Journal of Root Crops, 2008, Vol. 34 No. 1, pp. 21-25
Indian Society for Root Crops
ISSN 0378-2409

Host Range of Sri Lankan Cassava Mosaic Virus

Anitha Jose, T. Makeshkumar and S.Edison
Central Tuber Crops Research Institute, Sreekariyam
Thiruvananthapuram 695 017, Kerala, India
Corresponding author: T. Makeshkumar, e-mail: makeshkumar@yahoo.com

Abstract

Sri Lankan Cassava Mosaic Virus (SLCMV) causes mosaic disease in cassava in India and Sri Lanka. Among the 75 plant species belonging to seven families screened / tested against the virus, SLCMV was transmitted to 39 species belonging to Solanaceae (*Datura stramonium* and 38 species of *Nicotiana*) through sap inoculation. The incubation period for symptom appearance varied from 5-40 days depending on the species. Rest of the 36 species didn't exhibit any symptom of virus infection until 90 days. Presence or absence of SLCMV in the hosts was confirmed through PCR.

Keywords: Sri Lankan Cassava Mosaic Virus, host range, sap transmission, PCR

Introduction

Cassava (*Manihot esculenta* Crantz) is the staple or subsidiary food for about a fifth of the world's population (Edison, 2000) and raw material for starch based industries in many parts of the world. In India, it is grown in an area of 2,42,400 ha with an annual production of 76,20,200 tonnes (FAO, 2006) for both human and animal consumption as well 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 India the disease can cause a yield loss of 17-88% depending on the cultivars grown (Malathi et al., 1985).

Cassava mosaic disease is caused by a virus included in the genus *Begomovirus* (Family: *Geminiviridae*) which 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. In India, the cassava mosaic disease is caused by two viruses namely Indian cassava mosaic virus (ICMV) and Sri Lankan cassava mosaic virus (SLCMV) (Malathi et al., 1983; Dutt et al., 2005). However it is not possible to distinguish the viruses based on symptoms on cassava.

Since Cassava is vegetatively propagated, the cassava mosaic disease is carried from one crop cycle to the next, through the cuttings (setts) used as planting material and in the field mostly through whitefly transmission. The causal virus, ICMV was reported to be transmitted by whitefly *Bemisia tabaci* from cassava to cucumber (Menon and Raychaudhuri, 1970; Mathew and Muniyappa,1993) and cassava to cassava (Antony et al., 2006). ICMV was transmitted by mechanical inoculation from cassava to *Nicotiana benthamiana* and *N. glutinosa* (Malathi and Sreenivasan, 1983), *Datura stramonium*, *Manihot esculenta*, *M. glaziovii*, *Nicandra physalodes*, *Petunia hybrida* and 43 species of *Nicotiana* (Mathew and Muniyappa, 1993). Occurrence of SLCMV in cassava was first reported by Saunders et al. (2002) and till now there is no report on the host range and transmission. Survey of cassava mosaic disease in Kerala revealed that SLCMV is more common than ICMV (Dutt et al., 2005).

During our recent surveys in Kerala, up to 100% incidence of the mosaic disease was noticed in most of the cassava genotypes/ cultivars grown in Kerala. Like any other pathogens, SLCMV also requires different host plants for survival, multiplication and virulence. As there is no information is available on this aspect, the present study was undertaken to know the host range of SLCMV, which is essential for devising a possible management strategy to contain this disease.

Materials and methods

Virus culture

Plants showing cassava mosaic disease were collected from different parts of Kerala and the total DNA was isolated from these plants as per the procedure described by Lodhi et al. 1994. The presence of SLCMV infection was confirmed through multiplex PCR as described in Patil et al. 2004. SLCMV infected plant collected from Malappuram district was maintained in an insect proof glass house at CTCRI and utilized as the source of inoculum for the host range study.

Host range of SLCMV

Seeds of 75 plant species belonging to 7 families viz., Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Euphorbiaceae, Fabaceae, Malvaceae (seeds of plant species of these families obtained from Agricultural College, Vellayani, Thiruvananthapuram) and Solanaceae (seeds obtained from Central Tobacco Research Institute, Rajahmundry, Andhra Pradesh) were sown in pots kept an insect proof net house and the seeds germinated after two weeks. Four to five weeks old healthy seedlings (10 no.) were mechanically inoculated with sap. Plants were observed for symptom development up to twelve weeks.

Sap inoculation

SLCMV infected cassava plant maintained in the glass house was used as source of inoculam. The sap was extracted from the infected cassava leaves grinding in 0.1M sodium phosphate buffer pH 7.0 and inoculated on to carborundum (600 mesh) dusted leaves of test plants. Inoculation was done by rubbing the upper surface of the top most two or three fully opened leaves with a sterile absorbant cotton swab dipped in inoculum. Immediately after inoculation, the leaves were washed with tap water and the plants were kept in an insect proof net house for observation. Ten plants were used in each host species and in each case five plants per species were inoculated with phosphate buffer as control. Symptom development on these plants was recorded at five days interval up to 90 days.

PCR

Total DNA was isolated from all the host plants (Lodhi et al., 1994) to confirm the SLCMV infection through PCR amplificaton (Makeshkumar et al., 2005) using the primers specific to SLCMV.

Primers	Sequence (5' - 3')
SLCMV-A-F	TGT AAT TCT CAA AAG TTA CAG TCN
SLCMV-A-R	ATA TGG ACC ACA TCG TGT CN

Results

Out of 75 plant species inoculated, 39 species belonging to Solanaceae were infected. All the infected plants developed systemic symptoms within 5- 40 days after inoculation depending on the species (Table 1). Among the Solanaceous hosts used in the present study, *Datura stramonium* and different species of *Nicotiana* showed infection (Table 2). The symptoms observed on these plants include, chlorotic spots, curling of leaves, leaf distortion, reduction in leaf size, mosaic, stunting, vein clearing etc., (Fig.1). Among the *Nicotiana* species, *Nicotiana benthamiana* exhibited symptoms five days after inoculation. However *N. stimulans* took 30 days to express the symptoms. Most of them expressed the symptoms after 10 - 15 days of inoculation. Among all the *Nicotiana sp.* used in this study, *N. benthamiana, N. hybrid, N. clevelandii, N. rotundifolia, N. tabacum* cv. Jayasri showed 100% infection while other hosts showed 20 - 90 % infection. Among the different cultivars of *N. tabacum* tested, CTRI Special, Jayasri and Virginia Gold are highly susceptible to SLCMV as they had exhibited 100% infection.

Gomphrena globosa (Amaranthaceae), *Chenopodium album*, *C. amaranticolar*, *C. quinoa* (Chenopodiaceae), *Cucumis sativus, C. pepo, Citrullus lanatus* (Cucurbitaceae), *Acalypha indica, Euphorbia geniculata, E. hirta, Jatropha curcus, J. gossypifolia, Manihot esculenta*, *M. glaziovii, 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, Nicotiana amplexicaulis*, *N. benavidesii*, *N. naudicaulis, N. alata, N. excelsior*, *N. gluca, N. petunoides*, *N. otophora, N. plumbaginifolia and N. trigonophylla* (Solanaceae) did not show any symptom till 90 days period of observation and hence found to be not susceptible to SLCMV infection.

Table 1. Transmission and symptom characteristics of host range of solanaceous plants for SLCMV

Host	Transmission (%)	Symptoms	Incubation period in days
Datura stramonium	60	CS,M,LC	12-14
Nicotiana accuminata	30	CS,CPT,M,D	12-18
N. arentsii	40	CS,LC,M	12-22
N. attenuata	50	LC,M	10-18
N. benthamiana	100	LC,VC,M,CPT,ST	5-12
N. begelovii	50	M,ST	12-18
N. bonariensis	80	LC,D,M	12-16
N. cavicola	20	LC,D,ST	10-20
N. clevelandii	100	LC,D,M,ST,VC	10-14
N. cordifolia	20	M,D	10-20
N. corymbosa	20	M,D	10-20
N. debneyi	30	CS,M,D	12-22
N. forgetiana	50	CS,D,M,ST	14-24
N. glutinosa	60	CS,CPT,LC,ST,LS	10-20
N. goodspeedi	80	LC,M,D,ST,LS	10-20
N. gossei	50	CPT,LC,D,M	12-18
N. hybrid	100	LS,LC,M,CPT,ST	16-30
N. ingulba	50	D,M,ST	20-28
N. knightiana	20	LC,VC,CPT,ST	12-18
N. maritima	20	CS,M,D	10-20
N. megalosiphon	50	CS,D,M,LS	15-20
N. miersii	60	CS,D,M	12-20
N. nesophila	90	CS,D,M,LS	10-18
N. occidentalis	80	CS,D,M	15-22
N. paniculata	50	CPT,D,M,ST	12-20
N. pauciflora	50	CPT,D,LS,ST	12-18
N. raimondii	40	CPT,LC,D,M	28-40
N. repanda	90	CPT,LC,D,M, ST	12-18
N. rosulata	80	CS,M,LS	22-28
N. rotundifolia	100	CPT,LC,D,ST	20-25
N. rustica	40	LC,M,D,ST	12-22
N. simulans	50	LC,D,M	30-40
N. solanifolia	60	CS,CPT,D	23-32
N. stocktonii	50	CS,D,M	10-18
N. suaveolens	30	CS,CPT,LC,D	10-16
N. sylvestris	20	CS,LC,ST	20-30
N. tabacum cv. Jayasri	100	LC,CPT,D,ST	12-18
N. umbratica	60	CPT,D,M,ST	16-24
N. undulata	70	LC,D,M	11-14

Symbols used for symptoms: CS = Chlorotic spots; CPT = Curling of plant top; D = Leaf distortion;LC = Leaf curl; LS = Leaf stunting; M = Mosaic; ST = Stunting; VC = Vein clearing.

Table 2. Transmission and symptom characteristics of different cvs. of *N. tabacum* for SLCMV infection

N.tabacum cvs.	Transmission (%)	Symptoms	Incubation periods in days
Anand 2	50	D,LC	10-24
Anand119	50	M,D,LC	12-20
Anand 145	30	M,D,LC	10-20
CTRI special	100	LC,M,CPT,D	15-20
Delecrest	100	LC,M,CPT,D	12-24
FCV candel	50	LC,M,D	12-18
FCV -Florida— 2	50	LC,M,D	16-28
FCV-Hicks103	40	LC,M,D,CPT	12-20
Hicks special	60	LC,D,ST	14-20
Hirae	40	M,D,ST	12-20
HR 70/64	40	M,D,ST	12-20
GT4	50	LC,CS,ST	15-22
GT5	40	LC,CS,ST	12-20
GT6	50	LC,CS,ST	12-20
Jayasri	100	LC,CPT,D,ST	10-18
MDS-7	60	CPT,D,M,LS,ST	20-25
Oxford-3	40	LC,D,ST	12-20
PCT-7	60	LC,D,ST	12-20
Samsun	100	LC,D,ST	18-25
Virginia gold	100	LC,M,D,ST	16-22
White Burley	50	LC,CPT,D,ST	12-18
Xanthi	30	CS,LC,D,M,ST	14-20
Yellow gold	60	LC,D,ST	10-20

Symbols used for symptoms CS = Chlorotic spots, CPT = Curling of plant top, D = Leaf deformation, LC = Leaf curling, M = Mosaic, ST = Stunting

PCR was done using the total DNA isolated from all the host plants using SLCMV primer to diagnose the infection with SLCMV. Positive amplification was observed with ~600bp band only in plants with symptoms (Fig.2) and no amplification was observed with other hosts which did not exhibited symptoms.

Discussion

The present study revealed that by mechanical inoculation, the host range of SLCMV is limited to Solanaceae. Among 39 species of Solanaceae infected, except *D. stramonium,* all others were *Nicotiana* species. Among the *Nicotiana* species, *N. alata, N. excelsior*, *N. gluca*, *N. longiflora, N. otophora, N. petunoides, N. plumbaginifolia* and *N. trigonophylla* could not be infected by sap inoculation. ICMV was earlier reported to be transmitted to 43 species of *Nicotiana* plants by sap inoculation (Mathew et al., 1993).

ACMV was reported to be transmitted to 13 species of plants by sap inoculation (Bock et al., 1978; Walter, 1980). When compared to ICMV, host range of SLCMV was almost similar. However, in the present study *N. amplexicaulis*, *N. benavidesii*, *N. naudicaulis*, a host of ICMV was not infected by SLCMV and *Petunia hybrida*, *Nicandra physalodes*, *N. tomentosiforms*, *N. velutina* was not inoculated (Mathew et al., 1993). Mathew et al. (1993) failed to transmit ICMV to *N. longiflora* by sap inoculation, but in the present study it was infected with SLCMV. As compared to previous studies, in the present study

Fig.1. Different types of symptoms induced due to SLCMV infection in solanaceous hosts A. *Datura stramonium* – mosaic B. *Nicotiana tabcum* cv. Delecrest – Leaf distortion; C. *Nicotiana clevlandii* – Vein clearing D. *Nicotiana rependa* – Stunting; E. *Nicotiana rotundifolia* – curling of leaf

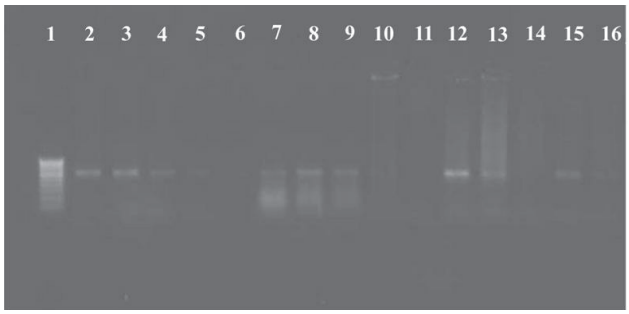

Fig.2. Diagnosis of SLCMV infection in hosts inoculated with SLCMV through PCR using SLCMV specific primers

Lane details: 1- 100bp marker 2- *N. benthamiana*
3-*N.* h*ybrid* 4-*N. bonariensis*
5- *N. cavicola* 6-*N. otophora*
7-*N. glutinosa* 8- *N. nesophila*
9-*N. rustica* 10- *N. alata*
11-*N. longiflora* 12-*N. tabaccum* cv. Jayasri
13-*N. paniculata* 14-*N. gluca*
15-*N. repanda* 16-*N. accuminata*

100 % transmission was obtained in *N. tabacum* cv. CTCRI special, Delecrest, Jayasri, Samsun and Virginia Gold by sap inoculation. Findings of the present study revealed that several species/cv. of *Nicotiana* are highly susceptible and can be used as propagative host for SLCMV. This information can be used in containing the disease.

Acknowledgment

Senior author is grateful to the Director, Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram for providing necessary facilities.

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