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Potential Hosts of Sri Lankan Cassava Mosaic Virus Evaluated Through Whitefly Inoculation

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Abstract

Sri Lankan Cassava Mosaic Virus (SLCMV) causes mosaic disease in cassava in India, Sri Lanka and south east Asian countries. Among the 98 plants species/ cultivars belonging to seven families screened/ tested against the virus, SLCMV was transmitted to 42 species of plants belonging to Solanaceae and 4 species of plants belonging to Euphorbiaceae through whitefly inoculation. The incubation period for symptom appearance varied from 6-49 days depending on the species. Presence or absence of SLCMV in the host was confirmed through PCR.

Key words: *Sri Lankan Cassava Mosaic Virus*, host range, whitefly transmission, PCR

Introduction

Cassava (*Manihot esculenta* Crantz) is a root crop that is grown widely throughout the tropics, primarily for its value as a starchy staple food. Although initially used as a food crop, most cassava in India and other Asian countries is now cultivated for industrial starch production. In India, it is grown in an area of 2,28,100 ha with an annual production of 46,50,100 tonnes (FAO 2018) for both consumption as well as for starch based industrial use. Mosaic disease of cassava is the most important disease which is a limiting factor for cassava production in the world. In Indian sub-continent, symptoms of cassava mosaic disease were first observed from southern India in the 1950s (Alagianagalingam and Ramakrishnan,1960), and subsequently from Sri Lanka in the 1980s (Austin, 1986)). Since 2015, the disease has spread more widely, affecting Cambodia and Vietnam in South-East Asia (Wang et al., 2016). Cassava mosaic disease is caused by a virus included in the genus Begomovirus (Family: Geminiviridae). As of now eleven cassava mosaic geminiviruses (CMGs) are currently recognized as distinct virus species causing cassava mosaic disease across the world (Legg et al., 2015; Legg and Winter, 2020). In India this disease could cause an yield loss of 17-88% depending on the cultivars grown (Malathi et al., 1985) and two different viruses viz., *Indian cassava mosaic virus* (ICMV) and *Sri Lankan cassava mosaic virus* (SLCMV). Among these two, the later one is more prevalent and wide spread (Malathi et al., 1985, Dutt et al., 2005; Anitha et al., 2008, 2011).

CMG has two genomic components viz., DNA-A (encodes for functions associated with viral replication and encapsidation) and DNA-B (encodes the movement protein functions). Both the components are required for infectivity.

Since cassava is vegetatively propagated crop, cassava mosaic virus is carried from one crop cycle to the next through the cuttings used as planting material and in the field through whitefly transmission. The causal virus was reported to be transmitted by whitefly, *Bemisia tabaci* from cassava to cucumber (Menon and Raychaudhuri,1970) and cassava to cassava (Antony et al., 2007). The virus could be transmitted by mechanical inoculation from cassava to *Nicotiana benthamiana* and *N. glutinosa* (Malathi and Sreenivasan, 1983). *Indian cassava mosaic virus* was transmitted through whitefly, *B. tabaci* and / or sap to 48 species of plants belonging to Euphorbiaceae and Solanaceae viz., *Datura stramonium*, *M. esculenta*, *M. glaziovii*, *Nicandra physalodes*, *Petunia hybrida* and 43 species of *Nicotiana* (Mathew and Muniyappa,1993).

During our surveys in Kerala, up to 100% incidence of the mosaic disease was observed in most of the cassava genotypes/ cultivars grown in Kerala (Anitha et al., 2011). Like any other pathogen, SLCMV also requires different host plants for survival, multiplication and virulence and also the vectors which acquire the virus from other host plants to the main host. As there is no information available on these aspects with respect to SLCMV, the present study was undertaken to know the potential hosts of SLCMV using whitefly transmission in order to understand the possibility of whitefly transmissibility to these hosts and also whether they can serve as source of inoculum by showing infection. This information is essential for devising a possible management strategy to contain this disease.

Materials and Methods

Collection of whiteflies

An aspirator consisting of a glass tube (30 cm length and 0.5 cm diameter) and a rubber tube of 40 cm length was used for collection of whiteflies. The whiteflies were collected from field by turning the leaves slightly upwards and then they were sucked into the glass tube, afterwards gently they were transferred to plastic tubes. Later they were released on healthy cassava plants for establishing pure cultures of *B. tabaci*.

Rearing cages for whiteflies

Wooden cages of size 45 × 45 × 30 cm was fabricated and muslin cloth was fixed on three sides and top with an adhesive fevicol. The front was covered with glass, which can be easily moved on the grooves made in the wooden frame work. This frame was kept on the wooden rectangular base (45.5 × 45.5 × 10 cm). In each cage healthy cassava plants grown in polythene bags were kept and pure cultures of *B. tabaci* was released.

Preparation of cages for inoculation of seedlings

Plastic tubes of 22 cm long and 6 cm in diameter were taken and the bottom portion was removed with the help of soldering rod. Muslin cloth was fixed to the removed portion which helps in avoiding the accumulation of excess moisture inside the cage. A small hole (0.5 cm) was made on the middle portion of the tube to release the whiteflies. The open end of the tube was plugged with cotton plug after inserting the young leaflets into the tube. The whiteflies were released through the small hole and closed the hole with cotton. The tube was tied to the bamboo stick with rubber band. This tube was used for inoculating slightly older plants. For inoculating young plants, small tubes of 7.5 × 2.5 cm size were prepared as described above. These tubes were covered over young plants and whiteflies were released through the small hole and plugged the hole with cotton.

Cages used for acquisition access

Whiteflies were collected from the colonies reared in the wooden cages with the help of an aspirator. Whiteflies were released into a round PVC bottle. The tube measured 20.2 cm long and 7.5 cm in diameter at one end and tapering towards the narrow end. The bottom portion was removed with the help of a soldering rod and was covered with muslin cloth. A small hole was made in the muslin cloth to release the whiteflies. The cassava mosaic disease infected (*Sri Lankan Cassava Mosaic Virus* infected) cassava branch was inserted into the tube and closed with cotton plug. The whiteflies were then released through the hole and allowed 24 hour acquisition access period. After the acquisition access period the viruliferous whiteflies were inoculated to healthy cassava seedlings at the rate of 25 flies per seedling.

Host range studies with whitefly inoculation

The study was undertaken to determine the host range of SLCMV as well as the main source of infestation of SLCMV. Seventy-five plant species belonging to 7 different families such as Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Euphorbiaceae, Fabaceae, Malvaceae (seeds of these families obtained from Agricultural College, Vellayani, Thiruvananthapuram) and Solanaceae (seeds obtained from ICAR-Central Tobacco Research Institute, Rajahmundry, Andhra Pradesh) were raised through seeds in insect proof glass house conditions. Twenty-three different cultivars of *N. tabacum* were also inoculated with SLCMV by *B. tabaci*. The whiteflies in large numbers were given an acquisition access period of 24 hours on diseased cassava. Afterwards 25 viruliferous whiteflies were released on each test plant and were given 24 hours inoculation access period. The test plants at 3-5 leaf stage were used for inoculation. After the inoculation access period the whiteflies were killed by spraying 0.0125% Dimethoate. The inoculated plants were maintained in

insect proof glass house for symptom production. Symptom development on these plants were recorded at five days interval up to 40 days. Each host species used for the present study was done with replication of ten plants. In each case, five plants of each species were maintained as control without whitefly inoculation. The symptoms exhibited by the host is also recorded.

PCR

All the plants inoculated were analysed by PCR to check for viral infection. The plants which did not show any symptom even 3 months after inoculation were also checked by PCR in order to confirm whether they are symptomless carriers. For this total DNA was isolated (Lodhi et al., 1994) and PCR amplification (Makeshkumar et al., 2005) was done to detect the presence of SLCMV infection in host plants using coat protein gene primer (CP-F:5'AAG CTT TTA ATT GCT GAC CGA3' and CP-R: 5'GGA TTC ATG TCG AAG CGA CCA3').

Results and Discussion

Host range by whitefly inoculation

In order to determine the host range of SLCMV, the plant species belonging to different families viz., Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Euphorbiaceae, Fabaceae, Malvaceae and Solanaceae were inoculated with SLCMV by *B. tabaci* as described under materials and methods. The inoculated plants were kept under observation for 3 months in the glass house.

The results of the host range studies are presented in Table 1. Out of 98 plant species/cultivars inoculated, four species belonging to Euphorbiaceae and 64 plant species/cultivars belonging to Solanaceae were infected. All the

Table 1. Symptoms induced in different hosts in response to whitefly inoculation of SLCMV

Host inoculated	Transmission (%)	Symptoms	Incubation period (days)
Euphorbia hirta	20	M, LC, ST	30-45
Jatropha curcus	10	M, N	33-49
Manihot esculenta	30	M, D, ST, LC, CS	10-20
Manihot glaziovii	20	M, D	22-34
Nicotiana accuminata	35	CS, CPT, M, D	16-22
N. amplexicza	40	CS, CPT, M, D, ST	13-25
N. arentsii	30	CS, LC, M	18-29
N. attenuata	65	LC, M	14-28
N. benthamiana	80	LC, VC, M, CPT, ST	9-13
N. begelovii	20	M, ST	19-25
N. bonariensis	55	LC, D, M	10-18
N. clevelandii	80	LC, D, M, ST	16-20
N. cordifolia	40	M, D	16-22
N. corymbosa	10	M, D	12-18
N. debneyi	20	CS, M, D	17-26
N. forgetiana	20	CS, D, M, ST	15-22
N. glutinosa	40	CS, CPT, LC, ST, LS	10-16
N. goodspeedi	55	LC, M, D, ST, LS	15-24
N. gossei	30	CPT, LC, D, M	14-22
N. hybrid	70	LS, LC, M, CPT, ST	22-40
N. ingulba	25	D, M, ST	28-36
N. knightiana	40	LC, VC, LTP, ST	16-20
N. longiflora	100	M, LC, D	18-29
N. maritima	15	CS, M, D	15-23
N. megalosiphon	25	CS, D, M, LS	12-18
N. miersii	35	CS, D, M	14-20
N. nesophila	70	CS, D, M, LS	6-12
N. occidentalis	60	CS, D, M	18-24
N. otophora	30	M, D, LS, ST	28-40
N. paniculata	35	CPT, D, M, ST	16-24
N. pauciflora	25	CPT, D, LS, ST	12-20
N. plumbaginifolia	40	M, D, CS	18-24
N. raimondii	30	CPT, LC, D, M	16-22
N. repanda	30	CPT, LC, D, M	12-20
N. rosulata	60	CS, M, LS	18-24
N. rotundifolia	100	CPT, LC, D, ST	22-28
N. rustica	30	LC, M, D, ST	14-20
N. simulans	25	LC, D, M	25-34
N. solanifolia	45	CS, CPT, D	20-28
N. stocktonii	20	CS, D, M	14-20
N. suaveolens	10	M, LC	16-24
N. sylvestris	10	CS, LC, ST	16-24
N. tabacum cv. Jayasri	100	M, D, LC	12-20
N. trigonophylla	50	-	20-30
N. umbratica	45	CPT, D, M, ST	16-23
N. undulata	50	LC, D, M	14-24

CS-Chlorotic spots; CPT-Curling of plant top; D-Leaf distortion; LC-Leaf curl; LS-Leaf smalling; M-Mosaic; ST-Stunting; VC-Vein clearing; N- Netting of leaves.

infected plants developed systemic symptoms within 6-49 days after inoculation depending on the species/ cultivars. SLCMV infected several species in Solanaceae (Fig.1 and 2), but in Euphorbiaceae it infected only cassava (Fig. 3), cera rubber (Fig. 4), *Euphorbia hirta* (Fig. 5) and *Jatropha curcus* (Fig. 6). In addition, 23 different cultivars of *N. tabacum* were also infected by whitefly inoculation (Fig. 7) Symptoms observed on these plants include chlorotic spots, curling of leaves, leaf distortion, leaf smalling, mosaic, stunting, vein clearing, netting of leaves etc. Among *Nicotiana* species, *N. nesophila* exhibited symptoms 6 days after inoculation and *N. benthamiana* exhibited symptoms 9 days after inoculation. However, *N. ingulba* and *N. otophora* took 28 days to express symptoms. Most of the *Nicotiana* species exhibited

Fig. 1. *N. rotundifolia* showing mosaic, severe leaf curling and severe stunting

Fig. 2. *N. hybrid* showing mosaic, curling of plant top and severe stunting

Fig. 3. *M. esculenta* showing mosaic

Fig. 4. *M. glaziovii* showing mosaic, mild upward curling

symptoms at 12-18 days after incubation. Among all the *Nicotiana* species used in this study, *N. longiflora* and *N. rotundifolia* had 100% infection while other hosts had 10-80% infection. Among the different cultivars of *N. tabacum* cvs. Jayasri, Samsun, Virginia gold, Delecrest and CTRI special were highly susceptible to SLCMV as they had exhibited 100% infection. (Table.2).

Among the Euphorbiaceae plants, *Jatropha curcus* expressed symptoms after 33 days and *Euphorbia hirta* expressed symptoms after 30 days. *Manihot esculenta* and

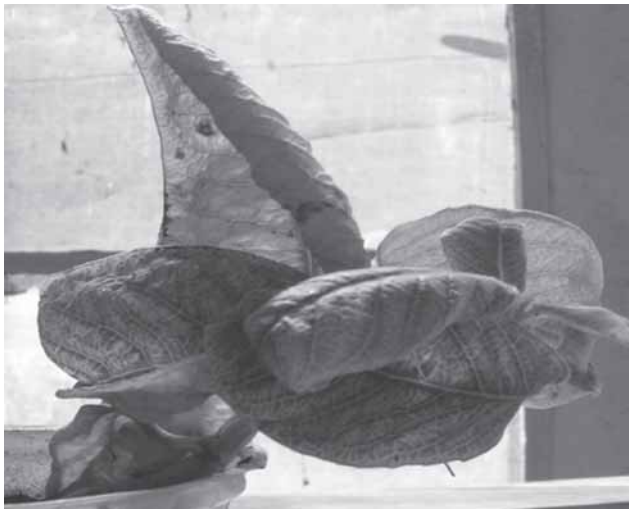

Fig. 5. *E. hirta* showing mosaic, vein clearing and severe leaf curling

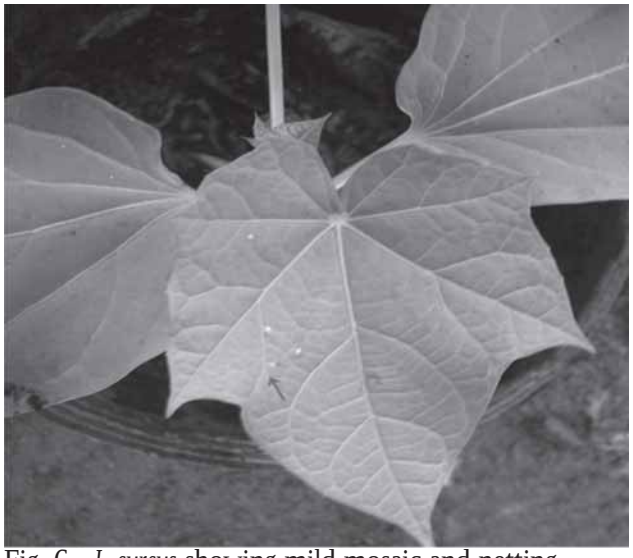

Fig. 6. *J. curcus* showing mild mosaic and netting

Fig. 7. *N. tabacum* cv. Samsun showing mosaic, leaf curling, stunting and leaf deformation

M. glaziovii expressed symptoms after 10 and 22 days respectively. Percent transmission was very low in *J. curcus* when compared to *E. hirta*, *M. esculenta* and *M. glaziovii*.

Gomphrena globose (Amaranthaceae); *Chenopodium album*, *C. amaranticolar*, *C.quinoa* (Chenopodiaceae); *Cucumis sativus*, *C. pepo*, *Citrullus lanatus* (Cucurbitaceae), *Acalypha indica*, *Euphorbia geniculata*, *J. gossypifolia*, *Ricinus communis* (Euphorbiaceae), *Cassia fistula*, *Phaseolus vulgaris*, *Vigna radiata* (Fabaceae); *Abelmoschus esculentus*, *Althaea rosea*, *Malvastrum coromandelianum* (Malvaceae), *Capsicum annuum*, *Lycopersicon esculentum*, *Solanum indicum*, *S. nigrum*, *S. tuberosum*, *Datura stramonium*, *N. benavidesii*, *N. naudicaulis*, *N. alata*, *N. excelsior*, *N. gluca*, *N. cavicola* and *N. petunoides* (Solanaceae) did not show any symptoms upon SLCMV infection by whitefly inoculation and it shows they are not the potential hosts for SLCMV.

PCR analysis using the total DNA isolated from all these host plants inoculated with SLCMV through whitefly transmission were done using primer for coat protein gene of SLCMV. Positive amplification was observed only with the plants exhibited symptoms except *Nicotiana trigonophylla* which did not exhibit any symptom but showed positive amplification in PCR.

Host range studies of SLCMV revealed that it is limited to plant species belonging Euphorbiaceae and Solanaceae. Altogether 69 different plant species/cultivars were infected with SLCMV in the host range studies. Out of the 69 species of plants inoculated with SLCMV by *B. tabaci*, 46 were susceptible, of which 42 belonged to Solanaceae and 4 belonged to Euphorbiaceae (*M. glaziovii*, *M. esculenta*, *Euphorbia hirta* and *Jatropha curcus*). In addition, 23 different cultivars of *N. tabacum* were also infected by whitefly inoculation (Table. 2). All the infected plants produced systemic symptoms. The incubation period in the host varied from 6 to 49 days depending on the host species involved.

Menon and Rayachaudhuri (1970) reported cucumber as a host of ICMV in India by whitefly inoculation. However, in the present study SLCMV was not transmitted to cucumber in spite of several attempts. This finding may be effectively exploited for using as host for getting pure culture of ICMV from cassava plants having mixed infection of ICMV and SLCMV. Several reports have showed that *M. glaziovii* and other *Manihot* spp. as the host of ACMV in Africa (Deighton,1926;

Table 2. Symptoms induced in different cultivars of *N. tabacum* in response to whitefly inoculation of SLCMV

Host inoculated	Transmission (%)	Symptoms	Incubation period (days)
Anand 2	45	D, LC	14-20
Anand 119	50	M, D, LC	16-22
Anand 145	35	M, D, LC	15-18
CTCRI special	100	LC, M, CPT, D	12-20
Delecrest	100	LC, M, CPT, D	18-24
FCV candel	45	LC, M, D	10-16
FCV -Florida-2	50	LC, M, D	18-26
FCV-Hicks103	45	LC, M, D, CPT	16-22
Hicks special	50	LC, D, ST	12-20
Hirae	30	M, D, ST	12-20
HR 70/64	40	M, D, ST	18-26
GT4	30	LC, CS, ST	14-20
GT5	40	LC, CS, ST	16-24
GT6	50	LC, CS, ST	12-22
Jayasri	100	LC, CPT, D, ST	12-20
MDS-7	45	CPT, D, M, LS, ST	18-28
Oxford-3	35	LC, D, ST	12-22
PCT-7	75	LC, D, ST	14-20
Samsun	100	LC, D, ST	15-26
Virginia gold	100	LC, M, D, ST	18-28
White Burly	60	LC, CPT, D, ST	15-20
Xanthi	30	CS, LC, D, M, ST	16-22

CS-Chlorotic spots; CPT-Curling of plant top; D-Leaf distortion; LC-Leaf curl; LS-Leaf smalling; M-Mosaic; ST-Stunting; VC-Vein clearing; N- Netting of leaves.

Kufferath and Ghesquire,1932; Golding,1936; Okusanya and Ekandem,1973; Singh,1975; Dubern,1979) and in the present study also showed *M. glaziovii* as host to SLCMV. Singh (1975) reported castor, *Ricinus communis* as a collateral host of ACMV. But in the present study, *R. communis* was not infected with SLCMV. This reveals that this is not a host for SLCMV even though this virus is one among CMGs. Dubern (1979) could not get transmission of ACMV to any plant species other than *Manihot* spp. by using *B. tabaci*, but the present study revealed that whitefly can successfully transmit SLCMV to many of the hosts tested. These findings clearly establish that there is wide host range variation among the CMGs.

In India, there is no information available on the host range of SLCMV transmitted through whiteflies and all the hosts reported here are the new ones. SLCMV was reported to be transmitted to 39 species of plants belonging to Solanaceae (*Datura stramonium* and 38 species of *Nicotiana*) through mechanical inoculation (Anitha et al 2008). ICMV was earlier reported to be transmitted to 48 different species and 23 different cultivars of *N. tabacum* by *B. tabaci* as well as through sap inoculation (Mathew et al 1993). When compared to ICMV, host range of SLCMV was almost similar but varied with per cent transmission which is evident from 100% transmission obtained in *N. tabacum* cvs. Jayasri, Anand 145, Candel, Florida, Virginia gold, Burley 21 by sap inoculation as well as through whitefly transmission. The findings of the present study becomes important as presence of these susceptible hosts in the cassava growing areas will serve as source of inoculum which can be easily transmitted to the main host (cassava) easily through whitefly which plays active role in field level transmission. Apart from this, the study also revealed that the highly susceptible species/cv of *N. tabacum* can be used as propagative host for all experimental purpose for studying SLCMV.

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