

IDENTIFICATION OF A NEW MOSAIC VIRUS DISEASE OF SNAKE GOURD IN SRI LANKA

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ABSTRACT

In 2001, a virus-like disease of snake gourd variety 'TA-2' was reported from the commercial seed production farm at Dambulla. Leaf mosaic with distortion, reduction of internode length and fruit distortion were prominent symptoms of the disease. The virus was mechanically transmitted to several cucurbitaceous hosts and was seed-borne in snake gourd. Leaves of virus-infected snake gourd with symptoms were examined under the electron microscope, which revealed the presence of high concentration of Tobamovirus in plant cells. Cucumber green mottle mosaic virus (CuGMMV) is the possible cause of the new disease of snake gourd. It is also evident that CuGMMV does not infect plants of Leguminosae, Caricaceae, Compositae, and Solanaceae families. Serological assay with polyclonal antibodies against PRSV-P and CMV antisera confirmed a mixed infection of CuGMMV with CMV and a virus serologically related to PRSV. Host-range studies showed *Trichosanthes cucumerina*, *Cucurbita maxima*, *Benincasa hispida* and *Momordica charantia* as susceptible to the PRSV related virus, while papaya varieties are resistant. This is the first record of CuGMMV and serologically PRSV related virus in snake gourd in Sri Lanka.

KEYWORDS: Cucumber green mottle mosaic virus, Papaya ring-spot virus.

INTRODUCTION

Cucurbits are popular vegetables belonging to the Cucurbitaceae family, cultivated in various agro-ecological zones of Sri Lanka. There are many varieties such as *Momordica charantia* (bitter gourd), *Trichosanthes cucumerina* (snake gourd), *Lagenaria siceraria* (bottle gourd), *Luffa acutangula* (sponge gourd/ridge gourd), *Cucurbita maxima* (pumpkin), *Benincasa hispida* (ash pumpkin), *Cucumis sativus* (cucumber), and *Cucumis pubescens* (kekiri). *Colocynthis citrullus* (watermelon) is a dessert type, while the others are consumed as vegetables in curries.

Viruses are the most common causes of diseases affecting cucurbits worldwide. As a result of virus diseases, yield losses and reduction in growth can occur. Virus diseases are responsible for distortion and mottling of fruits, making the products unmarketable. A complex of viruses is able to infect cucurbits and the most important viruses are cucumber mosaic virus (CMV) (Gibbs, 1970), watermelon mosaic virus -1 (WMV-1/ PRSV-P), watermelon mosaic virus-2 (WMV-2/ PRSV-W) (Purcifull *et al.*, 1984) and zucchini

mosaic virus (ZYMV) (Lisa, 1984). In Sri Lanka, CMV is the most prevalent virus affecting cucurbits.

During 1997 and 1999, it was reported that papaya ring spot virus type W (PRSV-type W) and ZYMV were the most frequently found viruses in cucurbit species cultivated in Brazil (Yuki *et al.*, 2000). These two viruses have been reported in squash too (Cardosa and Pavan, 1999). PRSV-type W, ZYMV, WMV-2 and CMV have been recorded in commercial melon cultivations and 5 weed species in Costa Rica (Sanches *et al.*, 1998). In Spain, PRSV-W has been reported in *Cucumis melo* cultivations in 1995, but it could not be detected in 1996 (Luis *et al.*, 1998). In 1998, host range and ELISA tests have been used for identification of PRSV types found in infected cucurbits in Taiwan (Wang *et al.*, 1998). Mixed infections of CMV, WMV-2 and PRSV have been reported in cucurbit spp. in the Czech Republic (Lebeda *et al.*, 1996).

Cucumber green mottle mosaic virus (CuGMMV) has been reported in snake gourd and cucumber in Europe, India, Japan and Taiwan (Singh, 1995). Fruits of infected plants show dark green warts and are severely malformed. Affected leaves of watermelon show slight mosaic and the flesh of fruits deteriorates. CuGMMV is a rigid, rod-shaped virus and a member of the Tobamovirus group. It has been reported that in India, the virus is transmitted by sap, seed and also by a coleopteran. Its host range is limited to cucurbits, *Chenopodium* spp. and *Nicotiana tabacum* (Singh, 1995). Natural host range of the virus is *Cucumis sativus* (cucumber), *Citrullus vulgaris* (melon) and *Lagenaria siceraria* (bottle gourd).

Recently, an unidentified virus-like disease was reported from watermelons and pumpkins cultivated in the Hambantota district. A similar incidence was also reported in snake gourd from the commercial seed production farm of Ceylon Agro Development Company Private Ltd. (CADC) at Dambulla. Transmission of the virus through infected seeds and sap can cause economic damage to the farmers, as well as to the seed industry. Several experiments were carried out to identify the new virus disease and its host range.

MATERIALS AND METHODS

Experiments were conducted both at the Horticulture Crop Research and Development Institute (HORDI), Gannoruwa and the Field Crop Research and Development Institute (FCRDI), Mahalluppallama from 2001 to 2004.

Virus isolation and Symptomatology

Leaves, fruits and seeds of infected snake gourds were collected from the CADC farm during 2001, to detect virus-like symptoms and to confirm the virus infections. Symptom expressions were recorded and inocula were prepared by grinding 1g of young symptomatic leaves, fruit flesh or seeds in 2 ml of 0.03 M phosphate buffer [pH 7.0, containing 1.7 g/l sodium diethyldithio carbamate (Na- DIECA), 0.5 g/l Na₂ CO₃ and 0.1 g/l activated charcoal]. In the greenhouse, four plants from each cucurbit variety (*Cucubits sativas*, *Cucubits pubescens*, *Cucubits maxima* and *Trichosanthes cucumerina*) were planted in plastic pots filled with sterilized soil. The test plants were kept for twenty-four hours in the dark, prior to inoculation, in order to enhance the effectiveness of the inoculation. Powdered carborundom was dusted on the leaves of the test plants prior to inoculation, in order to facilitate the entry of the virus. Test plants were inoculated at cotyledon stage, and rinsed with tap water to remove inhibitory deposits. Four plants from each cucurbit species were maintained as controls to compare with inoculated plants. Inoculated and control plants were kept in an insect-proof greenhouse and observations were made one week after inoculation. Some of the inoculated snake gourd plants were established in the field for further observations.

Host range studies

Fourteen plant species belonging to 3 families (Cucurbitaceae, Leguminosae and Solanaceae) were used to study the host range of the virus infecting snake gourd samples, at the Field Crops Research and Development Institute, Maha-Illuppallama. The host range study was repeated at the Horticulture Crop Research and Development Institute, Gannoruwa, using 34 plant spp. belonging to six families (Chenopodiaceae, Caricaceae, Compositae and the three families included in the previous study). Enzyme-linked Immunosorbent Assay was performed using PRSV and CMV antisera to test the host-range plants, prior to inoculating them, in order to confirm whether the plants were infected with the viruses. The virus inocula were prepared using infected leaves of maintenance host (snake gourd) and PRSV-type P infected papaya leaves in order to compare the reaction of PRSV related new virus, following the previously described inoculation procedure. Three plants from each plant species were kept as controls to compare with inoculated test plants. Plants were placed in an insect-proof cage and observations were recorded and the symptomless plants were tested using Double Antibody Sandwich-Enzyme-linked Immunosorbent Assay (DAS-ELISA) to confirm the virus infection.

Serological detection of the virus:

Serological assays were carried out at HORDI, Gannoruwa and FCRDI, Maha-illuppallama. DAS-ELISA was performed on diseased snake gourd samples (leaves, fruit flesh, seeds) collected from Dambulla. DAS-ELISA was also performed on basic seeds of snake gourd and different cucurbit species, after inoculating them with virus infected snake gourd samples collected from Dambulla according to the protocol of Agdia. Polyclonal antibodies were used (Agdia Inc. Ltd., U.S.A.) against CMV, tobacco mosaic virus (TMV), potato virus Y (PVY), potato virus X (PVX), potato virus M (PVM), potato virus S (PVS), potato leaf roll virus (PLRV), zucchini yellow mosaic virus (ZYMV), papaya ring-spot virus-P (PRSV-P) and cucumber green mottle mosaic virus (CuGMMV) to ascertain the presence of viruses (Noordam, 1973).

Seed transmission of the virus

Experiments were conducted at HORDI, Gannoruwa, to confirm the seed transmissibility of the virus. Seeds were collected from snake gourd fruits of virus-infected plants at Dambulla Ceylon Agro Development Company Private Ltd. (CADA). Some seeds were treated with 10% HCl solution to eliminate viruses on the seed coat. DAS-ELISA was performed on HCl treated and untreated seeds to confirm the presence of the above mentioned viruses. Eight seeds were sown and raised in pots containing sterile soil, in an insect proof green house. The seedlings were checked for symptoms of virus infection. Finally, virus infection was confirmed by performing DAS-ELISA to ascertain seed transmission of the virus.

Electron microscopic investigations:

Symptomatic leaf samples from snake gourd (maintenance host) were sent to the Global Plant Clinic, Diagnostic and Advisory Service, CABI Bioscience UK Center for identification of the virus.

RESULTS AND DISCUSSION

Virus isolation and Symptomology

Table 1 shows the reaction of cucurbit hosts after inoculation with different inocula prepared using leaf, fruit and seed samples of infected snake gourd plants. It is evident that the virus can be mechanically transmitted using leaf sap, fruit flesh and seeds of infected snake gourds. Isolated virus was maintained in snake gourd plants of 'variety TA-2' in the insect-proof greenhouse for subsequent virus identification. Disease symptoms were reproduced on fruits of inoculated snake gourd that were established in the

field. Initially, small, watery, dark green colored spots appeared on fruits of snake gourd and gradually the lesions enlarged, coalesced and ultimately, rotting of infected fruits were observed. The virus infected fruits showed distortion and size reduction. Leaf symptoms were severe mosaic with dark green blisters, leaf size reduction and distortion.

Table 1. Reaction of cucurbit hosts after inoculation with different inocula prepared using leaf, fruit and seed samples of infected snake gourd plants.

<i>Inoculated plant species</i>	<i>Source of inocula (snake gourd) and symptoms after inoculation</i>		
	<i>Infected fruit flesh</i>	<i>Infected leaves</i>	<i>Infected seeds</i>
<i>Cucumis sativas</i>	Sm	Sm	Sm
<i>Trichosanthes cucumerina</i>	Sm	Sm	Sm
<i>Cucumis pubescens</i>	Sm	Sm	Sm
<i>Cucurbita maxima</i>	Sm	Sm	Sm

Sm- systemic mosaic

Host range studies

Host range and reaction of the virus infected snake gourd plants after mechanical inoculation using inocula prepared by grinding leaves of the infected plant are summarized in Tables 2 and 3. Seven days after inoculation, mosaic symptoms were observed in all cucurbit species tested. Symptoms were not induced in hosts of the families Leguminosae, Caricaceae, Chenopodiaceae, Compositae and Solanaceae.

Table 2. Reaction of differential test plants to the snake gourd virus at Maha-Illuppallama.

<i>Family</i>	<i>Plant species</i>	<i>Symptoms</i>
Cucurbitaceae	<i>Cucumis sativus</i> - cucumber (LY-58)	Sm
	<i>Trichosanthes cucumerina</i> - snake gourd (TA-2)	Sm
	<i>Trichosanthes cucumerina</i> - snake gourd (MI-short)	Sm
	<i>Momordica charantia</i> - bitter gourd	Sm
	<i>Citrullus lanatus</i> - watermelon	Sm
	<i>Cucumis pubescens</i> - kekiri (local)	Sm
	<i>Cucurbita maxima</i> - pumpkin	Ssm
Leguminosae	<i>Vigna anguiculata</i>	ns
	<i>Vigna radiata</i>	ns
Solanaceae	<i>Capsicum annum</i>	ns
	<i>Nicotiana glutinosa</i>	ns
	<i>Nicotiana benthamina</i>	ns
	<i>Nicotiana tabacum</i> (White burley)	ns
	<i>Lycopersicon esculentum</i>	ns

Ssm- severe systemic mosaic *Sm*- systemic mosaic *ns* - no symptoms

Table 3. Reaction of differential test plants to the snake gourd virus at Gannoruwa.

Family	Plant species	Symptoms
Cucurbitaceae	<i>Cucumis sativus</i> - cucumber (LY-58)	Sm
	<i>Cucumis anguria</i> - gherkin (Laypso, Caroline, Napolian)	Lyl
	<i>Trichosanthes cucumerina</i> - snake gourd (TA-2)	Sm
	<i>Trichosanthes cucumerina</i> - snake gourd (MI-short)	Sm
	<i>Trichosanthes cucumerina</i> - snake gourd (MI-Long)	Sm
	<i>Trichosanthes cucumerina</i> - snake gourd (Thinnavelly)	Sm
	<i>Momordica charantia</i> - bitter gourd (MC-43)	Sm
	<i>Momordica charantia</i> - bitter gourd (Thinnavelly white)	Sm
	<i>Momordica charantia</i> - bitter gourd (Palee)	Sm
	<i>Citrullus lanatus</i> - watermelon (Sweet Lady, Thilini)	Sm
	<i>Cucumis pubescens</i> - kekiri (Local)	Lyl
	<i>Cucurbita maxima</i> - yellow pumpkin (Janani)	Sm
	<i>Cucurbita maxima</i> - yellow pumpkin (Ruhunu)	Sm
	<i>Benincasa hispida</i> - ash pumpkin (Local)	Slc
	<i>Luffa acutangula</i> - ridged gourd (Hercules)	Sm
	<i>Luffa acutangula</i> - ridged gourd (LA-33)	Sm
	<i>Lagenaria siceraria</i> - bottle gourd (Local)	Sm
Leguminosae	<i>Vigna radiata</i> - mung bean (MI-05)	ns
	<i>Phaseolus vulgaris</i> - bean (KWG)	ns
	<i>Vigna cylindrika</i> - mae (Hawari mae)	ns
	<i>Vigna unguiculata</i> - cowpea (MI-35)	ns
Chenopodiaceae	<i>Chenopodium amaranticolor</i>	ns
	<i>Chenopodium murale</i>	ns
Solanaceae	<i>Nicotiana glutinosa</i>	ns
	<i>Nicotiana benthamina</i>	ns
	<i>Nicotiana tabacum</i> (White burley)	ns
	<i>Lycopersicon esculentum</i> (Thilina)	ns
	<i>Petunia hybrida</i>	ns
Caricaceae	<i>Carica papaya</i> (Rathna)	ns
	<i>Carica papaya</i> (Red lady)	ns
Compositae	<i>Gomphrena globosa</i>	ns
	<i>Zinnia elegans</i>	ns

Ssm - severe systemic mosaic *Sm* - systemic mosaic *Lyl* - yellowing of inoculated leaves
Slc - systemic leaf cupping *ns* - no symptoms

Serological detection of the virus

According to the serological investigations, it is evident that the snake gourd samples and basic seeds of snake gourds collected from CADC Farm at Dambulla are co-infected with CMV and serologically PRSV-P related virus (Table 4). All the samples showed strong serological reaction with antiserum of PRSV-P from Agdia Inc. USA. Serological assay with polyclonal antibodies against TMV, PVY, PVS, PVM, PLRV, ZYMV and PVX gave negative results.

Table 4. ELISA performed on snake gourd samples from CADC Farm at Dambulla.

<i>Source of inoculum</i>	<i>Symptoms</i>	<i>CMV</i>	<i>PRSV-P</i>
Snake gourd basic seeds	-	+	+
Snake gourd seeds from infected fruit	-	+	+
Snake gourd infected fruit flesh	Green lesions	-	+
Snake gourd infected leaf	mosaic	+	+

+ = Positive reaction - = Negative reaction

Snake gourd, yellow pumpkin and ash pumpkin showed susceptibility to the PRSV-P when inoculated with inocula prepared from fruit flesh and leaves of infected snake gourd (Table 5). Mixed infection of the CMV and PRSV is evident in leaf and fruit samples of infected snake gourd plants. Cucumber green mottle mosaic virus, which belongs to the tobamo virus group, was detected in mechanically inoculated snake gourd plants (Table 5).

Table 5. Results of ELISA performed on different cucurbit species after inoculation with virus infected snake-gourd seeds, leaves and fruit flesh.

<i>Source of Inoculum</i>	<i>Inoculated host</i>	<i>Symptoms</i>	<i>LISA results</i>		
			<i>CMV</i>	<i>PRSV</i>	<i>GMMV</i>
Fruit flesh/ infected snake gourd	Snake gourd	Sm	+	+	nt
Leaf / infected snake gourd	Snake gourd	Sm	+	+	+
Leaf / infected snake gourd	Kekiri	Sm	+	-	nt
Leaf / infected snake gourd	Yellow pumpkin	Sm	-	+	nt
Leaf / infected snake gourd	Ash pumpkin	Slcu	-	+	nt
Seeds / infected snake gourd	Snake gourd	Sm	+	-	nt
Basic seed/Snake gourd	Snake gourd	Ns	-	-	nt
Basic seeds / snake gourd	Yellow pumpkin	Ns	-	-	nt
Seeds / Infected snake gourd	Yellow pumpkin	Ns	-	-	nt
Seeds / Infected snake gourd	Cucumber	Ns	-	-	nt
Seeds / Infected snake gourd	Kekiri	Ns	-	-	nt

Slcu = systemic leaf cupping, *Sm* = systemic mosaic, *nt* = not tested. *ns* = no symptoms

+ = Positive reaction, - = negative reaction

Seed transmission

Virus symptoms could be observed on snake gourd plants raised from the seeds of virus infected snake gourd fruits. ELISA with PRSV-P antiserum confirmed the presence of a virus serologically related to PRSV-P in HCl treated seeds. Plants germinated from untreated seeds also showed symptomatic reactions and showed a positive reaction to PRSV-P antiserum (Table 6).

Table 6. Symptom expression and ELISA results of snake gourd plants germinated from 10% HCL- treated and untreated infected seeds.

<i>Seed source</i>	<i>Symptoms of germinated snake gourd</i>	<i>PRSV -P</i>	<i>CMV</i>
Infected snake gourd seeds/ HCl treated	mosaic	+	-
Infected snake gourd seeds / HCl not treated	mosaic	+	-
Snake gourd basic seeds	mosaic	-	-

+ = Positive reaction, - = Negative reaction

Electron microscope observations:

The electron microscopic observations revealed that the leaves of infected snake gourd samples contained a high concentration of tobamovirus-like, rigid, rod-shaped 300nm particles, which were most probably cucumber green mottle mosaic virus (CuGMMV), which is a seed-borne virus (Phil Jones, Global Plant Clinic, Diagnostic and Advisory Service, CABI Bioscience, UK Center: personal communication). The virus can build up to high levels in the crops and farmers need to maintain their own healthy seed material to prevent losses caused by the tobamovirus. Seeds of virus infected cucumber, watermelon and bottle gourd transmit CuGMMV. The virus is transmitted by plant debris in soil, infected plant sap and seeds, but not by aphid vectors (Singh, 1995).

CONCLUSIONS

On the basis of symptom expression on infected plants, host-range studies and electron microscopic observations, the virus was identified to be a tobamovirus, which may be an isolate of cucumber green mottle mosaic virus. This is the first report of tobamovirus in snake gourd. This virus is transmitted through sap and seeds and the host range is confined to the cucurbitaceae. The snake gourd crop at Dambulla had mixed infections of CMV, CuGMMV and the PRSV-P related virus.

This disease can be managed by using virus free seeds. It is necessary to use appropriate hygienic practices such as disinfection of tools by immersing in 5% chlorine water or soap water solutions. Soaking the seeds for 15 min. in a solution of 10% tri-sodium orthophosphate will significantly reduce the virus concentration on the seed coat.

REFERENCES

- Cardosa, A.H. and M.A. Pawan. 1999. Evaluation of segregating squash populations to papaya ring spot virus type watermelon and zucchini yellow mosaic virus. *Summa Phytopathologica* 25: 3, 202-205.
- Zoysa, I.J. de, H.T.K. Liyanage, Liyanage and Vigananakulasinghum. 1995. Characterization of papaya viruses in Sri Lanka. *Proceedings of 51st Annual Session of SLAAS* 1:94-96.
- Gibbs, A.J. 1970. Cucumber mosaic virus. *CMI Descriptions of plant viruses*. No 1.
- Lebeda, A., S. Kozelska, E. Kristkova and R. Novotny. 1996. The occurrence of viruses on *Cucurbita* spp. in the Czech Republic and resistance of squash cultivars to CMV and WMV-2. *Zeitschrift-fur-Pflanzenkrankheiten-und-Pflanzenschutz* 103:455-463.
- Lisa, V. 1984. Zucchini yellow mosaic virus. *CMI descriptions of plant viruses*. No. 282.
- Luis- Arteaga, M., J.M. Alvarez, J.L. Alonso-Prados, F. Garcia-Arenal, A. Lavina, A. Batlle, and E. Moriones. 1998. *Plant Disease* 82:979-982.
- Noordam, D. 1973. Identification of plant viruses. *Methods and Experiments*. Oxford and IBH publishing Co., New Delhi. Pp 239-240.
- Purcifull, D., E. Hiebert and J. Edwardson. 1984. Watermelon Mosaic virus-2 CMI descriptions of plant viruses No. 293.
- Sanches, M.V., R. Agüero and C. Rivera. 1998. Host plants of the important viruses of melon infection, *Cucumis melo*. 1998. *Revista-de-Biologia-Tropical*.46:13-25.
- Singh, R.S. 1995. *Diseases of vegetable crops* (3rd edition). IBH Publishing Ltd., New Delhi. Pp 229-230.
- Wang, J., S.D. Yeh, J.J. Wang and S.D. Yeh. 1998. Characterization of papaya ring spot virus W type isolates collected from different areas of Taiwan by host reactions, immunodiffusion tests and RT-PCR. *Plant-Protection-Bulletin-Taipei* 40:383-395.
- Yuki, V.A., J.A.M. Rezende, E.W. Kitajima, R. Kuniyuki, and G.A. Groppa. 2000. Occurrence, distribution and relative incidences of five viruses infecting cucurbits in the state of Sao Paulo, Brazil. *Plant Disease* 84:516-520.