



Detection of Plant Viruses in Some Ornamental Plants That Act as Alternate Hosts

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Abstract

A study was conducted at the Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur to detect virus infecting ornamental plants. Enzyme-linked Immunosorbant Assay (ELISA) and symptomatology were used for detection. Five viruses namely TPVV (Tomato Purple Vein Virus), CMV-Y (Cucumber Mosaic Virus-Y), OYVCMV (Okra Yellow Vein clearing Mosaic Virus), MYMV (Mung bean Yellow Mosaic Virus), TYLCV (Tomato Yellow Leaf Curl Virus) were detected on *Tagetes erecta* (Marigold), *Salvia splendens* (Salvia), *Dahlia hybrida* (Dahlia), *Helichrysum bracteatum* (Straw flower), *Impatiens balsamina* (Garden balsam). CMV-Y caused mosaic of Dahlia and Leaf Curl of Marigold. MYMV caused Yellow Mosaic of *Dahlia hybrid*, while TYLCV caused mosaic of *Helichrysum bracteatum*. OYVCMV produced leaf chlorosis on *Salvia splendens*, and chlorotic spots on *Impatiens balsamina*. TPVV caused purple leaf on *Tagetes erecta*.

Keywords: Virus, ornamental plants, alternate hosts

1. Introduction

In Bangladesh, almost all crops have been reported to be infected by more than one virus causing severe damage to the yields of the crops (Akanda, 1991; Akanda *et al.*, 1991). Most of the plant viruses have weeds or other alternative natural hosts that provide a reservoir of viruses from which the economically important crop plants may become infected (Neeraj and Zaidi, 2008; Mathews and Dodds, 2008). Weeds, ornamental plants and wild plants near crop field seem to be infected with the viruses of cultivable plant species (Sivalingam and Varma, 2007) and play a key role in the development of virus disease epidemics, because the biotrophic nature of plant viruses obviously requires the alternate hosts to continue association of virus-host-

vectors association for the sustainability of the viral pathogens in nature in absence of original crop host. All these make the virus disease epidemiology more complex and hazardous as compared to any other diseases (Maramorosch and Harris, 1981). Since the alternate hosts contribute to the infection on crop plants in the field supplying inocula and act as important initial sources of infection from which the viruses spread into or within a crop, identification of initial foci of infection is important to formulate control measures or management practices against the virus diseases (Maramorosch and Harris, 1981).

Research on virus in Bangladesh has started very recently. As a result, the viruses of most crops remain unidentified and such plants may be the

alternate hosts of many crops of economic importance. Identification of initial source of infection is important for studying the epidemiological aspects of plant viruses and also to formulate control measures the viral diseases (Thresh, 1982). This study was therefore, undertaken to collect the ornamentals showing virus disease like symptoms under natural conditions at BSMRAU campus and to identify the viruses from the collected samples of ornamentals.

2. Materials and Methods

The survey was conducted in the Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur. The ornamentals plants showing typical virus disease like symptoms as described by Bos (1978) and Holmes (1964) were collected. The well-expanded leaves with distinct symptoms were considered. The samples were taken into polythene bags for each sample. The numbers of infected plants or leaves were counted, the symptoms were noted and the photographs were taken. The investigation was continued for two weeks.

2.1. Indicator plants test

Indicator host/test plants were grown in an insect proof net house. The seeds were sown in the plastic tray contained sterilized soils. After germination, the seedlings were transferred to the pots and each pot contained one seedling for each plant species. The plants were maintained with proper irrigation and intercultural management.

The inocula were prepared from the samples and were inoculated into the host and indicator plants. The leaf sample was macerated using buffer in a mortar and pestle. Four different buffers such as 0.02M sodium phosphate pH 7.1, 0.2M sodium phosphate buffers pH 7.1, 0.02M potassium phosphate buffers pH 7.1 and 0.2M potassium phosphate buffers pH 7.1 were used separately for each of the samples. The carborandum powder (600 mesh) was dusted on

the surface before inoculation. There were 4 replications for each sample. The inoculated plants were observed up to six weeks of inoculation. The expanded cotyledons were used to inoculate in case of Cucurbitaceous plants. The first leaf was inoculated in leguminous plants. In all other cases the expanded leaves were used for inoculation as mentioned by Noordam (1973).

2.2. Indirect Enzyme-linked Immunosorbant Assay (Indirect ELISA)

Indirect ELISA as described by Clark and Adams (1977) was employed for detecting the virus infection in some samples. The leaf sample of the ornamental hosts was macerated with 0.5 M carbonate buffer pH 9.6 of 10X dilution and the well of micro-titer plate (NuNC) was coated with the extracted sap at the rate of 200 μ l/well. The plate was incubated overnight at 4 °C and washed with washing buffer. After washing, 200 μ l I/well skim milk was used and incubated at 37 °C for one-hour. The diluted antiserum of CMV-Y at the rate of 200 μ l/well was added and the plate was incubated for 37 °C for 3 hours. After washing, the diluted conjugate (Antirabbit IgG conjugated with Alkaline Phosphatase) was poured into the micro-titer plate well following three hours of incubation at 37 °C. After incubation the plate was thoroughly washed using washing buffer and then substrate (P-nitrophenyl phosphate disodium salt) was added. After addition of substrate the micro titer plate was incubated for 30 minutes at room temperature and subjected to observe yellow color development for the positive reaction. The absorbance value at 405 nm was measured using a Micro Reader, Bio Red-450. Positive reaction indicated the presence of the virus.

3. Results and Discussion

3.1. Virus disease-like symptoms on five ornamental spices

Purple color on the leaves and curling of the infected plants were the major symptoms in

Tagetes erecta (marigold). Leaf rolling, narrowing, dentation and mild yellowing were also developed (Fig. 1. A-D). *Salvia splendens* (salvia) developed chlorosis. Older leaves showed clear symptoms than that of the younger leaves (Fig. 1. E-F). Yellow mosaic and mosaic symptoms (Fig. 1. G-I) appeared in *Dahlia hybrida* (Dahlia). Mosaic and mild yellowing (Fig. 1. J) was observed in *Helichrysum bracteatum* (straw flower). The chlorotic spots (Fig. 1. K) appeared as major symptoms in *Impatiens balsamina* (garden balsam).

The prevalence of virus disease-like symptoms recorded on the five different ornamental species are presented in Table 1. In all 25% and 75% prevalence of purple leaf and leaf curl symptom, respectively, were found to be prevalent in marigold; while 20% was noted in salvia. In case of Dahlia, 35% and 62% yellow mosaics and mosaic, respectively, were recorded. The strawflower produced 55% mosaic, while garden balsam showed 85% chlorotic spots under natural condition.

Chlorotic spot of garden balsam had the highest prevalence followed by leaf curl in marigold, mosaic in dahlia and straw flower, yellow mosaic in dahlia, purple leaf in marigold and leaf chlorosis in salvia.

3.2. Inoculation test of virus disease-like symptoms on five ornamental species

The results of inoculation test are presented in Table 2. The results demonstrated that all the symptoms except mosaic of dahlia (*Dahlia hybrida*) and leaf curl of marigold (*Tagetes erecta*) were mechanically non-inoculated in 25 test or indicator plants among the 42 test plants.

The dahlia mosaic and leaf curl of marigold were found to be mechanically inoculated on test plants. The symptoms so far obtained upon inoculation of dahlia mosaic and leaf curl of marigold are presented in Table 3. Systemic mosaic was produced on 17 test plants among the 42.

In *Dahlia hybrida* systemic mosaic and curling were produced, while necrotic local lesion was produced in *Chenopodium amaranticolor* and *Gomphrena giobosa*. *Cucumis sativus* produced chlorotic local lesion, which became systemic produced systemic yellow mosaic, while leaf curl was developed in *Tagetes erecta*. Yellow mosaic symptom was produced on *N. tabacum* cv. Orinico, *Cucumis melo*, and *Cucurbita moschata*. The symptom produced by the dahlia mosaic and leaf curl of marigold on different plants were found to be similar as described by Franki *et al.* (1979) and Neeraj and Zaidi (2008) for *Cucumber mosaic virus* (CMV).

Table 1. Prevalence of virus disease like symptoms developed on five ornamental species under natural condition

Ornamental Species	Major Symptom	Healthy Plants (%)	Infected Plants (%)
<i>Tagetes erecta</i> (Marigold)	1. Purple leaf	75	25
	2. Leaf curl	25	75
<i>Salvia splendens</i> (Salvia)	Leaf chlorosis	80	20
<i>Dahlia hybrida</i> (Dahlia)	1. Yellow mosaic	65	35
	2. Mosaic	38	62
<i>Helichrysum bracteatum</i> (Straw flower)	Mosaic	45	55
<i>Impatiens balsamina</i> (Garden balsam)	Chlorotic spots	15	85



Fig.1.A. Purpling and leaf rolling in *Tagetes erecta*



Fig.1.B. Leaf roll in *T. erecta*



Fig.1.C. Dentation and leaf curl in *T. erecta*



Fig.1.D. Leaf curl in *T. erecta*



Fig.1.E. Leaf chlorosis in *Salvia splendens*



Fig.1.F. Leaf chlorosis in *S. splendens*



Fig.1.G. Mosaic in *Dahlia hybrida*



Fig.1.H. Mosaic in *Dahlia hybrida*



Fig.1.I. Yellow mosaic in *Dahlia hybrid*



Fig.1.J. Mosaic in *Helichrysum bracteatum*



Fig.1.K. Chlorotic spot in *Impatiens balsamina*

Table 2. Reaction of inocula (plant virus) on indicator plants except mosaic of dahlia and leaf curl of marigold

SL. No.	Indicator/Test Plants Inoculated	Reaction	
		Symptoms Developed	Response
1	<i>Datura metel</i>	None	-
2	<i>D. stramonium</i>	„	-
3	<i>Nicotiana tabacum</i> cv. <i>Xanthi</i>	„	-
4	<i>N. rustica</i> v. <i>Xovnytl</i>	„	-
5	<i>Salanum torvum</i>	„	-
6	<i>Petunia hybrida</i>	„	-
7	<i>Physatis heterophyila</i>	„	-
8	<i>Cucumis sativus</i>	„	-
9	<i>Cucumis melo</i>	„	-
10	<i>Benincasa hispida</i>	„	-
11	<i>Citrullus lanatus</i>	„	-
12	<i>Luffa cylendrica</i>	„	-
13	<i>L. acutangula</i>	„	-
14	<i>Lagenaria siceraria</i>	„	-
15	<i>Trichosanthes anguina</i>	„	-
16	<i>Ageratum conyzoides</i>	„	-
17	<i>Blumea lacera</i>	„	-
18	<i>Helichrysum bracteatum</i>	„	-
19	<i>Synedrella nodiflora</i>	„	-
20	<i>Carica papaya</i>	„	-
21	<i>Salvia splendens</i>	„	-
22	<i>Impatiens balsamina</i>	„	-
23	<i>Jussiaea suffruticosa</i>	„	-
24	<i>Ficus hispida</i>	„	-
25	<i>Tetragonia expansa</i>	„	-

I-ELISA test proved that mosaic symptom of dahlia and leaf curl of marigold reacted positively against the polyclonal antibodies of *Cucumber mosaic virus-Y* (CMV-Y). All other symptoms were negative in I-ELISA test (Table 4). The results

proved that the mosaic symptoms of dahlia and leaf curl of marigold were infected by CMV-Y.

TPVV were identified as a possible cause of Purple leaf symptom of *Tagetes erecta* (Table

5). TYLCV was suspected as the cause of mosaic of *Helichrysum bracteatum*. Leaf chlorosis and chlorotic spots of *Salvia splendens* and *Impatiens balsamina* were possibly caused by OYVCMV. The symptoms so far noted on the ornamental plants seemed to be identical to TYLCV, TPVV, MYMV and OYVCMV as described by Green and Kalloo (1994) for TYLCV, Gupta (2000) for TPVV, Green and Kim (1991) and Capoor and

Verma (1950) for OYVCMV in the respective host and other hosts including weeds. All these Geminiviruses (TYLCV, TPVV, MYMV and OYVCMV) were found to be prevalent in Bangladesh and several studies were conducted on all those viruses at BSMRAU (Gupta, 2000; Begun, 2002; Alam, 2001 and Chowdhury, 2001; Sivalingam and Varma, 2007).

Table 3. Response of indicator/test plants inoculated with mosaic symptom of dahlia and leaf curl symptom of marigold

SL. No.	Indicator/Test Plants Inoculated	Reaction	
		Symptoms Developed**	Response*
1	<i>Nicotiana tabacum</i> cv. NN	smos	+
2	<i>N. tabacum</i> cv. Samson	smos	+
3	<i>N. tabacum</i> cv. White barley	smos	+
4	<i>N. tabacum</i> cv. Bright yellow	smos	+
5	<i>N. tabacum</i> cv. Virginia gold	smos	+
6	<i>N. tabacum</i> cv. Orinico	y mos	+
7	<i>N. ghitinosa</i>	smos	+
8	<i>Lycopersicon esculentum</i>	smos	+
9	<i>Physalis floridana</i>	cll, y mos	+
10	<i>Cucurbita moschata</i>	y mos	+
11	<i>Dolichos lablab</i>	y mos	+
12	<i>Phaseolus vulgaris</i>	y mos	+
13	<i>Pisum sativum</i>	y mos	+
14	<i>Tagetes erecta</i>	lc	+
15	<i>Dahlia hybrida</i>	mos, lc	+
16	<i>Chenopodium amaranticolor</i>	nll	+
17	<i>Gomphrena globosa</i>	nll	+

* -, Negative Response, '+', Positive Response

** cll (Chlorotic Local Lesion), y mos (Yellow Mosaic), lc (Leaf curl), mos (Mosaic), nll (Necrotic Local Lesion), smos (Systemic Mosaic)

Table 4. Response of virus disease like symptoms against antiserum in I-ELISA

Symptoms	Color Developed*	OD
Mosaic of Dahlia	+	0.724
Yellow Mosaic of Dahlia	-	0.823
Healthy Plant of Dahlia	-	0.337
Purple Leaf of Marigold	-	0.748
Leaf curl Marigold	+	1.027
Healthy Plant of Marigold	-	0.627

* '-', (Negative Response) and '+', (Yellow color)

Table 5. Identification of viruses infecting virus disease like symptoms developed on five ornamental species based on symptoms and inoculation test and I-ELISA test

Ornamental Species	Major Symptom	Causal Virus/Possible causal Virus ^a
<i>Tagetes erecta</i> (Marigold)	1. Purple leaf	TPVV
	2. Leaf curl	CMV-Y
<i>Salvia splendens</i> (Salvia)	Leaf chlorosis	OYVCMV
<i>Dahlia hybrida</i> (Dahlia)	1. Yellow mosaic	MYMV
	2. Mosaic	CMV-Y
<i>Helichrysum bracteatum</i> (Straw flower)	Mosaic	TYLCV
<i>Impatiens balsamina</i> (Garden balsam)	Chlorotic spots	OYVCMV

^a TPVV(Tomato Purple Vein Virus), CMV-Y(Cucumber Mosaic Virus-Y), OYVCMV(Okra Yellow Vein clearing Mosaic Virus), MYMV (Mung bean Yellow Mosaic Virus), TYLCV(Tomato Yellow Leaf Curl Virus)

4. Conclusions

The mosaics of dahlia and leaf curl of marigold were caused by *Cucumber mosaic virus* (CMV-Y) as detected by inoculation test and I-ELISA test. The other four viruses, such as *Mungbean yellow mosaic virus* (MYMV) for yellow mosaic of *Dahlia hybrida*, mosaic of *Helichrysum bracteatum*, leaf chlorosis of *Salvia splendens*, and purple leaf of *Tagetes erecta* were identified as possible causal viruses.

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