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REACTION OF CHILLI ACCESSIONS TO LOCAL ISOLATES OF CUCUMBER MOSAIC AND CHILLI VEINAL MOTTLE VIRUSES

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ABSTRACT

Cucumber mosaic virus (CMV) and chilli veinal mottle virus (CVMV) are the most important viruses which cause yield losses in chili cultivation. These viruses are transmitted by aphids and by sap inoculation. Development of resistant varieties is the only feasible measure to manage virus diseases. Studies were conducted at Field Crops Research and Development Institute to identify the local isolates and resistant sources for CMV and CVMV during 1999- 2000. Field surveys indicated that, CMV and CVMV were prevalent in Anuradhapura and Mahaweli-H areas and mixed infection of both viruses was common. Two isolates of CMV and one isolate of CVMV were identified by host range and serology. Fourteen and eighteen chilli accessions received from AVRDC, Taiwan tested by sap inoculation, eight and eleven accessions were free of infection and also gave a negative reaction to DAS-ELISA test for two isolates of CMV and CVMV isolate respectively. The identified resistant sources could be used in breeding programmes to develop resistant chilli varieties with desirable agronomic characteristics.

KEYWORDS: Cucumber Mosaic Virus, Chilli Veinal Mottle Virus, Resistant Chilli Cultivars

INTRODUCTION

Chilli (*Capsicum annum* L.) is one of the major condiments grown in Sri Lanka. Farmer's average yield of chilli is lower (around 1 t/ha) than the potential yield of recommended varieties (3 t/ha). Low levels of chilli yield are attributed to many factors including virus diseases. So far, 40 different viruses have been reported from chilli throughout the world (Green and Kim, 1991). In Sri Lanka, the most economically important viruses in chilli are leaf curl and mosaic types (Sujiura *et al.*, 1975). Field surveys conducted under South Asian Vegetable Research Network (SAVERNET) Phase-1 have shown that Cucumber Mosaic Virus (CMV) and Chilli Veinal Mottle Virus (CVMV) were prevalent in major chilli growing areas followed by leaf curl virus (Anonymous, 1996). Shivanathan (1977) reported that CMV has been isolated rom over 20 host species in Sri Lanka. These viruses are transmitted by aphids and also by sap inoculation. CMV infects more than 770 plant species belonging to 85 families (Franki, 1979). Symptoms of CMV infection in chilli are mosaic, mottle, yellow discoloration, vein clearing, leaf narrowing and leaf deformation while symptoms in CVMV are dark green mottle, vein banding, and reduce leaf size, leaf distortion and small fruits. However, the host range of CVMV is confined to the solanaceae (Ong *et al.*, 1980). Development of resistant varieties is the only feasible measure to manage virus diseases. However, information on the local strains and disease management is lacking in Sri Lanka. In view of this the present investigation was carried out to identify local isolates of CMV and CVMV and to identify resistant cultivars to these viruses.

MATERIALS AND METHODS

Survey for CMV and CVMV of chilli

A survey was carried out in two chilli- growing areas of Anuradhapura and Mahaweli-H during July September 1998 and February 2000 for isolation of CMV and CVMV respectively. In each area, 3-5 locations were visited and samples were collected from plants showing symptoms of mosaic, mottling, leaf narrowing and vein banding etc. The samples were tested according to the Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA) technique (Clark and Adams, 1977) using polyclonal antisera specific to CMV, CVMV, Potato virus Y (PVY), Tobacco mosaic virus (TMV) obtained from Asian Vegetable Research and Development Center (AVRDC).

Isolation of CMV and CVMV of chilli

Field samples positive only for CMV and CVMV antisera were selected in 1998 and 2000 respectively. Isolation of CMV and CVMV was carried out according to AVRDC protocol (Green, 1997). The pure isolates of CMV was obtained by a series of single lesion inoculation from *Chenopodium amaranticolor* and subsequent inoculation to the systemic host, *Nicotiana glutinosa* while CVMV was purified by single lesion inoculation from *N. tabacum* white burlay to *N. glutinosa.* Pure cultures of CMV and CVMV were maintained in *N. glutinosa* and *Capsicum annum* in the insect proof green house at FCRDI, Maha Illuppallama, respectively. The purity of CMV and CVMV isolates was re-confirmed using DAS- ELISA test from time to time.

Host range studies and symptomatology

Host range of local isolates of CMV and CVMV determined by mechanical inoculation of 11 plant species belonging to 4 families. Inocula were prepared by grinding 1 g of infected leaves from maintenance host of isolates CMV and CVMV with 4 ml 0.03M phosphate buffer (pH 7.0) containing 1.7 g/l sodium diethyldithiocarbamate (Na-DIECA), 0.5 g/l Na_2CO_3 and 0.1 g/l activated charcoal. Each inoculum was gently rubbed onto carborundum-dusted leaves of four plants of each host species with the broad end of the pestle and rinsed with tap water to remove inhibitory deposits. To detect latent infections, plant showing no symptoms were assayed for the presence of the virus using DAS- ELISA three weeks after inoculation.

Resistance screening against CMV and CMV

Eleven chilli lines received from AVRDC along with three local varieties (MI-2, KA- 2 and Arunalu) were screened against CMV isolate 1 and 2 in 1999. Fifty seeds of each line was sown on sterilized soil in clay pots (1 seed/pot) and kept in the insect-proof greenhouse. Ten to twenty seedlings of each line at five leaf stage were inoculated with extracts of both isolates

(CMV-1 and CMV-2) prepared from *N. glutinosa* leaves (maintenance host). AVRDC protocol was followed for inoculation and to assign disease rating for test plants (Green, 1997). Visual symptoms of CMV were observed 1 and 2 weeks after the 1^{si} inoculation and plants were tested according to DAS-ELISA using polyclonal antisera specific to CMV. The plants which showed negative results were re-inoculated and retested using DAS-ELISA test 2 weeks after the 2nd inoculation and the number of infected plants was counted.

CVMV isolate obtained from Mahaweli-H area was used for CVMV resistant screening in 2000. Fifteen AVRDC chilli lines and three local chilli varieties were tested. Ten seedlings of each line at 3-leaf stage were inoculated with extracts of CVMV isolates prepared from chilli leaves (maintenance host) and ground in 0.03 M Na–phosphate buffer (same buffer described earlier). Each chilli line was replicated 4 times and all 3 leaves were inoculated with the extracts 50 days after planting. Visual symptoms of CVMV were recorded after 2 weeks and tested with DAS-ELISA using polyclonal antisera specific to CVMV confirmed the 1st inoculation and infection. The plants which showed negative results were re-inoculated and retested with DAS-ELISA 2 weeks after the 2nd inoculation and the numbers of infected plants were counted.

RESULTS AND DISCUSSION

Survey for CMV and CVMV of chilli

Of the 20 samples tested from Mahaweli-H area, 10 were positive to CMV (Table 1). Mix infection of CMV and CVMV was only detected in Mahaweli H area (30- 37%). CVMV incidence was higher (50%) in February 2000 as compared with the 1998 (10%) in Mahaweli H area (Table 2). The cause of this increase (40%) is not well understood but might be due to a number of factors such as climatic conditions, vector activity, susceptibility of chilli crop, presence of alternative host. Out of 8 locations, two places in Anuradhapura (Rajangane and Mihintale) were CVMV free. The incidence of CMV in Anuradhapura was 80% during July - September, 1998 while in the February 2000 it was 10%. PVY and TMV were not detected in any of the samples. It is obvious from this observation that CMV and CVMV are still the most prevalent viruses associated with chillies and mix infection of both viruses was common in Sri Lanka.

Table 1. Chilli viruses detected using DAS – ELISA test from different locations in Anuradhapura and Mahaweli-H area in 1998

Location	*No. of Samples Tested*	*No of Samples Positive*		
		CMV	*CVMV*	*CMV + CVMV*
Mahaweli-H area				
Galnewa	6	4	-	2
Maha Illuppallama	2	-	1	-
Eppawela	6	3	1	2
Thabuththegama	3	1	-	1
Usgalasiabalangamuwa	3	2	-	1
Anuradhapura				
Rajangane	5	5	-	-
Galenbidunuwewa	5	3	2	-

Isolation of CMV and CVMV of chilli

Two isolates of CMV obtained, one from Anuradhapura designated as CMV-1, another from Mahaweli-H area designated as CMV-2 were maintained on *N. glutinosa* in pure form separately, in the insect-proof greenhouse. Only one isolate of CVMV obtained from Mahaweli H area was maintained on *C. annum* in pure form in the insect-proof greenhouse and all isolates were used for resistant screening studies.

Table 2. Chilli viruses detected using DAS – ELISA test from different locations in Anuradhapura and Mahaweli-H area in 2000

Location	*No. of Samples Tested*	*No of Samples Positive*		
		CMV	*CVMV*	*CMV + CVMV*
Mahawelli-H area				
Galnewa	9	-	4	4
Maha Illuppallama	4	-	3	-
Eppawela	8	-	3	5
Thabuththegama	4	-	2	1
Usgalasiabalangamuwa	5	-	3	1
Anuradhapura				
Rajangane	8	2	-	-
Galenbidunuwewa	8	-	3	-
Mihintale	4	-	-	-

Host range studies and symptomatology

The reactions of the different plant species to the two isolates of CMV and CVMV isolates are summarized in Table 3. Both isolates of CMV serologically more related but isolate CMV-1 was found to be more severe of many host species. All plant species were susceptible to both isolates of CMV, showed local or systemic symptoms. Cucumber exhibited symptoms 7 days of inoculation and was the only host, which responded differently to both CMV

isolates Unlike CMV, infection of CVMV isolate mostly confined to solanaseae family. However, *N. glutinosa* and *N. tabacuum* (white burly) did not show any symptoms, but DAS- ELISA test indicated symptomless infection for the CVMV isolate.

Table 3. Host range of CMV and CVMV isolates[1]

Plant Species	*CMV Isolate*		*CVMV Isolate*
	CMV-1	*CMV-2*	
Solanaceae			
Nicotiana glutinosa	SM	M	LS
Nicotiana tabacum (white burly)	SM	M	LS
Nicotiana tabacum	NL(IL)	NL(IL)	-
Nicotiana benthamina	LN	LN	-
Capsicum annum	M	M	VB
Lycopersicum esqulentem	LN	LN	-
Chenopodiaceae			
Chenopodium amaranticolor	LL	LL	NL(IL)
Chenopodium murulae	NL(IL)	NL(IL)	-
Chenopodium quinoa	NL(IL)	NL(IL)	-
Cucubitaceae			
Cucumis sativas	SM	VC	-
Trichosanthes cucumerina	CS	CS	NT
Leguminaceae			
Vigna unguiculata	NL(IL)	NL(IL)	-

[1] CS- chlorotic spot, IL - inoculated leaf, LN- leaf narrowing, LS - symptomless infection, M - mosaic, NL - necrotic lesion, NT- Not tested, SM - severe mosaic, VB - vein banding, VC- vein clearing, - no infection

Resistance screening for CMV

The results of greenhouse screening of chilli lines against isolates of CMV-1 and CMV-2 are given in Table 4 and 5. Eight out of the eleven AVRDC chilli lines did not show any symptoms and ELISA negative for both CMV-1 and CMV-2 isolates. Theses lines were regarded as highly resistant against local isolates of CMV infection. The susceptible check PBC370-2-2-1 mainfested severe mosaic and leaf deformation that gave strong reaction to CMV antiserum (scored visually) and rated as highly susceptible to both isolates. Line PBC521-2-1-1 was found to have 45% infection (visually) for CMV-1 but DAS- ELISA results confirmed 100% infection. According to DAS-ELISA results, 55% of the plants were symptomless carriers of CMV-1. All three local varieties were susceptible (16% - 100% infection) for both CMV-1 and CMV-2.

Table 4. Disease reaction of chilli accessions against CMV-1 isolate from Anuradhapura in 1999

Line	*Name*	*Infected Plants Deleted (%)*		*Type of Symptoms*[1]	*Disease Reaction*[2]
		Visually	*ELISA*		
VC16aNo5-1-1-1	PerennialHDV	0	0	NS	HR
VC41aNo3-1-1-5	HDV295	0	0	NS	HR
VC236-2 (5-5)	Toom	20	20	M, D	MS
VC232-1-4	P-3	0	0	NS	HR
PBC148A-1	Punjab Lal	0	0	NS	HR
PBC455-1	-	0	0	NS	HR
PBC518	PSP 11	0	0	NS	HR
PBC521-2-1-1	Tiwari 2	45	100	SM	HS
PBC549-3-2-1	LV2722	0	0	NS	HR
PBC569-5-2-1	-	0	0	NS	HR
PBC370-2-2-1(S.ck.)	Thai Bird's eye	100	100	SM, LD	HS
MI-2		75	75	M	HS
KA-2		100	100	M	HS
Arunalu		70	70	M	HS

1. M - mosaic, LD - leaf deformation, D - distortion, NS - no symptoms, SM - severe mosaic
2 HR - Highly resistant (0%), MR - Moderately resistant (1% - 10%), MS – Moderately susceptible (11- 20%)
S - Susceptible (21-50%), HS - Highly susceptible (51% and above)

Table 5. Disease reaction of chilli accessions against CMV-2 isolate from Mahaweli H area in 1999

Line	*Name*	*Infected Plants Detectec*		*Type of Symptoms*[1]	*Disease Reaction*[2]
		Visually	*ELISA*		
VC16aNo5-1-1-1	PerennialHDV	0	0	NS	HR
VC41aNo3-1-1-5	HDV295	0	0	NS	HR
VC236-2 (5-5)	Toom	10	10	M,D	MR
VC232-1-4	P-3	0	0	NS	HR
PBC148A-1	Punjab Lal	0	0	NS	HR
PBC455-1	-	0	0	NS	HR
PBC518	PSP 11	0	0	NS	HR
PBC521-2-1-1	Tiwari 2	17	17	M	MS
PBC549-3-2-1	LV2722	0	0	NS	HR
PBC569-5-2-1	-	0	0	NS	HR
PBC370-2-2-1(S.ck)	Thai Bird's eye	67	67	M,LD	HS
MI-2		25	25	M	S
KA-2		NT	NT	-	-
Arunalu		16	16	M	MS

1. M – mosaic, LD - leaf deformation, D – distortion, NS - no symptoms, NT- not tested
2 HR - Highly resistant (0%), MR - Moderately resistant (1% - 10%), MS - Moderately susceptible (11- 20%)
S - Susceptible (21-50%), HS - Highly susceptible (51% and above)

Table 6. Reaction of chilli accessions to CVMV isolate from Mahaweli H area in 2000

Accessions	*Name*	*Infected Plants Detected*		*Type of Symptoms*[1]	*Disease reactions*[2]
		Visually	*ELISA*		
VC16No.1-4-1	Perennial HDV	0	0	NS	HR
VC58a-8	HDV832	0	0	NS	HR
VC160a-1	PSP-11	0	0	NS	HR
VC185a	Saegochu	0	0	NS	HR
VC240-1-1	Panjab Lal	0	17	NS	MS
VC241-1-1	Taiwan83-168	0	0	NS	HR
PBC521-1-1	BG-1	0	0	NS	HR
PBC549	LV2722	100	100	Mo,VB,LD	HS
VC237-1-1	Hybrid Huareu	0	0	NS	HR
PBC524-2-1	Serrano Huasteco	0	0	NS	HR
PBC491	Yolo wonder	0	0	NS	HR
VC36aNo.5-1	HDA249	0	14	NS	MS
VC235-5	Lek Haeng	0	0	NS	HR
VC236-3	Toom	0	0	NS	HR
VC239-1-1	Chinda1-2	30	30	Mo,VB,LD	S
MI-2		27	27	Mo,VB	S
KA-2		10	10	MO	MR
Arunalu		3	3	MO	MR

1 MO – mottle, LD - leaf deformation, NS - no symptoms, VB - vein banding
2 HR- Highly resistant (0%), MR - Moderately resistant (1% - 10%), MS – Moderately susceptible (11- 20%) S- Susceptible (21-50%), HS- Highly susceptible (51% and above)

Resistance screening of chilli for CVMV

The results of greenhouse screening of chilli against CVMV isolate given in table 6. Eleven AVRDC lines were found free of infection on the basis of visual observation and also had negative DAS- ELISA reactions for CVMV isolate. Eventhough chilli lines VC240-1-1 and VC36a No5-1 were symptomless, they were positive for CVMV infection by DAS-ELISA. Line PBC549 appeared as the highly susceptible with highest infection rate (100%). It produced severe mottling and vein banding symptoms after 10-12 days of the first inoculation and also gave a very strong reaction to the DAS-ELISA test against CVMV isolate. All local varieties (Arunalu and KA-2) except MI-2 showed moderately resistant to CVMV isolate.

CONCLUSION

Chilli lines selected as resistant to CMV and CVMV could be used as donor sources for the development new chilli varieties having desirable agronomic charactristics and resistance to both viruses.

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