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Characterisation of Viruses Affecting Weeds - I. Mosaic Diseases*

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The weeds *Amaranthus viridis* and *Trianthema decandra* harboured the amaranthus mosaic virus and the virus was transmitted by the aphids *Aphis gossypii*, *A. craccivora* and *Myzus persicae*. From the physical properties, host range and serological tests, these two virus isolates were identified as strains of amaranthus mosaic virus. The weeds *Solanum nigrum* and *T. portulacastrum* harboured the chilli mosaic virus and the aphids *A. gossypii*, *A. evonymii*, *A. craccivora* and *M. persicae* were able to transmit the virus. These two virus isolates were identified as strains of potato virus Y. The present study indicated the possibility of the weeds serving as vital links in the disease cycle.

Several weeds showing symptoms of infection have been reported to be sources of the virus diseases on cultivated plants (Hein, 1953; Sakimura, 1953; Simons, 1956a; Anderson, 1959; Pontis and Feldman, 1963; Brcak and Polak, 1966; Tomlioson *et al*, 1970; Feldman and Olga Gracia, 1972). The weeds growing in the garden lands around Coimbatore very often exhibited symptoms similar to the virus diseases of crop plants. In order to understand the relationship of the mosaic diseases occurring on the weeds and cultivated plants the present study was undertaken.

MATERIALS AND METHODS

The garden land areas around Coimbatore were visited periodically and the weed plants showing obvious disease symptoms were collected and established in the glass house for use as source plants. Transmission tests were made by different methods viz., graft, sap inoculation and aphids. The possible transmission of viruses through seeds was also tested.

Antiserum for the *A. viridis* mosaic virus was prepared by immunizing a rabbit. The sap extracted with 0.1 M phosphate buffer from diseased *A. viridis* plants was purified by differential centrifugation first at 4000 rpm and then at 40,000 rpm. The precipitate was suspended in distilled water and again purified under low and high speed centrifugation. The concentrated virus suspension was tested for its infectivity on *A. viridis*. After ascertaining the infectivity the virus suspension was mixed with 0.86 per cent saline and injected into the rabbit intravenously. Six injections of 2 ml

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each were given at weekly intervals. A fortnight after the last injection the animal was bled and the blood was collected and the serum separated. The serum was centrifuged at 2000 rpm to eliminate the blood cells. The serum was mixed with the sap of the healthy *A. viridis* plant for the cross absorption of the antibodies induced by the host protein, if any. The sap-serum mixture was centrifuged at 2000 rpm for 30 minutes. The supernatant antiserum was preserved at 5°C with sodium azide 0.01 per cent for further use.

RESULTS

From the survey it was noticed that 45 species of weeds belonging to 15 families grew periodically around Coimbatore Mosaic disease was noticed on 14 species of weeds (Table I). The transmission of these virus isolates from the weeds were tested by graft, sap inoculation, through insects (6 species of aphids) and through seeds. The transmission of *L. mollis* mosaic through sap but not by grafting may be due to failure of graft establishment. Out of these none of them was transmitted through seeds. Two of them were transmitted through graft and sap, one by graft only and three of them by aphids only. The remaining eight mosaic virus isolates were transmitted through graft, sap and by aphids. Out of these, four of them (viz., mosaic diseases of *A. viridis*, *S. nigrum*, *T. decandra* and *T. portulacastrum* infec-

TABLE I. Transmission of the mosaic diseases of weeds

Mosaic virus isolates from the weeds	Transmission to their natural host							Transmission to crop plants				
			By aphids									
	By graft	By sap	*Aphis craccivora*	*Aphis evonymii*	*Aphis gossypii*	*A. malidis*	*A. nerii*	*Myzus persicae*	Through seed	*Amaranthus caudatus*	*Capsicum annuum*	*Nicotiana tabacum*
Amaranthus viridis	+	+	+	−	+	−	−	+	−	+	−	−
Boerhaavia diffusa	0	−	+	−	−	−	−	−	−	−		−
Digera arvensis	+	+	−	−	−	−	−	−	−	−	−	−
Datura fastuosa	+	+	−	−	+	−	−	+	−	−	−	−
D. metel	+	+	−	−	+	−	−	+	−	−	−	−
Gynandropsis pentaphylla	0	−	−	−	+	−	−	−	−	−	−	−
Lagasea mollis	0	+	−	−	−	−	−	−	−	−	−	−
Ocimum canum	+	+	−	−	−	−	−	−	−	−	−	−
Peristrophe bicalyculata	+	+	+	−	+	−	−	+	−	−	−	−
Solanum nigrum	+	+	+	+	+	−	−	+	−	−	+	+
Trianthema decandra	+	+	+	−	+	−	−	−	−	+	−	−
Trianthema portulacastrum	+	+	+	+	+	−	−	+	−	−	+	+
Tridax procumbens	0	−	−	−	+	−	−	−	−	−	−	−
Vernonia cinerea	+	+	−	−	+	−	−	−	−	−	−	−

+ Transmission positive; − Transmission negative; 0 Failure of graft transmission due to difficulty of graft establishment

ted the cultivated plant species viz., *A. gangeticus*, *Capsicum annuum* and *Nicotiana tabacum* (Table I).

Symptomatology: The symptoms exhibited by the four mosaic virus isolates that infected the cultivated plants were as follows: Symptoms of vein clearing, mild chlorosis, downward rolling of margins, reduction in size of the leaf and plant were evident in all the four weeds. However, the leaves were conspicuously pale green and the inflorescence was short and sparse in *A. viridis*. The stems were slender in *T. decandra*. Puckering of interveinal areas with chlorotic rings or flecks were common on the leaves of *T. portulacastrum*. Clear symptoms of mosaic and blisters were seen on the leaves of *S. nigrum*.

Host range: In order to study the host range of the four mosaic virus isolates, 41 species of plants were inoculated by sap inoculation. The results showed that 16 of them were susceptible (Table II). Mosaic iso-

TABLE II. Host range of the mosaic virus isolates from the weeds

Species of Plants susceptible to the mosaic virus isolates	Mosaic virus isolates from the weeds			
	Amaranthus viridis	*Trianthema decandra*	*Trianthema portulacastrum*	*Solanum nigrum*
Amaranthus caudatus L.	VC, MM, C, RL, S	MM, C, RL, S	—	—
A. gangeticus L.	VC, MM, C, RL, S	MM, C, RL, S	—	—
A. viridis L.	VC, MM, RL, S, MRD	VCIMM, RL, S, MRD	—	—
Celosia cristata L.	MM, RL, S	—	—	—
Gomphrena globosa L.	C, S, MM	C, S, MM	—	—
Capsicum annuum L.	—	—	VC, MM, RL, CS	VC, VB, M M, C, F,R,S
Nicotiana glutinosa L.	—	—	MM, F, RL	MM, F, RL
N. tabacum L. var. White Burley	—	—	MM	MM
N. tabacum L. var. *xanthi*	—	—	MM, VC, C, S	MM,VC,C,S
Nicandra physaloides (L) Pers.	—	—	MM, C, S	MM, C, S
Petunia hybrida Vilm.	MM, RL, S, D, MRD	MM, RL, S, MRD	MM, RL, F	MM, RL, F
Physalis floridana Rydb.	—	—	MM, RL, S	MM, RL, S
Solanum nigrum L.	—	—	MM, VC	VC, MM, RL, C
S. tuberosum LXS *demissum* Lindl. Hybrid A 6	—	—	DNL	DNL
Trianthema decandra L.	MM	MM, C, RL, MRD,S	—	—
T. portulacastrum L.	—	MM, RL, S, F, MRD	MM, RL, S, F, MRD	C, S, CR, MM, RL

C-Chlorosis; D-Distortion; F-Filiformity; CR-Crinkling; DM-Dark necrotic lesion; MM-Mosaic mottling; RL-Reduction in leaf size; P-Puckering; S-Stunting; VC-Vein Clearing; MRD-Downward rolling of leaf margins; VB-Vein banding

iate from *A. viridis* infected six species viz., *A. caudatus, A. gangeticus, C. cristata. G. globosa, P. hybrida* and *T. decandra.* The mosaic virus isolate from *T. decandra* infected the above hosts and *T. portulacastrum* except *C. cristata.* The mosaic virus isolates from *T. portulacastrum* and *S. nigrum* infected eight species of plants (viz.. *C. annuum, N. glutinosa N. tabacum* var. White Burely, *N. tabacum* var. *xanthi, N. physaloides, P. hybrida, P. floridana* and potato hybrid A-6) besides their natural hosts. A brief description of the symptom on some of the important vegetable crops is given below :

A. gangeticus: The mosaic virus isolates from *A. viridis* and *T. decandra* produced mosaic mottling symptom on the newly produced leaves 13-15 and 20-22 days after inoculation respectively.

Capsicum annuum: Virus isolates from *T. portulacastrum* and *S. nigrum* infected this plant species and produced vein clearing, mosaic mottling and mild chlorosis on the newly produced leaves, 10–15 days after inoculation. Besides mild crinkling and blistering were induced by the virus isolate from *T. portulacastrum,* while vein banding and filiformity were common on the plants infected by the mosaic isolate from *S. nigrum.*

Physical properties : The physical properties of the mosaic virus isolates were studied using the respective host plant species on test plants since no local lesion host was available (Table III).

TABLE III. Physical properties

Virus isolates	Dilution end pbint				Thermal inactivation point				Longevity *in vitro*							
	1	2	3	4	1	2	3	4	Age of the sap	1		2		3		4	
										a	b	a	b	a	b	a	b
Dilution	% transmission				Temperature °C					% transmission							
Standard sap	80	70	80	90	Standard sap	53	60	80	80	Fresh sap	—	80	—	60	—	80	—	80
1 : 10	53	60	70	70	40	47	50	60	60	8 hrs	60	50	60	50	70	60	70	60
1 : 100	27	50	40	40	45	40	40	40	30	1 day	40	30	60	40	50	40	40	40
1 : 500	20	50	40	30	50	20	30	20	20	2 days	20	20	40	30	30	20	20	20
1 : 1000	7	30	30	20	55	20	20	20	10	3 ,,	10	0	40	20	30	10	20	20
1 : 2000	0	10	20	0	60	13	20	10	0	4 ,,	0	0	20	10	20	0	10	—
1 : 2500	0	0	0	0	65	0	10	0	0	5 ,,	0	0	10	0	0	0	—	—
1 : 4000	0	0	0	0	75	0	0	0	0	6 ,,	0	0	10	0	0	0	—	—
1 : 8000	0	0	0	0	75	0	0	0	0	7 ,,	0	0	0	0	0	0	—	—
										10 ,,	0	0	0	0	0	0	—	—
										15 ,,	0	0	0	0	0	0	—	—

1. Mosaic of *A. viridis* 2. Mosaic of *T. decandra* 3. Mosaic of *T. portulacastrum*
4. Mosaic of *S. nigrum* a=at 5°C b=at 28°C

Dilution end point : The infectivity was retained by the mosaic virus isolates from *A. viridis* and *S. nigrum* at 1:1000 dilution. The virus isolates from *T. decandra* and *T. portulacastrum* remained infective at 1:2000 dilution.

Thermal inactivation point: The thermal inactivation point of the mosaic virus isolates from *A. viridis* and *T. portulacastrum* was found to lie between 60-65°C. The virus isolates from *T. decandra* and *S. nigrum* remained infective when subjected to a temperature of 65 and 55°C respectively.

Longevity in vitro: The infectivity of the sap from diseased *A. viridis* was retained for 3 and 2 days when stored at 5° and 28-30°C respectively. The longevity *in vitro* of the virus isolate from *T. decandra* was 6 and 4 days respectively at 5° and 28-30°C. The virus isolates from *T. portulacastrum* and *S. nigrum* had remained infective when stored *in vitro* up to 4 and 3 days respectively at 5° and 28-30°C (Table III).

Serological tests : Two fold serial dilutions of the antiserum of the *A. viridis* mosaic virus were prepared with normal saline. The antigenicity of the mosaic virus isolates from *A. viridis*, *T. decandra* and *A. gangeticus* was tested by precipitation test. The virus isolates and the antiserum were mixed in equal volumes (1 ml each) and kept in a water bath at 40°C for 30 minutes. The positive precipitation reaction indicated that they had antigenic group(s) in common.

DISCUSSION

The importance of weeds as potential sources of virus infection has been underscored by many earlier investigators. The present study revealed the presence of four mosaic virus isolates from weeds capable of infecting crop plants. It is imperative, therefore, to establish the identity of virus isolates which could infect amaranthus, chillies and tobacco.

Ramakrishnan *et al.* (1971) reported the transmission of amaranthus mosaic virus by *A.gossypii*. The mosaic isolate from *A. viridis* causing identical symptoms on *A. gangeticus* was transmitted by *A. gossypii*, *A. craccivora* and *M. persicae*. The mosaic virus isolate from *A. viridis* infected plants belonging to Amaranthaceae, Aizoaceae and Solanaceae (Table II) The host range and the physical properties (Tables II and III) were similar to the amaranthus mosaic virus described by Ramakrishnan *et al.* (1971). The antiserum of the *A. viridis* mosaic virus gave positive reaction with the amaranthus mosaic virus indicating the relationship between them. From these characters the virus is identified as amaranthus mosaic virus. The natural occurrence of amaranthus mosaic virus on *A. viridis* and *A. blitum* has also been reported from Delhi (Phatak, 1965). The virus isolate from *T. decandra* showed some difference in transmission (Table I), host range (Table II) and physical properties (Table III). However, the virus reacted positively with the antiserum of *A. viridis* mosaic virus (Table IV). Hence, the virus iso-

TABLE IV. Antigenicity of mosaic virus isolates indicating *A. gangeticus*

Test sap	Dilution of antiserum (AMV)						
	0	1:2	1:4	1:8	1:16	1:32	1:64
Sap from healthy *A. viridis*	—	—	—	—	—	—	—
Infective sap from *A. virdis*	+++	+++	++	++	+	—	—
Infective sap from *T. decandra*	++	++	+	—	—	—	—
Infective sap from *A. gangeticus*	++	++	+	—	—	—	—

Intensity of precipitation: + Trace ++ Moderate +++ High

late from *T. decandra* could be a strain of amaranthus mosaic virus.

The occurrence of chilli (pepper) mosaic virus on *S. nigrum*, a common weed, was reported from Florida by Simons (1956) and Anderson and Corbett (1957). The transmission of the mosaic virus isolate from *S. nigrum* by *A. gossypii* was reported by *A. gossypii* (Simons, 1959). Besides, the above three species of aphids viz., *A. craccivora, A. evonymii* and *M. persicae* transmitted the mosaic virus isolates from *S. nigrum* (Table I). The present virus was found to differ (Table III) from the earlier reported virus which had a dilution end point of 1 : 10,000. The mosaic virus isolates from *T. portulacastrum* and *S. nigrum* had a similar mode of transmission. These two virus isolates had the same host range infecting plants belonging to Solanaceae and Aizoaceae. They produced local necrotic lesions on the detached leaves of A-6 potato an indicator host for the potato virus Y (Kohler, 1953). Hence, they are identified as a strain of potato virus Y. The present study clearly indicated that the amaranthus mosaic virus could be harboured by the weeds *A. viridis* and *T. decandra* and the chilli mosaic virus (potato virus Y) could be harboured by the weeds *S. nigrum* and *T. portulacastrum* and that these weeds might serve as vital links in the disease cycle.

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