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Detection of Viruses Infecting Pumpkin

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Abstract: A field experiment was conducted at the experimental field of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur during October 2010 to April 2011 to detect different viruses in 26 pumpkin breeding lines. The test lines were evaluated under natural condition for virus reaction. Different types include in pumpkin leaves of the test lines, of which the most prevalent symptoms were fern leaf, mosaic, leaf distortion, chlorosis, and vein banding and chlorotic spot. Four different viruses were detected in those of symptomatic pumpkin leaves by Double antibody Sandwich ELISA (DAS-ELISA) techniques. Detected viruses included *Papaya ringspot virus*-watermelon strain (PRSV-W), *Watermelon mosaic virus 2* (WMV2), *Cucumber mosaic virus* (CMV) and *Zucchini yellow mosaic virus* (ZYMV). These viruses caused fern leaf, mosaic, chlorosis and vein banding and leaf distortion symptoms, respectively. ELISA results showed PRSV-W and ZYMV were the most prevalent virus followed by CMV and WMV2 related to number of infected lines. Among the lines, seven (Pk13-1-1, Pk20-2-1, Pk02-2-1, Pk19-4-1, Pk54-4-12, Pk01-10-9-4 and Pk106) did not react to any of the four antisera tested. Of the rest line, five (Pk55-2-2, Pk05-1-2, BARI mistikumra 1, BARI mistikumra 2 and Pk101) were positive to PRSV-W; five (Pk05-4-1, Pk05-8-2, Pk75-1, Pk07-4-7 and Pk102) ZYMV, two (Pk34-4-3 and Pk67-1-9) CMV, and only one (Pk105) WMV2. Six lines (Pk31-2-4, Pk37-1-4, Pk61-1-1, Pk04-7-12-3, Pk05-7-11-8 and Pk107) showed positive reaction to *Potyvirus* group while negative to four antisera tested. Fourteen indicator plants were mechanically inoculated with four detected viruses (PRSV-W, WMV2, CMV and ZYMV). Results of the host range test suggested that PRSV-W, WMV2, CMV and ZYMV were sap transmissible to plant species belonging to Cucurbitaceae, Chenopodiaceae, Solanaceae, Leguminosae and Amaranthaceae family. Cucurbitaceous and Solanaceous host plants were also the systemic host for all viruses.
Keywords: *Cucurbita moschata*, *Papaya ringspot virus*-watermelon strain (PRSV-W), *Watermelon mosaic virus 2* (WMV2), *Cucumber mosaic virus* (CMV)

INTRODUCTION

Pumpkin (*Cucurbita moschata*: Cucurbitaceae) is a very popular vegetable in many tropical and sub-tropical countries. In Bangladesh, the areas under cultivation of pumpkin are 27,602 acre and production 3, 41,000 mt [1]. It is very nutritious due to high content of vitamin A and can play a vital role in meeting the vegetable shortage and nutritional problem.

Viral diseases cause important economic losses throughout the world. More than 35 viruses have been isolated from cucurbits [2, 3]. Most commercial pumpkin varieties are susceptible to the viral pathogens. Potyviruses form the largest and the most economically significant group of plant viruses [4]. Severe losses in pumpkin production areas are due to potyvirus infection. Identification of pumpkin virus diseases by farmers and their advisors is difficult because the diseases cannot be identified reliably by their symptoms. CMV, PRSV-W, WMV and ZYMV may exhibit different symptoms at times, and at other times have overlapping symptoms. In addition different isolates of a virus may result in different symptoms [5]. The most important virus diseases of pumpkin are *Papaya ringspot virus*-watermelon strain (PRSV-W, formerly *Watermelon mosaic virus*-1), *Watermelon mosaic virus* (WMV, formerly *Watermelon mosaic virus*-2), *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic* (ZYMV) and *Squash mosaic virus* (SQMV) etc. cause serious damage though out the world [5-11]. Infected pumpkin plants may show vine decline, reduced or absent yield and fruit quality defects. In Bangladesh four plant viruses from 21 samples of various cucurbitous crops were identified [12]. The present investigation was conducted to detect types of viruses in the population of lines of pumpkin.

MATERIALS AND METHODS

Plant Culture

The detection experiment was conducted during November 2010 to April 2011. The test entries

consisting of 26 selected pumpkin lines in the present experiment. They were collected from Vegetable division, Horticultural Research Centre (HRC), Bangladesh Agricultural Research Institute (BARI), Gazipur. Twenty to twenty eight days old seedlings of the test entries, earlier raised in polybags, were planted individually in pits of 45 cm x 45 cm x 40 cm sizes in unit plots of 2.0 m and 2.0 m spacing. There were three replicated 78 plots and each plot contained 4 pits for each of the test entries. Standard cultural practices and recommended doses of fertilizers were applied according to Bhuyan [46]. Bait traps were placed in field for controlling fruit flies [13].

Identification of viruses

Pumpkin plants grown in the experimental field were checked at 55 days after transplanting. The recorded symptoms include fern leaf, mosaic, leaf curling, chlorosis, leaf distortion, and smaller leaflets of plants. Individual plants showing visible symptoms of virus diseases were recorded. Photographs of the symptoms were taken and compared with standard literatures [14].

Serological detection of viruses using DAS-ELISA

Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) method [15] was followed to detect the virus (es) serologically from the collected samples. Polyclonal antisera against PRSV-W, WMV2, CMV and ZYMV were used for the serodiagnosis of the collected samples. Antisera and alkaline phosphatase enzyme conjugate of PRSV-W, WMV2, CMV and ZYMV were obtained from Agdia Inc. USA.

The assay was carried out in polystyrene micro titer plates (NUNC- Immuno ^{TM} Plate) having 96 wells. All reagents were used in the ELISA at volume of 200 µl per well. The micro plates were coated sequentially with 10 fold diluted sap extract of infected pumpkin leaf samples in 0.05M carbonate-bicarbonate buffer with pH 9.6. The plates were incubated at 4C for overnight. The plates were washed with 0.02 M phosphate buffered saline (PBS) containing 0.05% tween-20 (PBS-T) for three times. After washing, the unoccupied binding sites of the plates were saturated by incubation with 0.1% skim milk in 0.02M PBS, pH 7.4 for 1.5 hour to avoid non-specific reaction. The plates were washed three times with PBST. After washing, the plates were successively incubated with (1) antigen (leaf samples) in 0.005 M carbonate buffer, pH 9.6, for 12 hours at 4C; (2) virus specific antiserum diluted in PBST for 2 hours at room temperature; (3) enzyme-labeled conjugate in 0.02 M PBS, pH 7.4 for 2 hours at room temperature and (4) 1 mg/ml of *p*-nitrophenyl phosphate (Sigma-Aldrich Co.) in 10% of diethanolamine buffer, pH 9.8 and absorbance (405 nm) monitored using ELISA reader model MTP-120 after 1 hour substrate incubation.

The absorbance value of wells for each sample was used to evaluate virus infection. Healthy plants and leaf tissue extracts from infected plants consisted of leaf tissue from uninfected plants of each of the lines tested were used as negative controls and positive controls, respectively. Based on preliminary assays, a sample was considered virus positive if the absorbance value (405 nm) was greater than twice of that of the healthy plant. Specimens showed positive reaction in DAS-ELISA test was selected for further test to confirm the detection.

Host range test

The host range of the viruses (PRSV-W, WMV2, CMV and ZYMV), inoculation test was conducted in an insect proof net house to avoid insect contamination. The Pumpkin leaf samples showed positive results in DAS-ELISA were used for host range tests**.**

Fourteen plant species belonging to 5 dicotyledonous plant families (Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Leguminosae and Solanaceae) were used in the host range test [47]. The test plants were *Nicotina tabaccumm* and *N. globodera* (Tobacco), *Chenopodium amaranticolor* and *Chenopodium quinoa* (Bathua), *Gompherena globosa* (Button flower*), Datura stramonium* (Datura), *Pisum sativum* (Pea), *Lagenaria siceraria* (Bottle gourd), *Trichosanthes anguina* (Snake gourd), *Luffa acutangula* (Ridge gourd), *Luffa cylindrica* (Sponge gourd), *Benincasa hispida* (White gourd), *Cucurbita moschata* (Pumpkin) and Petunia (*Petunia hybrida*). The virus inocula (pure isolates of PRSV-W, WMV2, CMV and ZYMV) were prepared by grinding of virus infected leaf samples in 0.01 M potassium phosphate buffer having pH 7.4 and containing 0.1% mercaptoethanol. The test plants were dusted with 600 mesh carborundum powder and the sap diluted with buffer (0.01 M, pH 7.4) was rubbed on it by finger. The inoculated test plants were kept in the net house and observed three weeks for development of symptoms. Inoculated and subsequently developed leaves were back inoculated to pumpkin for confirming virus infection.

RESULTS AND DISCUSSION

Detection of viruses by visual symptoms observation

Various types of symptoms developed on pumpkin lines due to infection with different viruses are shown in Table 1. Virus symptoms showed only on young leaves of the plants. The observed symptoms were classified into 10 symptom categories. They were fern leaf, mild to severe mosaic, chlorosis and vein banding, leaf distortion, chlorotic spotting and mottling, yellow-green mosaic, yellowing of leaves, deformation and blistering of leaf lamina. In many cases, mixed symptoms were recorded on the same plant.

The symptoms recorded from the experiment were compared with symptoms presented in standard literature and based on visible symptoms the viruses were identified as PRSV-W, WMV2, CMV, ZYMV and other *potyviruses* (BgMV). Photographs of virus infected leaves and healthy leaves showing typical symptoms were taken and presented in Plate 1 (A-F).

Table 1: Categories of symptoms on infected plants in the field

Symptoms category	Description of the symptoms
1	fern leaf
2	mild mosaic
3	chlorosis and vein banding
4	Leaf distortion
5	chlorotic spotting
6	chlorotic mottling
7	yellow-green mosaic
8	yellowing of leaves
9	blistering of leaf lamina
10	deformation of leaf lamina

Fern leaf symptoms

The symptom appeared as the deformation of the leaf blades leading to the formation of fern leaf or shoe string like structure. Mild mosaic having vein clearing and vein banding were also developed on the infected leaves as associated symptoms. In later stage of development totally deformed leaves with reduced size was observed. The flowering behavior was usually as usual but the infected plants produced deformed small sized fruits having green raised spot scattered on the surface. The older leaves were small and deformed fern leaf like appearance (fig-1 A).

The symptoms so far noted on pumpkin and named as fern leaf were identical with the symptoms produced by *Papaya ringspot virus* both watermelon strain or papaya strain (PRSV-W/P) in papaya and cucurbits as reported by Purcifull *et al.* [16]. It was reported that symptoms produced by PRSV-W may be as mottling, mosaic, vein clearing [17-19, 2, 8], and chlorosis, distortion and leaf deformation [20, 21]. Considering the symptoms of PRSV as per literature and the symptomatological observation noted on pumpkin lines in the experiment suggest that the causal virus might be PRSV. However, identification of this virus was confirmed following DAS-ELISA virus detection method.

Mosaic symptoms

In initial stage, mosaic symptoms were observed in growing leaves. Then vein clearing appeared from the edge of the leaf. Further development of the symptoms was characterized by chlorosis or yellowing started from leaf tip and edge of the leaves resulting yellowing/chlorosis of the most of the area of the infected leaves. The infected leaves became twisted and reduced in size. The plants became stunted or ceased to grow and the infected plants yielded small size fruits (fig-1 B).

Lovisolo [2] and Purcifull *et al.* [22] reviewed the *Watermelon mosaic virus* 2 (WMV2) in different aspects including its symptoms and host range. They concluded that WMV2 has a wide range of host specially infects many kinds of cucurbits. In their review the symptoms what they described for WMV2 in different cucurbits seemed to be identical with the symptoms recorded from pumpkin plants in the present experiment lead to suspect the virus as WMV2. For confirmation of identification the virus serological test was performed.

Chlorosis and vein banding symptoms

The first symptoms were yellow green spots with mottling. There were alternative yellow green patches on leaves, which enlarged rapidly and covered the entire leaf. With the aged of the plant, the infected leaves developed chlorosis, vein banding, yellow patches and distortion (fig-1 C). The older plants showed small and deformed leaves. The plants were stunted; mosaic and stunting [23] may be for CMV. Serological test confirmed the identification of the virus.

Leaf distortion symptoms

Pumpkin leaf showed mosaic symptom at early stage of infection. But at later stage of infection leaves showed yellow mosaic with vein banding and leaf distortion. Especially fern leaf and shoestring type leaf distortion was appeared at later stage of infection when pumpkin plant was infected by ZYMV [24, 25] (fig-1 D). However, identification of the virus was confirmed by serological test.

Chlorotic spot symptoms

Different sized chlorotic spots appeared scatter on the leaves as initial symptom (fig-1 E). The further development was started as chlorosis from the leaf margin followed by downward curling. Chlorosis was also observed in the main veins. The fruits yielded by the infected plants were deformed and usually small in

size compared to healthy plants. Komm and Agrios [26] reported that a severe strain of bottle gourd mosaic virus (BgMV) causing chlorotic leaf spotting on yellow summer squash. Provvidenti and Uyemoto [27] reported the occurrence of BgMV causing chlorotic spots on cucurbitaceous crops. The results of the symptomatological study suggested that the chlorotic spots appeared on pumpkin might be due to the infection BgMV.

The symptoms described under the present investigation were confirmed with the symptoms of PRSV-W, WMV2, CMV and ZYMV and other *potyvirus* (BgMV) as mentioned by different researchers on various cucurbits [2, 16, 18, 22].

Fig-1: Various types of symptoms appeared on pumpkin lines under natural condition

Serological detection of different viruses from pumpkin breeding lines

The reactions and values of the plant samples of pumpkin leaves in DAS-ELISA test are presented in Table 2.

Table 2: Response of 26 pumpkin breeding lines against different viruses by DAS-ELISA

Lines	*CMV*	*PRSV-W*	*ZYMV*	*WMV2*	*Poty Virus group*	Reaction
Healthy	-	-	-	-	-	
Positive control	0.40	0.16	0.41	0.21		
Negative control	0.14	0.07	0.11	0.12		
Pk05-4-1	-	-	0.14	-	-	S
Pk31-2-4	-	-	-	-	+	S
Pk05-8-2	-	-	0.15	-	-	S
Pk34-4-3	0.25	-	-	-	-	S
Pk75-1	-	-	0.16	-	-	S
Pk67-1-9	0.22	-	-	-	-	S
Pk37-1-4	-	-	-	-	+	S
Pk07-4-7	-	-	0.15	-	-	S
Pk13-1-1	-	-	-	-	-	R
Pk20-2-1	-	-	-	-	-	R
Pk02-2-1	-	-	-	-	-	R
Pk19-4-1	-	-	-	-	-	R
Pk55-2-2		0.11	-	-	-	S
Pk61-1-1	-	-	-	-	+	S
Pk54-4-12	-	-	-	-	-	R
Pk05-1-2	-	0.11	-	-	-	S
Pk04-7-12-3	-	-	-	-	+	S
Pk05-7-11-8	-	-	-	-	+	S
Pk01-10-9-4	-	-	-	-	-	R
BARI mistikumra1	-	0.12	-	-	-	S
BARI mistikumra 2	-	0.10	-	-	-	S
Pk101	-	0.12	-	-	-	S
Pk102	-	-	0.14	-	-	S
Pk105	-	-	-	0.14	-	S
Pk106	-	-	-	-	-	R
Pk107	-	-	-	-	+	S

R= Resistant; S=Susceptible

Serological test of healthy and diseased leaves of 26 pumpkin lines was performed using antisera against PRSV-W, WMV2, CMV and ZYMV. Out of 26 leaf samples 13 samples **(**Pk34-4-3, Pk55-2-2, Pk05-1-2, Pk07-4-7, Pk75-1, Pk67-1-9, Pk05-4-1, Pk05-8-2, BARI mistikumra1, BARI mistikumra 2, Pk101, Pk102 and Pk105**)** showed positive reaction against four antisera which were used for detection of viruses. Among them, five lines (Pk55-2-2, Pk05-1-2, BARI mistikumra1, BARI mistikumra 2 and Pk101) having titer values of 0.10-0.12 showed positive reaction against the PRSV-W. One (Pk105) with titer value of 0.14 showed positive against the WMV2, two (Pk34-4-3 and Pk67-1-9) showed positive reaction against the CMV having titer values 0.22-0.25 and five (Pk05-4-1, Pk75-1, Pk05-8-2, Pk07-4-7 and Pk102) with titer values of 0.14-0.16 showed positive reaction against the ZYMV. Samples of rest of the lines did not show any reaction in DAS-ELISA against four antisera namely PRSV-W, WMV2, CMV and ZYMV. Six lines (Pk31-2-4, Pk37-1-4, Pk61-1-1, Pk04-7-12-3, Pk05-7-11-8 and Pk107) showed positive reaction to *Potyvirus* group while negative to four antisera tested which antisera were not used to identify. The results of ELISA test, out of 26 lines 13 lines showed positive against four antisera, rest of lines negative against were termed as susceptible and resistant, respectively. Based on results of DAS-ELISA in the present study indicate that plants of pumpkin lines were infected with at least four viruses namely PRSV-W, ZYMV, CMV and WMV2.

The collected 26 samples, which showed virus disease-like symptoms in the field i.e, symptomatic, but showed negative reaction in DAS-ELISA might be due to abiotic agents [28-30] or may either have been infected with other viruses for which antiserum was not used [31] or infection with an unidentified virus as yet not characterized or suffering from physiological or nutritional disorder [32].

Watermelon mosaic virus (WMV) by Gray *et al.* [33] and *Cucumber mosaic virus* (CMV) by Kobori *et al.* [34] reported that virus involve cellular membrane changes that impede the diffusion or transport of infective virus particles from cell to cell, and/or inhibit virus particle replication in the leaf tissue of resistant host plants or restriction of CMV movement at the interface of cell.

Similar work was conducted by Kader *et al.* [35] found out of six four samples were positive to PRSV-P and two were found positive against the antisera of PRSV-W and WMV2, Yilmaz and Sherwood [36] for detection of *Cucumber mosaic virus* (CMV), *Papaya ringspot virus* type W (PRSV-W), *Squash mosaic virus* (SqMV),*Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV) by using formats of protein-A ELISA (PAS-ELISA), antigen-coated plate ELISA (ACP-ELISA), and indirect ELISA kit. Cheng *et al.* [37] for PRSV and PRSV-W in Taiwan by ELISA and Shaifullah [38] for CMV-Y and PRSV-W viruses in an I-ELIZA test of 51 pumpkin leaf samples collected from Gopalgonj, Faridpur and Khulna areas.WMV-2 which has been reported to cause severe damage to cucurbits in India [39] was detected only from pumpkin.

This study suggests that DAS-ELISA may provide a more definite criterion than visual inspection in the selection for virus diseases resistance. The result of the present study suggests that PRSV-W and ZYMV are the major virus disease of pumpkin in Bangladesh. Emphasis should be given to develop pumpkin variety resistant to PRSV-W and ZYMV.

Host range test

The results of host range test are presented in Table. 3 with a comparison of the host range of different PRSV-W, WMV2, CMV and ZYMV isolates. Based on the reaction of test plant species provoked by the isolated viruses, it could be concluded that the tested plant material was infected by PRSV-W, WMV2, CMV and ZYMV. Most of the cucurbitaceous plants used in the host range test were infected and produced different kinds of systemic symptoms.

Necrotic local symptoms typically appeared about 5-7 days after inoculation of CMV and WMV2 isolates, except for local chlorotic spots caused by the isolates of ZYMV and PRSV-W on *Chenopodium quinoa* and *Chenopodium amaranticolor*, which appeared considerably late, 10 days after inoculation. On *Chenopodium quinoa*, CMV caused local spots that changed to necrosis rapidly, in a few days. PRSV-W, ZYMV and WMV2 showed the identical host range, but could be differentiated from each other by the reaction on *Chenopodium quinoa* and *Chenopodium amaranticolor*. On *Chenopodium quinoa*, WMV2 caused chlorotic local spots and easily distinguishable mosaic combined with slight deformation of the leaf lamina. At the same time WMV2 caused local chlorotic spots on *Chenopodium amaranticolor* which turned to necrosis after a few days, contrary to ZYMV which provoked chlorotic spots that remained chlorotic till the full collapse of the leaf.

CMV caused systemic mosaic, mottling and vein clearing of growing leaves of *Nicotiana glutinosa* L. Similarly systemic reaction in the form of vein clearing, mosaic, and deformation of young leaves of infected *N. tabacum*. On the other hand PRSV-W, ZYMV and WMV2 were not infectious for this genus but WMV2 produced local mosaic symptom on inoculated leaves.

CMV infection developed the most conspicuous symptoms on the leaves of *Datura stramonium* diffusive changeable light and dark green areas vein clearing and crinkling. CMV isolates developed necrotic local lesion on *Gompherena*

globosa, whereas PRSV-W isolates initially produced chlorotic local lesion and finally necrotic local lesion but other viruses did not produced any reaction.

All the isolates produced systemic infection in cucurbitaceous plant but only the exception was *L. siceraria* (bottle gourd), where local lesion and latent symptom was developed by CMV, PRSV-W, WMV2 and ZYMV. Taskeshita *et al.* [40] also reported that CMV produced pin point pointed local lesion in *L. siceraria*. PRSV-W developed mild mosaic symptoms on *C. moschata* (pumpkin), *L. acutangula* (ribbed gourd) *and L. cylindrica* (Sponge gourd). All virus isolates produced systemic symptom on pumpkin. Mostly they were mosaic and deformation of leaves in PRSV-W, CMV, and ZYMV, except WMV2, produced local chlorotic lesion on leaves.

CMV produced chlorotic lesion, mottling and necrosis systemic symptoms on *Pisum sativum* where as PRSV-W produced systemic mosaic, ZYMV and WMV2 produced local lesion by mechanical inoculation of viruses. No visible symptoms were showed in *Petunia hybrida* by all inoculated viruses.

The investigation reported in this result confirms the presence of PRSV-W, ZYMV, CMV and WMV2 in our country. These viruses had been described previously in other countries [41]. Based on the species of host plants and characteristic symptoms it is possible to make a biological characterization of mechanically transmissible viruses of cucurbits.

Table 3: Reaction of host plants to mechanical inoculation with CMV, PRSV-W, ZYMV and WMV2

Host plant		Symptom*							
Scientific name	Common name	CMV		PRSV-W		ZYMV		WMV2	
		Local	Systemic	Local	Systemic	Local	Systemic	Local	Systemic
Amaranthaceae									
Gompherena globosa	Button flower	LLn	--	LLc	NS	NS	NS	NS	NS
Chenopodaceae									
Chenopodium amaranticolor	Bathua	LLn	--	LLc	--	LLc	--	LLn	--
Chenopodium quinoa	Bathua	LLn	--	LLc	--	LLc	--	LLn	M, D
Cucurbitaceae									
C. moschata	Pumpkin	--	M,D	--	SM,D	--	M,D	LLc	--
Luffa acutangula	Ribbed gourd	--	M	--	SM	--	M	--	M
Luffa cylindrika	Sponge gourd	--	M	--	SM	--	M	--	M
Lagenaria siceraria	Bottle gourd	M	--	M	SM	M	--	M	--
Trichosanthes anguina	Snake gourd	--	M	--	M	--	M	--	M
Benincasa hispida	White gourd	--	M	--	Lc	--	M	--	M
Leguminosae									
Pisum sativum	Pea	--	LLc,Mo	--	SM	L	--	L	--
Solanaceae									
Nicotiana tabacum L.	Tobacco	--	M, Vc,	--	--	--	--	M	--
Nicotiana glutinosa L.	Tobacco	--	M, Vc, Mo	--	--	--	--	M	--
Datura stramonium	Datura	--	Vc, Cr	--	--	--	--	--	--
Petunia hybrida	Petunia	NS	NS	NS	NS	NS	NS	NS	NS

***--**/NS = no symptoms. LLc=Chlorotic local lesions, LLn= Necrotic local lesions, M – mosaic, D= Deformed of leaves/leaf distortion, V_C – vein clearing, Mo – mottling, Cr – Crinkling, SM=systemic mottle or mosaic

The isolates of viruses obtained in this study tended to cause the same symptoms as those previously described in literature [8, 42-45]. In spite of the fact that virus caused numerous and destructive diseases on the cultivated species of the family Cucurbitaceae, in our country little attention has been paid to these viruses in

the past. In view of the intensified incidence of pumpkin viruses and their growing economic importance in Bangladesh, it is necessary to continue this study, focusing the attention on PRSV-W, one of the most destructive viruses of pumpkin.

CONCLUSION

The viruses of different pumpkin lines were PRSV-W, WMV2, CMV and ZYMV as detected by visual symptoms, DAS-ELISA and host range test.

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