Cucumber Mosaic Virus in Bangladesh

Akanda, Abdul Mannan
Laboratory of Plant Pathology, Faculty of Agriculture, Kyushu University

Tsuno, Kazunori
Laboratory of Plant Pathology, Faculty of Agriculture, Kyushu University

Maeda, Takanori
Research Institute for Bioresources, Okayama University

Wakimoto, Satoshi
Laboratory of Plant Pathology, Faculty of Agriculture, Kyushu University

https://doi.org/10.5109/23960

出版情報：九州大学大学院農学研究院紀要. 35 (3/4), pp.151-159, 1991-02. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., 35 (3•4),151-159 (1991)

Cucumber Mosaic Virus in Bangladesh

Abdul Mannan Akanda, Kazunori Tsuno, Takanori Maeda* and Satoshi Wakimoto

Laboratory of Plant Pathology, Faculty of Agriculture,
Kyushu University, Fukuoka 812, Japan
*Research Institute for Bioresources, Okayama University, Kurashiki 710

(Received October 30,1990)

As many as 92 different samples belonging to 15 botanical families, showing virus disease-like symptom were collected from various locations of Bangladesh in 1986-87. Plant samples were lyophilized or dried with calcium chloride and preserved at 4°C. Since inactivation of most of the samples was observed in mechanical inoculation to original or closely related host plants in 1989, the dried samples were subjected to double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and dot-immunobinding assay (DIBA) for detecting cucumber mosaic virus (CMV). Two, three, two and one of the samples of cucumber, chilli, pepper and tomato, respectively, were found to be positive against the antiserum of CMV in DAS-ELISA. Of these eight samples, six reacted positively in DIBA. Simplified double-diffusion (SDD) test was also applied to detect CMV from dried samples. All these three methods DAS-ELISA, DIBA and SDD test were found to be useful for the detection of CMV from dried samples. Results demonstrated that the antigenicity of CMV retained at least for 2-3 years after drying even if they had completely lost their infectivity. The results also suggested the occurrence of CMV on cucurbitaceous and solanaceous plants in Bangladesh, but its distribution frequency seemed not so high.

INTRODUCTION

Bangladesh, a predominately agro-based country, grows many varities of crops such as legumes, vegetables, fibres, spices etc. as major food and cash crops (Rabbani, 1984 ;Rashid, 1976 ;Rashid, 1981). High prevalence of plant diseases has been reported to limit the yield and quality of crops there (Talukder, 1974 ; Ahmed, 1984). Fakir (1984) reported that hot-humid-tropical climate is one of the main reasons which favours the catastrophic occurrence of plant diseases on various crops in Bangladesh. Among these, the diseases caused by viruses have been found to be high on the basis of the symptomatological observation (Ahmed, 1984). The actual cause of these diseases have not yet been identified due to lack of proper facilities and trained manpower to investigate the plant viruses. However, the identification of viruses infecting various crops is highly important to combat the diseases.

Cucumber mosaic virus (CMV) has been reported to be one of the most economically important aphid-borne viruses infecting a wide variety of hosts including herbaceous and woody plants all over the world (Franki et *al*.,1979). It has been reported that 775 plant species belonging to 85 families are said to be susceptible to CMV and the highest number of susceptible hosts are listed in Cruciferae, Solanaceae, Compositae, Leguminosae and Cucurbitaceae (Lovisolo, 1981). The virus has been given special attention due to its complex epidemiology as it is transmitted by several

aphid species and through seeds of some hosts and also due to its serious damage to crop production (Cohen, 1982 ; Jones, 1988 ; Conti and Lovisolo, 1982 ; Martelli and Quacgarelli, 1982 ; Phillips and Burnt, 1985). Although, Lovisolo (1981) reported the severe occurrence of CMV on many crops specifically in the temperate zone, its occurrence on various crops has also been reported from the south and south east, tropical asian countries (Kajiwara and Konno, 1986 ; TARC, 1977).

This study was, therefore, undertaken to make a serological survey on the occurrence of CMV on different crops in Bangladesh by using dried specimens.

MATERIALS AND METHODS

Plant sample used

In all 92 samples representing 39 plant species of 15 botanical families were collected from various fields located in seven different adminstrative districts of Bangladesh in 1986-87 (Table 1). the leaves of plants showing virus disesae-like symptom were collected and dried by lyophilization or with calcium chloride. The dried samples were preserved at 4°C until use.

Inoculation test

All the samples were inoculated to the original hosts and some common local lesion hosts such as *Chenopodium quinoa, C. amaranticolor, Gomphrena globosa, Nicotiana tabacum, N. glutinosa, Cucumis sativus, Pisum sativum* etc. following the

Table 1. List of the samples collected in Bangladesh.

No.	Plant samples	Symptom	Location
	CUCURBITACEAE		
*1	Bottlegourd	Mosaic	Nurbag[1], Gazipur[2]
2	Bottlegourd	Vein-clearing	Mouckak, Gazipur
3	Bottlegourd	Mosaic	Gazipur, Gazipur
*4	Bottlegourd	Mosaic	Kashimpur, Gazipur
5	Bottlegourd	Yellowing	Kashimpur, Gazipur
6	Bittergourd	Mosaic, Curl	Joydebpur, Gazipur
7	Bittergourd	Mosaic	Kashimpur, Gazipur
*8	Cucumber	Mosaic	Kashimpur, Gazipur
9	Cucumber	Yellowing	Kashimpur, Gazipur
10	Cucumber	Mosaic	Jessore, Jessore
*11	Pumpkin	Mosaic	Kashimpur, Gazipur
12	Pumpkin	Mosaic	Salna, Gazipur
13	Round cucumber	Mosaic	Joydebpur, Gazipur
14	Spongegourd	Mosaic, Vein-clearing	Charpolisha, Jamalpur
15	Sweetgourd	Mosaic	Katabari, Jamalpur
16	Whitegourd	Mosaic	Kashimpur, Jamalpur
17	Zucchini	Mosaic	Joydebpur, Gazipur
	SOLANACEAE		
18	Chilli	Curl	Kashimpur, Gazipur
19	Chilli	Yellowing, Curl	Joydebpur, Gazipur
20	Chilli	Curl	Ragunathpur, Jamalpur
*21	Chilli	Vein-clearing	Kashimpur, Gazipur
22	Chilli	Mosaic	Salna, Gazipur
23	Eggplant	Mosaic	Nutonhat, Jessore

24	Eggplant	Mosaic	Kashimpur, Gazipur
25	Eggplant	Yellowing	Kashimpur, Gazipur
26	Eggplant	Vein-clearing	Kashimpur, Gazipur
27	Eggplant	Mosaic	Salna, Gazipur
28	Pepper	Curl	Gazipur, Gazipur
29	Pepper	Enation	Kashimpur, Gazipur
30	Tomato	Curl	Joydebpur, Gazipur
31	Tomato	Purple	Joydebpur, Gazipur
32	Tomato	Yellowing	Kashimpur, Gazipur
33	Tomato	Curl	Salna, Gazipur
34	Tomato	Necrosis	Salna, Gazipur
35	Tomato	Purple	Salna, Gazipur
36	Tomato	Crinkle	Salna, Gazipur
37	Tomato	Yellowing	Salna, Gazipur
	LEGUMINOSEAE		
38	Asparagus bean	Yellowing	Salna, Gazipur
39	Blackgram	Mosaic	Pars, Jamalpur
40	Blackgram	Mottle	Pars, Jamalpur
41	Blackgram	Yellow mosaic	Rahmatpur, Barisal
42	Blackgram	Mosaic, Chlorosis	Nashopur, Dinajpur
43	Country bean	Mosaic	Salna, Gazipur
44	Cowpea	Mosaic, Vein-clearing	Jessore, Jessore
45	Cowpea	Mosaic	Salna, Gazipur
"46	Cowpea	Mosaic	Joydebpur, Gazipur
47	Hyacinth bean	Mosaic	Bhaorber, Jessore
48	Longbean	Mosaic	Kashimpur, Gazipur
49	Longbean	Necrosis	Kashimpur, Gazipur
50	Longbean	Shrinking	Kashimpur, Gazipur
51	Longbean	Yellow spots	Kashimpur, Gazipur
52	Longbean	Yellowing	Kashimpur, Gazipur
53	Mungbean	Curl, Mottle	Gabua, Patuakhali
54	Pigeonpea	Mosaic	Ragunathpur, Jamalpur
55	Soybean	Mosaic	Salna, Gazipur
56	Soybean	Mosaic	Ishurdi, Pabna
57	Soybean	Mosaic	Kashimpur, Gazipur
58	Yard long bean	Vein-clearing	Joydebpur, Gazipur
59	Yard long bean	Mosaic	Joydebpur, Gazipur
	PAPILIONACEAE		
60	Groundnut	Yellowing	Rahmatpur, Barisal
61	Groundnut	Mosaic	Loknathpur, Chuadanga
	CARICACEAE		
*62	Papaya	Fern leaf	Kashimpur, Gazipur
*63	Papaya	Mosaic	Joydebpur, Gazipur
64	Papaya	Mosaic	Kashimpur, Gazipur
*65	Papaya	Vein-clearing	Salna, Gazipur
*66	Papaya	Mosaic	Patuakhali, Patuakhali
67	Papaya	Mosaic	Salna, Gazipur
	CRUCIFERAE		
68	Cauliflower	Mosaic	Salna, Gazipur
69	Chinese cabbage	Mosaic	Kashimpur, Gazipur
70	Chinese cabbage	Yellowing	Kashimpur, Gazipur
71	Chinese cabbage	Curl	Kashimpur, Gazipur
72	Chinese cabbage	Chlorosis	Kashimpur, Gazipur
73	Radish	Mosaic	Salna, Gazipur
	MALVACEAE		
74	Cotton	Mosaic	Basherhat, Dinajpur
75	Cotton	Vein-clearing	Basherhat, Dinajpur

76	Cotton	Chlorosis	Basherhat, Dinajpur
77	Okra	Mosaic	Kashimpur, Gazipur
78	Okra	Vein-clearing	Salna, Gazipur
79	Okra	Mosaic	Rahmatpur, Barisal
80	Okra	Mosaic	Salna. Gazipur
	TILIACEAE		
81	Jute	Mosaic	Ragunathpur, Jamalpur
82	Jute	Vein-clearing	Ragunathpur, Jamalpur
83	Jute	Mosaic	Charpolisha, Jamalpur
	ARACEAE		
84	Colocasia	Vein-clearing	Ragunathpur, Jamalpur
85	Colocasia	Mosaic	Salna, Gazipur
	UMBELLIFERAE		
86	Carrot	Necrosis	Kashimpur, Gazipur
87	Coriander	Necrosis	Joydebpur, Gazipur
	COMPOSITAE		
88	Lettuce	Chlorosis	Kashimpur, Gazipur
	LILIACEAE		
89	Garlic	Mosaic	Joydebpur, Gazipur
	ASTERACEAE		
90	Safflower	Mosaic	Joydebpur, Gazipur
	PIPERACEAE		
91	Betel leaf	Mosaic	Rahmatpur, Barisal
	MUSACEAE		
92	Banana	Bunchy-top	Kahmatpur, Barisal

[1] : Name of collection site
[2] : Name of adminstrative district

methods of Hill (1984). Inoculations were done in a temperature controlled greenhouse (20-25°C).

Antiserum and virus

Polyclonal rabbit antiserum to the yellow strain of CMV (CMV-Y) was used in all the experiments. The antiserum was absorbed with insolubilized healthy tobacco leaf proteins before use. For DAS-ELISA, y-globulin was purified from the absorbed antiserum by ammonium sulfate precipitation followed by DEAE cellulose colum chromatography (Clark and Bar-Joseph, 1984). In DIBA test, y-globulin solution was absorbed with extract of healthy tobacco leaves to eliminate trace amount of antibodies against healthy components. The antiserum of CMV-Z (serotype P) was also used in SDD test.

Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA)

DAS-ELISA was performed essentially as described by Clark and Adams (1977) with slight modifications. Wells of polystyrene microtiter plates were coated with y-globulin ($2\mu g/ml$) for 3 hr at 30°C. The crude extract was triturated ca. 0.05 g dried samples in 0.5 ml of sample buffer on Parafilm by using glass rods. An amount of $30\mu l$ of homogenate was poured in each well of plates containing 120 μl of sample buffer and incubated overnight at 4°C. The enzyme conjugate (1 : 800 dilution) was reacted for 3 hr at 30°C. Artificially infected tobacco leaves with CMV-Y (dried

samples) were used as positive control. The ELISA values were measured by using an ELISA analyzer (Immuno Reader NJ-2000, Inter med) at the wavelength of 405 nm.

Dot-immunobinding assay (DIBA)

With some simple modifications, DIBA procedures were carried out following the method described by Powell (1987). Dried samples were macerated in TBS (0.02 M Tris-HCI, 0.5 M NaCl, pH7.5) on Parafilm with glass rods. Nitrocellulose membrane (NCM, Transfer Blot, Bio Rad) was immersed in distilled water and placed on filter paper for 5 min to dry. Two μl of extract was spotted on the NCM and dried for 10 min. The NCM was put in blocking solution consisted of 2 % BSA (bovine serum albumin) and 2 % Triton X-100 in TBS for 1 hr, followed by washing with TBST (TBS containing 0.05 % Tween 20). The NCM was placed in a plastic box and 20 μl/grid of y-globulin (2μg/ml) diluted with 1 % healthy tobacco leaf extract in TBST containing 0.2 % BSA and 2 % polyvinylpyrrolidone (antibody and conjugate buffer) was added and the membrane was incubated for 2 hr at room temperature. The NCM was washed three times with TBST. The membrane was then incubated with goat anti-rabbit IgG-alkaline phosphatase conjugate diluted 2,000 times with the antibody buffer and washed as described earlier. The NCM was finally, incubated for 30–60 min in color development solution prepared by mixing fast red TR salt (Sigma) and naphthol AS-MX phosphate (Sigma) as recommended by Banttari and Goodwin (1985). The reaction was stopped by washing the NCM in distilled water after 30 min incubation and then air dried for visual observation.

Simplified double-diffusion (SDD) test

Some of the samples positive in DAS-ELISA and DIBA were tested by simplified double-diffusion test for sero-diagnosis of CMV. SDD test was performed as described by Shohara and Inoue (1978). The crude extract was prepared by macerating dried samples in 24 % Noble Agar (Difco), 0.85 % NaCl and 0.2 % sodium azide in 0.2 M Tris-HCI buffer (pH7). The antiserum used in the experiment was diluted to, 1 : 2. Results were recorded after 22 hr incubation at 30°C. Hexagonal arrangement of wells was applied.

Virus purification

Two infective samples-cucumber (8) and chilli (21) propagated on cucumber and chilli, respectively were used for the purification of the virus. The virus was partially purified following the method described by Maeda et al. (1983) with some modifications. Infected leaves of cucumber and chilli were seperately used for purification. For each 100 g of leaves homogenized with mortar and pestle in 200 ml of 0.5 M citrate buffer (pH 6.5) containing 0.025 M EDTA. Homogenate was filtered through double-layered cheese cloth. The filtered extract was stirred for 20 min at 4°C with 1/5 volume of chloroform followed by centrifugation at 5,000 g for 15 min and the supernatant was collected. The supernatant was stirred with 1 % Triton-X 100 for 20 min and then 8 % PEG was added. The mixture was centrifuged at 8,000 g for 20 min and collected the pellet. The pellet was resuspended in 0.005 M sodium borate buffer (pH 8.0) containing 0.005 M EDTA followed by centrifugation at 8,000 g for 20 min and the supernatant was collected. A small amount of 20 % sucrose was layered onto the

borate-EDTA buffer follwing layering of virus suspension. This was then centrifugrd at 87,650 g in Hitachi 65 P ultracentrifuge (RPS 65 T rotor) for 90 min. The pellet resuspended in the borate-EDTA buffer contained the partially purified virus.

Electron microscopy

The partially purified virus was fixed with 2 % formaldehyde and negatively stained with 2 % uranyl acetate or 2 % phosphotungstic acid (pH 6.0). The particle size was measured by a transmission electron microscope (JEM-100 S, JEOL Ltd.).

RESULTS

Inoculation test

Ten different samples marked with asterisk in Table 1 were found to be infective when inoculated to their original hosts in the greenhouse. All others remained inactive in repeated inoculation. Among these ten infective samples, one cucumber (Sample No. 8) and one chilli (Sample No. 21) were later confirmed as CMV. All other infective samples were identified as viruses other than CMV (our unpublished data).

On sap inoculation, both the virus isolates (cucumber, Sample No. 8 and chilli, Sample No. 21) produced local lesions on *Chenopodium quinoa, C. amaranticolor, Gomphrena globosa and Pisum sativum* typical for CMV. The isolates also induced mosaic symptoms typical for CMV on the leaves of inoculated *Nicotiana tabacum* (White burley, Xanthi and Samsun NN), N. *glutinosa,* cucumber and chilli (Jones, 1988 ; Phillips and Rrunt, 1985 ; Tobias *et al.,* 1982).

DAS-ELISA

Out of 92 samples tested, eight samples were found to be CMV-positive in DAS-ELISA (Table 2). Positive samples included the samples representing the four plant species such as cucumber, chilli, pepper and tomato among the 39 different plant species tested.

DIBA

All the samples tested by DAS-ELISA were included in the DIBA. The positive reactions were recognized by the appearence of well-defined purple red spot on the

Table 2. Samples positive in DAS-ELISA, DIBA and SDD test.

Plant samples	DAS-ELISA	DIBA	SDD test
*Cucumber (8)[a)]	+[b)]	+	+
Cucumber (9)	+	+	+
Chilli (18)	+		rlt
Chilli (20)	+	+	+
*Chilli (21)	+	t	+
Pepper (29)	+	+	
Tomato (30)	+	+	nt
Tomato (32)	+		

*) : Infective sample in inoculation test.
a) : Sample number in table 1.
b) : + : Positive reaction, − : Negative reaction, nt : Not tested.

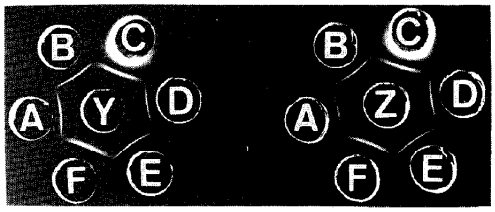

Fig. 1. Simplified double-diffusion (SDD) test by using dried sample. Y, Antiserum of CMV-Y ; Z, Antiserum of CMV-P ; A, Chilli (Sample No. 20 ; B, CMV control sample; C, Cucumber (Sample No. 8); D. Chilli (sample No. 21), E, CMV control sample; F, Cucumber (Sample No. 9).

nitrocellulose membrane. Results of DIBA are summarized in Table 2. In total six samples were found to be positive in DIBA. Two samples positive in DAS-ELISA were reacted negtatively in DIBA. Appearence of non-specific reactions has been reported as only problem in DIBA was successfuly eliminated by absorbing antisera with 1 % healthy leaf extracts.

SDD test

As shown in Table 2 and Fig 1, four different samples out of six tested, produced distinct precipitin bands in SDD test. Two samples found to be positive in DAS-ELISA and DIBA did not produce any precipitin bands in SDD test.

Electron microscopy

The isometric virus particles measuring about 30 nm in diameter were observed under electron microscope from partially purified cucumber and chilli samples.

DISCUSSION

The results of this study demonstrated the occurrence of CMV on cucurbitaceous (cucumber) and solanaceous (chilli, pepper and tomato) plants in Bangladesh. Several other samples of different plant species including Leguminosae and Cruciferae reacted negatively, though these two families reported to have the highest numbers of hosts susceptible to CMV (Franki *et al., 1979* ; Lovisolo, 1981). Moreover, the frequency of CMV positive samples even in cucurbits sand solanaceous plants were not so high (8/37) in this study. Hot-humid-tropical climate of Bangladesh might be one of the reasons of low prevalence of the virus there, as Lovisolo (1981) reported the high prevalence of CMV specially in the temperate zone.

Three serological methods, DAS-ELISA, DIBA and SDD test, were found to be useful for the detection of CMV from dried samples. The loss of infectivity of the virus during storage did not interfere with the detection of antigenicity in serodiagnosis. The highest number of samples, eight were found to be CMV positive in DAS-ELISA followed by six in DIBA and four in SDD test. Results suggested DAS-ELISA was the highest in sensitivity of DAS-ELISA as compared to two other methods used. The superiority of DAS-ELISA as a serological method over any others has been reported

by many researchers (Clark, M. F. 1981; Clement *et al., 1986).* However, Ozyanar and Sako (1987) reported the equal sensitivity of DAS-ELISA and DIBA in detecting CMV, when they used either purified virus or crude leaf extracts of artificially inoculated plants in which the antigen concentrations and specificity were expected to be high compared to field samples used in our experiments. SDD test was found to be less sensitive than either of the methods.

Six CMV positive samples out of eight were found to be inactivated in storage within 223 years. McKinny et al. (1965) reported that CMV remained infective up to 15 years in storage. However, it has been reported that the longevity of virus infectivity varied with many factors such as host plants, drying methods, storage conditions etc (McKinny and Silber, 1968). Moreover, in our case, drastic change of temperature condition for several days during transportation of the samples from Bangladesh to Japan might be one of the main cause of inactivation of the virus.

In our study, the samples were irregular in number and limited in respect of plant species and areas surveyed. The high prevalence of CMV in Bangladesh may be expected in winter crops as the virus was reported to be common in the temperate zone.

However, to draw a sound conclusion on the occurrence of CMV in Bangladesh requires extensive survey considering the epidemiology and host range of the virus. Results of our study was suggestive, rather than conlusive to take well-planed research on CMV in Bangladesh.

ACKNOWLEDGEMENT

The authors are thankful to Dr. T. Kawarabata, Kyushu University for his technical help and advises during experiments. Thanks are due to Dr. S. H. Khan and Dr. I. H. Mian, Institute of Post-Graduate Studies in Agriculture, Bangladesh for cooperating at the time of sample collection in Bangladesh.

REFERENCES

Ahmed, H. U. 1984 Disease problems of pulse and oil-seed crops in Bangladesh. A paper presented in First Biennial Conference (held on 13-14) of Bangladesh Phytopathological Society, 18 pp

Ahmed, M. U. 1984 Diseases of vegetables and fruit plants. A paper presented in First Biennial Conference (held on December 13-14) of Bangladesh Phytopathological Society, 18 pp

Banttari, E. E. and P. H. Goodwin 1985 Detection of potato virus S, X, and Y by enzyme-linked immunosorbent assay on nitrocellulose membrane (DOT-ELISA). *Plant Dis., 69* : 202 205

Clark, M. F. 1981 Immunosorbent assays in plant pathology. *Ann. Rev. Phytopath.,* 19 : X3-106

Clark, M. F. and A. N. Adams 1977 Characteristics of microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol., 34:* 475-483

Clark, M. F. and M. Bar-Joseph 1984 Enzyme immunosorbent assays in plant virology. *Meth. Virol., 7:* 51 *85*

Clement, D. L., R. M. Lister and J. E. Foster 1986 ELISA-based studies on the ecology of barley yellow dwarf virus in Indiana. *Phytopathology '76* : 86-92

Cohen, S. 1982 Resistance to transmission of aphid-borne non-persistent viruses in vegetables. *Acta Hort.,* 127: 117-124

Conti, M. and 0. Lovisolo 1982 Virus problems in protected vegetable crops. *Acta Hort.,* 127 : 83-100

Fakir, G. A. 1984 Plant disease problems of Bangladesh. A Keynote paper presented in First Biennial Conference (held on December 13-14) of Bangladesh Phytopathological Soc., 9 pp

Francki, R. I. B., D. W. Mossop and and T. Hatta 1979 Cucumber mosaic virus. CMI/AAB Descriptions of Plant Viruses No. 213, CMI, KEW, Surry, England 6 pp

Jones, R. A. C. 1988 Seed-borne cucumber mosaic virus infection of narrow-leafed lupin *(Lupinus angustifolius)* in western Australia. *Ann. app. Biol., 113* :507-518

Lovisolo, 0. 1981 Virus and viroid diseases of cucurbits. *Acta Hurt., 88* : 33-90

Maeda, T., S. Wakimoto and N. Inouye 1983 Serological properties of cucumber mosaic virus in Japan. *Ann. Phytopath. Soc. Japan 49* :10-17

Martelli, G. P. and A. Quacqarelli 1982 The present status of tomato and pepper viruses. *Acfa Hurt.*, 127 : 39-64

McKinny, H. H., G. Silber 1968 Methods of preservation and storage of plant viruses. *Meth. Virol.*, 4 : 491-501

McKinney, H. H., G. Silber and L. W. Greeley 1965 Longeivity of some plant viruses stored in chemically dehydrated tissues. *Phytopathology 55* : *1043 1044*

Mclaughlin, M. R. and R. D. Ensign 1889 Viruses detected in forage legumes in Idaho. *Plant Dis., 73* : 906-909

Ozyanar, F. and N. Sako 1987 The detection of cucumber mosaic virus strains by enzyme-linked immunosorbent and dot-immunobinding assays. *Bull. Fac. Agri., Saga Univ., 62* :109-115

Phillips, S. and A. A. Brunt 1985 Occurrence of cucumber mosaic virus and asparagus virus II in asparagus *(Aparagus officinalis* L. var. *officinalis)* in Britain. *Plant Pathol., 34* : *440-442*

Powell, C. A. (1987). Detection of three plant viruses by dot-immunobinding assay. *Phytopathology 77* : 306-309

Rabbani, A. K. M. G. (Ed.) 1984 Yearbook of Agricultural Statistics of Bangladesh. Govt. of Bangladesh, Dhaka, Bangladesh 505 pp

Rashid, H. 1981 An Economic Geography of Bangladesh, University Press Litd. Red Cross Building, 114/Motijiheel C. A., Dhaka, Bangladesh 276 pp

Rashid, M. M. 1976 Vegetables in Bangladesh (in Bengali). BARI, Joydebpur, Bangladesh 409

Shohara, K. and T. Inouye 1978 Simplified immunodiffusion tecniques in agar gel for detection and serodiagnosis of cucumber mosaic virus. *Ann. Phytopath. Soc. Japan 44* : 619-625

Talukder, M. J. 1974 Plant diseases in Bangladesh. *Bangladesh J. Agri. Res.* 1 :61-86

TARC (Tropical Agriculture Research Center) 1977 Symposium on the virus diseases of tropical crops. Trop. Agri. Res. Ser. No. 10, TARC, Ibaraki, Japan 208

Tobias, I., D. Z. Maat, and II. Huttinga 1982 Two Hungarian isolates of cucumber mosaic virus from sweet pepper *(Capsicum annum)* and melon *(Cucumis melo)* : identification and antisera preparation. *Netherland J. PI. Pathol., 88* : *171-183.*