the disease is considered as a monopartite begomovirus.

Keywords: Mesta plants, Disease incidence, whitefly (*Bemisia tabaci*) and dodder

Introduction

Mesta (*Hibiscus sabdariffa* L.) belongs to Malvaceae family and it is one of the important fibre crop grown in the country. It is believed to be originated from Afro-Asian countries. It is being cultivated in larger parts covering areas from Karnataka to Tripura including Maharashtra, Andhra Pradesh, West Bengal, Bihar, Orissa and Meghalaya. In Tripura and Meghalaya, the crop is grown either as a pure or as mixed with rice.

The tender leaves are used as a vegetable, which is easily digestible with high nutritive value. Fiber is obtained from fully ripened and dried fruits which has multiple uses. The quality and yield of this crop is getting reduced in recent days due to the pests and diseases. This crop is known to be affected by many diseases, among them, powdery mildew, leaf spot, root rot, yellow vein mosaic and leaf curl disease (Paul *et al.*, 2006)^[12], are important. In India, begomoviruses have emerged as major constraint in field crop cultivation. Recently, leaf curl disease of mesta caused by begomoviruses are becoming the major constraints for its production. The emergence of several plant viruses in crop species poses a threat to food security and economic progress in different parts of the world. Currently, there is no information of begomoviruses infecting mesta from Southern India. With this backdrop, the present study on the survey and biological characterization was taken up.

Materials and methods

Disease incidence

A roving survey was conducted in different places of mesta growing areas of Raichur, and Bidar districts during *khari* and *rabi* season of 2015-16 to assess the incidence of leaf curl disease of mesta and whitefly population. The disease incidence was recorded in each field by visual examination of 1000 plants following a 'W' pattern (by crossing the rows) as a sampling procedure. During the survey, two taluks from each district and five villages from each district were selected the disease incidence was recorded. The incidence of the disease in each location was calculated by using following formula, apart from this, type of symptoms, surrounding plants, whitefly population and sources of inoculum were recorded. Further infected leaf samples along with non-symptomatic plants were collected.

$$\text{Disease Incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

Biological characterization

Mesta plants exhibiting different kinds of symptoms were collected from different formers

field may contaminate by more than one virus, to avoid this following repeated artificial transmission was carried out under controlled condition, leaf curl disease symptom expression remained the same after every case of transmission on mesta. This indicate the possible absence of mixed infection in the source material. In this way the pure culture of the virus was maintained at glasshouse used for biological and molecular characterization.

Results

Disease incidence

Mesta leaf curl disease was found to be prevalent in the areas surveyed. Among the taluks of Raichur district surveyed, the prevalence of the disease was ranged from 34.99 to 81.66 per cent with a mean incidence of 56.52 per cent in *kharif*. The mean maximum disease incidence (73.88%) was recorded in Raichur taluk followed by Manvi (39.16%). The highest incidence was noticed at Yeragera (81.66%) village of Raichur taluk (Fig.1) and least incidence was observed in Atanur (34.99%) village of Manvi taluk. However, no disease was observed in Bidar district in *kharif*. (Table 1). However in case of *rabi* 2015-16, survey across the villages of two taluks of Raichur district reveals that, the leaf curl disease incidence (70.25%) was less as compared to *kharif* in Raichur taluk, similarly in Manvi taluk also the disease incidence observed was more in *kharif* than *rabi*. The average incidence of 47.83 and 32.18 per cent was recorded in Raichur and Bidar districts respectively (Table 2).

The leaf curl disease during *rabi* 2015-16 was more severe in Yergera (80%) village of Raichur taluk and least was in Neermanvi (22.10%) village of Manvi taluk of Raichur district. However the magnitude of the disease incidence ranged from 22.10 to 80.00 per cent in Raichur district. The mean incidence in Raichur and Manvi taluks were 70.25 and 25.40 per cent respectively. In Bidar district, the disease incidence ranged from 16.23 to 52.70 per cent. The maximum disease incidence was observed in which Nawadagi (52.70%) village of Bhalki taluk and least incidence was noticed in Janawada (16.23%) village of Bidar district. Whereas the maximum disease incidence of 44.85 per cent was observed in Bhalki taluk followed by Bidar taluk with 19.52 per cent with an average incidence of 32.18 per cent in Bidar district (Table 2).



Fig 1: Incidence of leaf curl disease of Mesta in Sidrapura (North Karnataka)

Table 1: Prevalence of leaf curl disease of mesta in Raichur and Bidar districts of Karnataka during *kharif* 2015

District	Taluk	Location	No. of fields visited	Crop plants and weeds observed	No. of whiteflies/plant	Disease incidence (%)	Mean
Raichur	Raichur	Yergera	5	Cotton, <i>Parthenium</i> , <i>Cynadon</i> sp., <i>Cyperous</i> sp. and <i>Tridax</i> sp.	7-8	81.66	73.88
		Sidrapura	3	Chilli, tomato, brinjal, pigeonpea, <i>Cassia</i> sp., <i>Parthenium</i> and <i>Cyperous</i> sp.	5-7	73.33	
		Tuntapura	4	Cotton, brinjal, tomato, <i>Parthenium</i> and <i>Cyperous</i> sp.	6-8	66.66	
	Manvi	Neermanvi	2	Pigeonpea, cotton, chilli, <i>Croton</i> sp., <i>Ageratum</i> sp. and <i>Cyperous</i> sp.	2-6	43.33	39.16
		Atanur	2	Cotton, bhendi, <i>Parthenium</i> , and <i>Cyperous</i> sp.	0-2	34.99	
	Mean						
Bidar	Bidar	Mailoor	4	Sugarcane, pigeonpea, <i>Parthenium</i> , <i>Tridax</i> sp. and <i>Cyperous</i> sp.	0	0	0.00
		Janwada	5	Sorghum, Sugarcane, <i>Parthenium</i> , <i>Cynadon</i> , sp. and <i>Cassia</i> sp.	0	0	
	Bhalki	Nawadagi	5	Sugarcane, maize, pigeonpea, <i>Croton</i> sp., <i>Euphorbia</i> sp. and <i>Malvastrum</i> sp.	0	0	0.00
		Mavinhalli	3	Greengram, blackgram, Pigeonpea, <i>Parthenium</i> sp. and <i>Cyperous</i> sp.	0	0	
		Kapalapur	2	Greengram, blackgram, sorghum, pigeonpea, <i>Parthenium</i> , <i>Cyperous</i> sp. and <i>Malvastrum</i> sp.	0	0	
	Mean						

Table 2: Prevalence of leaf curl disease of mesta in Raichur and Bidar districts of Karnataka during *rabi* 2015-16

District	Taluk	Location	No. of fields visited	Crop plants and weeds observed	No. of whiteflies/plant	Disease incidence (%)	Mean
Raichur	Raichur	Yergera	5	Cotton, brinjal, <i>Parthenium</i> , <i>Cynadon</i> sp., <i>Cyperous</i> sp. and <i>Tridax</i> sp.	10-20	80.00	70.25
		Sidrapura	3	Chilli, tomato, cluster bean <i>Cassia</i> sp., <i>Parthenium</i> , and <i>Cyperous</i> sp.	10-15	71.07	
		Tuntapura	4	Cotton, tomato, <i>Parthenium</i> and <i>Cyperous</i> sp.	8-12	59.69	
	Manvi	Neermanvi	2	Cotton, chilli, sunflower, <i>Croton</i> , <i>Acanthospermum</i> sp. and <i>Cyperous</i> sp.	2-5	22.10	25.40
		Atanur	2	Cotton, sunflower, bhendi, <i>Acanthospermum</i> sp., <i>Parthenium</i> and <i>Cyperous</i> sp.	8-10	28.70	
	Mean						
Bidar	Bidar	Mailoor	4	Sugarcane, sorghum, chickpea <i>Parthenium</i> , <i>Tridax</i> sp. and <i>Cyperous</i> sp.	2-5	22.80	19.52
		Janawada	5	Sorghum, sugarcane, <i>Parthenium</i> , <i>Cynadon</i> sp. and <i>Cassia</i> sp.	0-4	16.23	

	Bhalki	Nawadagi	5	Sugarcane, tomato, brinjal, sorghum, <i>Croton</i> sp, <i>Euphorbia</i> sp. <i>Parthenium</i> and <i>Malvastrum</i> sp.	1-6	52.70	44.85
		Mavinhalli	3	Chickpea, wheat, <i>Parthenium</i> and <i>Cyperous</i> sp.	1-2	45.70	
		Kapalapur	2	Sorghum, <i>Parthenium</i> , <i>Cyperous</i> sp. and <i>Malvastrum</i> sp.	0-2	36.15	
	Mean						32.18

Biological characterization and symptamatology

The leaf curl infected mesta plants under field conditions exhibited characteristic symptoms like vein clearing, vein thickening, upward curling of leaves with twisting of petioles, leathery and small sized leaves and further the infected plants showed stunted growth. The mesta plants were experimentally inoculated with whiteflies exhibited the similar kind of symptoms as it was observed under natural conditions. The virus inoculated plants under artificial condition took a minimum of 18 days to express the initial symptoms like vein clearing and vein thickening. An incubation period of 45-48 days was required by the plants to express complete symptoms of leaf curl, reduction in leaf size, enations, and stunted growth. The leaf curl virus infected plants produces less or no flowers with poor pod formation (fig. 3). The biological characterization of mesta leaf curl disease was recorded to know the different symptoms exhibited over a period of time, through different modes of transmission viz., whiteflies, dodder, grafting and sap. The observations were recorded at a three days interval on expression of different kind of symptoms (Table.3).



(c) Vein thickening (d) Twisting of Petioles



(e) Severe curling (f). Enations



(a) Vein clearing (b) Upward curling



Fig 3: Manifestation of leaf curl disease infected mesta plants

Table 3: Characterization of symptoms of leaf curl disease of mesta through different means of transmission

Observations at DAI	Symptoms observed in different modes of transmission			
	Whiteflies*	Dodder	Grafting	Sap
3	-	-	-	-
6	-	-	-	-
9	-	-	-	-
12	-	-	-	-
15	-	-	-	-
18	Vein clearing	-	-	-
21	Slight Vein thickening	-	-	-
24	Vein thickening	-	-	-
27	Chlorosis of leaves	Vein clearing	-	-
30	Slight curling of leaves	Vein thickening	-	Vein clearing
33	Twisting of petioles	Slight curling of leaves + chlorosis	Vein clearing	Vein thickening
36	Leathery leaves	Twisting of petioles, leathery leaves	Vein thickening	Slight curling of leaves
39	Upward curling of leaves	Severe curling Upward curling	Slight curling of leaves, chlorosis	Twisting of petioles
42	Stunted growth of the plants	Severe curling	Twisting of petioles, leathery leaves	Leathery leaves
45	Severe curling	Severe curling, stunted growth of the plant	Severe curling, stunted growth of the plant	Severe curling
48	Reduction in leaf size, enations	Reduction in leaf size, enations	Reduction in leaf size, enations	Stunted growth

DAI: Days after inoculation, * 20 whiteflies were used per plant

Whitefly transmission

In case of whitefly transmission, the first symptoms of vein clearing was observed at 18 days after inoculation, followed

by vein thickening, slight curling, chlorosis of leaves, twisting of petioles, leathery leaves, severe upward curling, reduction in leaf size enations and stunted growth of

the plants were recorded between 18 to 48 days (fig. 2 a).

Dodder transmission

In case of dodder transmission, the initial symptoms of vein clearing was observed at 27 days after dodder twining (inoculation), and the further symptoms like vein thickening, slight curling, chlorosis of leaves, twisting of petioles, leathery leaves, severe curling, reduction in leaf size, enations and stunted growth of the plants were observed at between 27 to 48 days (fig. 2 b).

Graft transmission

In case of graft transmission, the initial symptoms of vein clearing was observed at 33 days after grafting, and the further symptoms like vein thickening, slight curling, chlorosis of leaves, twisting of petioles, leathery leaves, severe curling, reduction in leaf size and stunted growth of the plants were found between 33 to 48 days of virus inoculation (fig. 2 c).

Sap transmission

In case of sap transmission, the initial symptoms observed was vein clearing at 30 days after sap inoculation, and the further symptoms like vein thickening, slight curling, chlorosis of leaves, twisting of petioles, leathery leaves, severe curling, reduction in leaf size and stunted growth of the plants were observed from 30 to 48 days of virus inoculation (fig. 2 d).

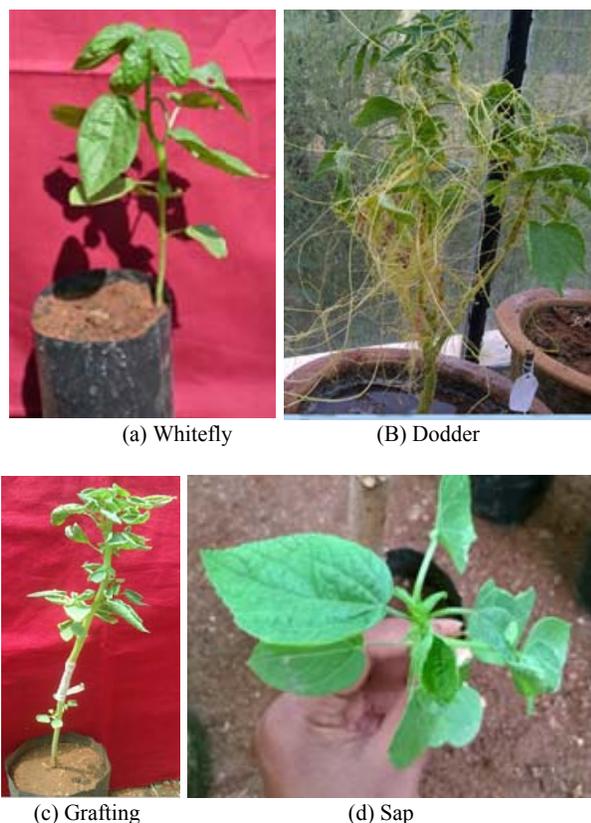


Fig 2: Biological characterisation of leaf curl disease of mesta

Discussion

Naturally virus infected mesta plant exhibited symptoms such as vein clearing, chlorosis, vein thickening, upward curling of leaves, reduction in the leaf size, enations on the lower surface of the leaves, twisting of petioles, leathery leaves,

reduced leaf size and stunted growth of the plant. The similar kind of symptoms were observed by Paul *et al.* (2006)^[12] and Aswathanarayana *et al.* (2016)^[11].

In both the seasons *kharif* 2015 and *rabi* 2015-16, the maximum incidence of mesta leaf curl disease was recorded in Raichur district, but no incidence of disease was observed in Bidar district in *Kharif* 2015-16. The disease incidence was ranged from 19.52 to 73.88 per cent. The high incidence of the disease in Raichur district was mainly due to continuous staggered cultivation of mesta for leafy vegetable and accessibility of primary source of inoculum, infected *Parthenium* and *Cassia* species in the fields (Aswathanarayana *et al.*, 2016)^[11], and also occurrence of whitefly biotype complex and availability of alternate hosts (cotton and brinjal) under irrigated conditions adjoining to the mesta crop. There was no incidence of disease was observed in Bidar district during the *kharif* which may be due to, non-availability of alternate host (cotton, brinjal, sunflower, *Parthenium*, *Cassia*), whitefly population and favourable environmental conditions for disease development. There was no whitefly population on mesta and surrounding host plants and virus reservoirs such as, leaf curl infected *Parthenium*, tomato, brinjal and *Cassia*.

The differences in the incidence of disease in areas surveyed might be due to the variation in the source of virus inoculum (ToLCV Karnataka clone IKH12) (Vanitha, 2012)^[18], vector population, climatic conditions and area under the crop. Interestingly, in the areas where maximum disease incidence was recorded, the vector population was apparently high with a favourable environmental conditions. *B. tabaci* is readily capable of establishing to extreme population levels with higher temperature, particularly crops grown under irrigated and arid field conditions and greenhouse systems. In addition to this, whitefly has the potential to colonize a wide range of dicotyledonous species, among which are primarily vegetable and fiber species.

Recent studies indicated that, there are numerous populations of *B. tabaci* that, vary in their capacity to develop high population densities and cause direct feeding damage to the crop plants. The whitefly has wide host range and in the efficacy with which they can transmit begomoviruses. Availability of source of inoculums of begomoviruses on the hosts like *Croton bonplandianum*, *Euphorbia geniculata*, *Nicotiana benthamiana*, *Nicotiana glutinosa*, *Nicotiana tabacum* (Rajeshwari *et al.*, 2005)^[14] and *Acanthospermum hispidum*, *Ageratum conyzoides*, *Amaranthus viridis*, *Cassia tora*, *Croton bonplandianum*, *Datura stramonium*, *Euphorbia geniculata*, *Euphorbia hirta*, *Parthenium hysterophorus*, *Phyllanthus niruri* (Vindyashree *et al.*, 2015). These hosts also found around the mesta crop might have increased the incidence. In addition, introduction of B-biotype complex of whitefly has been attributed for epidemics and emergence of new viruses in Southern parts of Karnataka (Banks *et al.*, 2001)^[2, 5]. Similar observations were made by Saikia and Muniyappa (1991)^[10] and Ramappa (1993)^[15]. In recent years there have been wide spread occurrence of begomoviruses on many crop plants as well as ornamental plant species *Hibiscus* (Rajeshwari *et al.*, 2005)^[14], zinnia (Shivakumar 2010)^[16] and *Croton* (Mahesh *et al.*, 2010)^[8].

The natural occurrence of leaf curl disease on kenaf in West Bengal is emerging as a major threat due to their increasing incidence and the severity of the diseases they cause in many economically important crops. Evolution of newer viruses and variants of the existing ones and occurrence of mixed infections with different begomoviruses and satellite

molecules has evinced the adaptability to new hosts by these begomoviruses (Paul *et al.*, 2006)^[12]. Similarly emergence or infection of *Begomovirus* in sunflower was reported by Govindappa *et al.* (2011)^[6] at MARS, Raichur, Karnataka to an extent of 40 per cent.

Further, Vanitha (2012)^[18] made a survey in five districts of Northern and Southern Karnataka. The studies revealed that the incidence of SuLCV in sunflower ranged from 0-58 per cent and highest incidence of 58 per cent was recorded at Main Agriculture Research Station, University of Agricultural Sciences, Raichur. However there was no incidence in Thagarigunte and Malangi villages of Tumkur district, Vishwanathapura, Budigere, Chikkahalli and Battaramarahalli villages of Bengaluru Rural district and University of Agricultural Sciences, Bengaluru.

As per the survey, it is clearly showed that Raichur has recorded highest incidence of the disease and it can be one of the “hot spot” for mesta leaf curl disease.

The causal agent of leaf curl disease of mesta was characterized for symptom expression on mesta through different modes of transmission *viz.*, whiteflies, dodder, grafting and sap. The virus inoculated through whiteflies, seedlings exhibited most of the symptoms such as vein clearing, chlorosis, vein thickening, upward curling, reduced leaf size, twisting of petioles, and stunted growth which are similar to the symptoms observed under natural conditions. Similar symptoms were observed by Aswathanarayana *et al.* (2016)^[11].

The leaf curl disease of mesta was transmitted by wedge grafting from mesta to mesta and observed the development of symptoms from 33-48 days after grafting. The virus was also successfully transmitted to mesta through dodder *C. subinclusa*, a natural complete stem parasite on mesta. The virus was also transmitted through sap. The present findings are supported by the reports of Kim *et al.* (1987)^[7], Aswathanarayana *et al.* (2006)^[4] and Aswathanarayana *et al.* (2006)^[4], Michael, (2013)^[9] and Sohrab *et al.* (2014)^[17].

The time taken for the expression of symptoms varied with mode of transmission. Early symptoms were observed in whiteflies transmission followed by dodder, grafting and sap due to changes in uptake of the virus and preference of whiteflies for the host in case of whitefly transmission, establishment of dodder on healthy from infected plants will take some time for haustoria formation, so expression of symptoms were late. Delay in expression of symptoms in graft transmission depends upon the time taken for compatible establishment of the root stock and scion and uptake of the virus (Aswathanarayana *et al.*, 2007)^[3]. In sap transmission the virus particles should establish compatibility with the host and cell to cell movement of virus particle. Hence it took more time than other means of transmission for symptom expression (Kim *et al.*, 1987)^[7]. The expression of symptoms might have also varied due to the host biochemical composition which interferes with the multiplication of virus. Similarly several *Begomovirus* were characterized symptomatologically by many scientists worked on tomato leaf curl disease (Muniyappa *et al.*, 2000)^[11], jatropa mosaic disease (Aswathanarayana *et al.*, 2007)^[3], kenaf leaf curl disease (Paul *et al.*, 2009)^[13], sunflower leaf curl disease (Govindappa *et al.*, 2011)^[6], bhendi yellow vein mosaic disease (Venkataravanappa *et al.*, 2012)^[19]. Therefore the present investigations proved that, the leaf curl disease of mesta is associated with one of the *Begomovirus* infecting mesta crop.

Conclusion

As per the survey in *kharif* 2015, showed prevalence of leaf curl disease of mesta in Raichur district ranging from 34.99 to 81.66 per cent. There was no incidence of leaf curl disease of mesta in Bidar district. During *rabi* 2015-16, occurrence of leaf curl disease of mesta was ranging from 22.10 to 80.00 per cent and 16.23 to 52.70 per cent in Raichur and Bidar districts respectively.

Naturally leaf curl disease infected mesta plants under field conditions and artificially inoculated plants exhibited characteristic symptoms specific to the leaf curl viruses of *Begomovirus*. The important symptoms exhibited are vein clearing, chlorosis, vein thickening, upward curling of leaves, reduction in the leaf size, enations on the lower surface of the leaves, twisting of petioles, leathery leaves, reduced leaf size and stunted growth of the plant. Biologically symptoms were characterized by artificial transmission of virus to mesta seedlings by various means such as whitefly, dodder, grafting, sap and seeds. The inoculated plants exhibited same symptoms as it was observed in natural conditions. Therefore the virus belong to the leaf curl virus of *Begomovirus* group.

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