Journal of Agricultural Technology 2014 Vol. 10(1):277-287
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Detection of cucumber green mottle mosaic tobamovirus (CGMMV) in three growth stages of Japanese cucumber in the highland area of Northern Thailand

Sutasinee Nontajak[1,2], Sarunya Vulyasevi[3], Nuchnart Jonglaekha[2] and Prasartporn Smitamana[1*]

[1]Biotechnology Program, Graduate School, Chiang Mai University, Chiang Mai 50200, Thailand, [2]The Royal Project Foundation, Chiang Mai 50200, Thailand, [3]Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

Nontajak, S., Vulyasevi, S., Jonglaekha, N., Smitamana, P. (2014) Detection of cucumber green mottle mosaic tobamovirus (CGMMV) in three growth stages of Japanese cucumber in the highland area of Northern Thailand. Journal of Agricultural Technology 10(1):277-287.

This study was conducted at three different locations in the highland area of Northern Thailand. Two of the locations, Huai Luek (greenhouse production) and Mae Tha Nuea (field production), are in the main cucumber growing areas and the third location, Thung Roeng (field production), had not been previously cropped with cucumber. The research was carried out to investigate seed transmission of CGMMV and detection of the virus at different crop growth stages (seedling, flowering and 1 month after fruit-set). Polyclonal antibodies (ELISA kits, Agdia Inc., Elkhart IN., USA) were used to detect the virus. CGMMV was not detected in whole ground cucumber seeds, endosperm, seed coat, cotyledons or leaves in 1600 seeds from the seed lot tested. The virus was also not detected in any of the growth stages of cucumber at Thung Roeng where the crop had never been grown before. However, CGMMV was detected in each of the three stages at the other two sites at incidences ranging from 7.3 to 30%, except in the seedling stage at Mae Tha Nuea. Total marketable cucumber yield was significantly higher at Thung Roeng (21.25 kg) than at Huai Luek (16.75 kg) and Mae Tha Nuea (15.87 kg). These results suggest that soil transmission of CGMMV was likely and that the virus played a role in the reduction of cucumber yields.

Keywords: virus transmission, virus survey, CGMMV

Introduction

Cucumber (*Cucumis sativus* L.) belongs to the Cucurbitaceae, also comprising melons and squash. Japanese or Suhyo type cucumber is the most important cucurbit crops grown in Northern Thailand under the Royal Project Foundation extension program. Suhyo growers are faced with many problems mostly from diseases and insect pests that caused serious losses in both quality

* Corresponding author: Prasartporn Smitamana; e-mail: sporn@gmail.com

and quantity. Especially important are viral diseases which commonly cause damage to this crop. More than 30 different viruses were reported to infect cucumber crops worldwide (Mazereeuw *et al*., 2010; Ko *et al*., 2007; Yuki *et al.*, 2000). Cucumber green mottle mosaic virus (CGMMV), a member of the genus Tobamovirus, is an economically significant seed transmitted virus which has been reported to cause yield losses of about 15% in cucurbitaceous crops (Antignus *et al*., 2001; ISTA, 2010; Shang *et al*., 2011). CGMMV can be transmitted through water and soil contamination, and sap of the plants infected with the virus. Seed and soil transmissions are recognized as the primary sources for epidemic development of CGMMV disease (Tan *et al*., 2000; Choi, 2001; Mandal *et al*., 2008; Liu *et al*., 2013; Nematollahi *et al.*, 2013). However, the early spread pattern in cucumber, which is different in the fruit-harvesting stage, shows that infected plants are scattered throughout the field (Choi, 2001; Mandal *et al*., 2008). The most common symptoms of infected cucumber are leaf mosaic and mottling, growth stunting, wilting and leaf and fruit malformation (Kim and Lee, 2000; Kim *et al.,* 2003; Moradi and Jafarpour, 2011; VKM 2008). CGMMV was first reported in the United Kingdom in 1935 (Ainsworth, 1935). Subsequently, it had been reported in Europe (e.g. Denmark, Finland, Germany, Greece, Holland, Norway, Russia, and Spain), United stated, Asia (e.g. China, India, Indonesia, Japan, Korea and Pakistan), and the Middle East (Iran, Israel and Saudi Arabia) (Varveri *et al*., 2002; Moradi and Jafarpour, 2011; Yoon *et al*., 2008; Zhou *et al*., 2008). In 2008, Thonmo and Thummabenjapone found CGMMV in 25% of cucurbits samples which were collected from cucurbit seed production fields in Northeastern Thailand. The current methods of detecting CGMMV include symptomotological observations, host range, mechanical inoculation, nucleic acid hybridization, reverse transcription polymerase chain reaction (RT-PCR) and reverse transcription loop-mediated isothermal amplification (RT-LAMP) assays (Adkins *et al*., 2003; Sevik and Arli-Sőkmen, 2003; Qin *et al.*, 2005; Bananej and Vahdat, 2008; Liu *et al*., 2009; Li *et al*., 2013; Nematollahi *et al*., 2013; Gumus and Paylan, 2013). The ELISA method is routinely used worldwide for detection of CGMMV in field surveys because of its sensitivity and it enables the handling large number of samples (Yuki *et al*., 2000; Varveri *et al*., 2002 and Hossain *et al*., 2007). ELISA can be the preferable method for the detection of plant viruses than PCR because of it dose not requiring extensive training and is more economical (Gumus and Paylan, 2013). Therefore, double antibody sandwich ELISA (DAS-ELISA) is a common diagnostic technique used to detect CGMMV (Moradi and Jafarpour, 2011; Shim *et al*., 2006; Siriyan *et al*., 2006; and Yoon *et al*., 2008). The current study was carried out to investigate possible seed transmission of CGMMV and detection of the virus in different cucumber growth stages.

Materials and methods

Seed materials and experimental conditions

Commercial seeds of Japanese cucumber (*C. sativus*) cultivar Pretty Swallow 279 (cucumber F1 hybrid, lot No. EA25061) were used throughout this study. The sample and subsample sizes tested followed ISTA procedures (2010). Five seed groups were separated. Group 1, consisted of 400 whole seeds; group 2, only the endosperm of 400 seeds and group 3, the seed coat of 400 seeds; These samples were soaked in 1X PBS buffer [0.02 M PBST (phosphate buffered saline with Tween 20, 1:10 w/v)] for a minimum of 1 h 4^{o}C. Solids settled to the bottom and the light-colored upper layer was used for CGMMV detection. With group 4 and 5, 200 seeds were sown in plastic trays filled with a growing medium and placed in an insect proof greenhouse. The cotyledons of group 4 were collected for processing after the seedlings germinated. The leaves of group 5 were randomly collected to be examined after the seedlings reached the trifoliate stage 10 days after planting (10 dap.). Cotyledons/leaves were individually ground in PBS buffer and centrifuged (10000xg) for 5 min. The supernatants of ground cotyledon/leaf tissue were used to detect CGMMV.

Collection of infected cucumber samples

Eight-days-old seedlings of Japanese cucumber were planted at the three Royal Project Development Centers; Mae Tha Nuea where field cultivation was used, Huai Luek where the crop was grown in a greenhouse (Mae Tha Nuea and Huai Luek are the main cucumber growing areas in Northern Thailand) and Thung Roeng an area not previously grown in cucumber where field cultivation was used, and grown from May to July 2011. Leaf samples were taken from at least 100 plants at three different growth stages; seedling, flowering and fruiting stages (1 month after fruit set), and symptoms were visually categorized as mosaic, mottle, chlorosis, leaf deformation and stunting. CGMMV detection was performed using DAS-ELISA kits (Agdia Inc., Elkhart IN, USA) as previously described.

Effects on fruit production

The effect of CGMMV on fruit production was evaluated in cucumber planted at the three different locations used in the study. Fruit were collected every 1 or 2 from 100 plants for 12-14 after fruit set. Marketable fruit sizes were classified using three standard grades; grade 1was 15-20x 3.0-3.5 cm, grade 2 was 15-20 x 2.5-3.5 cm. and under grade was>15 cm. Others fruit were rejected as unmarketable.

Detection of CGMMV

DAS-ELISA method was used to detect CGMMV (Agdia Inc., Elkhart IN, USA) followed the protocol described by the company. Duplicate wells were used for each sample. Healthy and diseased tissues were also included as controls. Each well of the ELISA plates (certified Nunc-Immuno Plates MaxiSorp F96) was coated with 100 µl IgG at 1:1000 dilutions in coating buffer and incubated at 30^{o}C for 4 h. The plates were washed three times with PBST and let stand for 3 min. 100µl of the sap samples were pipette into each well and incubated overnight at 4 ^{o}C. The plates were then washed again as described. 100 µl of alkaline phosphatase IgGs at 1:1000 dilutions in conjugate buffer were pipette into the wells and incubated at 30^{o}C for 5 h. The plates were washed as described before and 100 µl of 1 mg/ml p-nitro phenyl phosphate in substrate buffer were pipette into the wells. The plates were incubated at room temperature for 60 min to obtain a clear reaction. The reaction was colorimetrically detected at A405 nm using an ELISA reader (Sunrise basic TECAN, Austria).

Results

Detection of CGMMV in Japanese cucumber seed

Based on the DAS-ELISA result, the CGMMV was not detected in the commercial seeds of Japanese cucumber cultivar Pretty Swallow 279 (cucumber F1 hybrid, lot No. EA25061). The virus was not detected in the sap of whole ground cucumber seeds, endosperm, seed coats, cotyledons or true leaves (10 dap.).

Detection of CGMMV in cucumber plants

A total of 398 disease samples were collected showing various symptoms that could have been induced by virus infection. Common symptom found in the disease samples were mottle, mosaic, and deformed leaves (Figures 1 and 2). Based on DAS-ELISA (Table1), CGMMV was not detected in the cucumber samples collected at Thung Roeng where the crop had never been grown before. However, CGMMV was detected in samples collected from the two main cucumber growing areas. CGMMV was detected at an incidence of 30%in cucumber at the seedling stage in Huai Luek where greenhouse culture was used, but was not detected in seedlings from Mae Tha Nuea where the crop was field-grown. CGMMV was detected at flowering stage at incidences of 20.83 and 7.32 percent at Huai Luek and Mae Tha Nuea, respectively. CGMMV incidence at Huai Luek and Mae Tha Nuea at 1 month after fruit set was 12.66 and 8.95 percent, respectively.

Fig. 1. Symptoms of cucumber seedlings stage showing leaf blister and deformation at Mae Tha Nuea (A), mottle and blistered leaves at Huai Luek (B), and blistered and deformed leaves at Thung Roeng areas (new to cucumber production) (C).

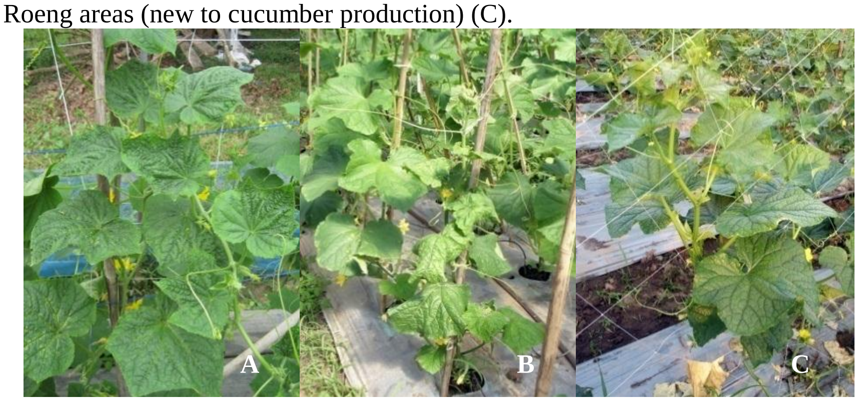

Fig. 2. Symptoms in cucumber at the flowering stage showing blistering and vein banding of leaves at Mae Tha Nuea (A), curling and blistering of leaves at Huai Luek (B), and interveinal chlorosis in leaves at Thung Roeng (C).

Table 1. Incidence of Cucumber Green Mottle Mosaic (CGMMV) in Japanese cucumber at three growth stages in the highland of Northern Thailand in samples collected in May to July 2011

Cultivated areas	CGMMV incidence (%) at three growth stage[1\]			Total CGMMV incidence (%)
	seedling	flowering	1 month after fruit set	
1. Mae Tha Nuea (field-grown)	0 (0[a]/12[b])	7.32 (3/41)	8.95 (6/67)	7.50 (9/120)
2. Huai Luek, (greenhouse-grown)	30 (6/20)	20.83 (10/48)	12.66 (10/79)	17.69 (26/147)
3. Thung Roeng (field-grown, new to cucumber)	0 (0/21)	0 (0/40)	0 (0/70)	0 (0/131)
Total	11.32 (6/53)	10.08 (13/129)	7.41 (16/216)	8.79 (35/398)

[1\] CGMMV detection by DAS-ELISA (Agdia Inc., Elkhart, In, USA)
[a]=Number of infected plant
[b]=Number of collected plant

Effects of CGMMV on cucumber yield

Analysis of variance followed by mean separation using Duncan's Multiple Range Test (DMRT) indicated a number of significant yield differences between the three cucumber-growing areas studied (Table 2). The standard grade 1 fruit weight was significantly higher at Thung Roeng and Huai Luek, 8.75 and 7.75 kg, respectively, than at Mae Tha Nuea, 5.25 kg. The yield of standard grade 2 cucumbers was significantly higher at Thung Roeng (9.62 kg) than Huai Luek (6.25 kg), with Mae Tha Nuea's yield (7.75 kg) being intermediate between the two. Total marketable yield was significantly higher at Thung Roeng (21.25 kg) than at Huai Luek (16.75 kg) and Mae Tha Nuea (15.87 kg). No significant difference in the yield of unmarketable fruit and total fruit yield (maketable fruit + unmarketable fruit) was observed between the three areas studied.

Table 2. Cucumber yield in the highland of Northern Thailand between May to July, 2011

Cultivated areas	Graded cucumber yield/100 plants (kg.) [1/]					Total cucumber yield (kg.)
	1	2	Under grade	Total marketable	Rejected/unmarketable	
Mae Tha Nuea[2/](field-grown)	5.25[b2/]	7.75[ab]	2.875[a]	15.875[b]	19.875[a]	35.750[a]
-Huai Luek[2/] (greenhouse-grown)	7.75[a]	6.25[b]	2.75[a]	16.125[b]	21.800[a]	37.925[a]
Thung Roeng (field-grown, new to cucumber)	8.75[a]	9.625[a]	2.875[a]	21.25[a]	20.675[a]	41.925[a]
LSD 0.05[4/]	2.0186	2.7921	1.0693	2.2197	5.8310	7.0946
CV)%([3/]	16.09	20.49	21.81	7.23	16.22	10.64

[1/]Mean of standard grade of cucumber product /100 plants (kilograms)
[2/]Mae Tha Nuea and Huai Luek are the main cucumber-producing areas in Northern Thailand.
[3/]Means followed by the same letter are not significantly different by DMRT at P = 0.05
[4/]CV(%) = coefficient of variation 95%

Discussion

Japanese cucumber is one of the most popular cucurbits in the highland of Northern Thailand. Viral diseases are one of the most serious problems for cucumber production considering the fact that there are many reports of plant viruses affecting the crop in Thailand such as CMV, SMV, MNSV, PRSV-W, and Tospovirus (Coolhapitagtam and Hongprayoon, 2004; Siriyan *et al*., 2006; Thonmo and Thummabenjapone, 2008; and Nontajak *et al*., 2012). CGMMV is a newly emerging virus affecting cucumber production in the country. Accurate identification and detection of CGMMV are the first steps in successful management of the disease. CGMMV was studied because this virus can be seed–borne, survive in the soil, be transmitted mechanically during agricultural practice and through water and has the potential of wide distribution throughout the world. Seed, pollen, and soil transmissions are important factors as primary sources in epidemic development of CGMMV diseases (Tan *et al*., 2000; Choi, 2001; Mandal *et al*., 2008; Liu, *et al*., 2013; Nematollahi *et al.*, 2013). CGMMV is physically very stable and accumulates in high concentrations in the infected tissue of host crops and is easily transmitted by plant sap. Plant viral diseases are diagnosed by applying a combination of methods, and relying only on symptoms may be misleading because different viruses such as ZYMV and SqMV can cause similar or identical symptoms for example leaf mosaic, leaf distortion and stunting. Our observations of the disease symptoms were based on samples collected from the main cucumber growing areas and from a location in which cucumber had never been grown before. Common symptoms observed included mottle, mosaic, and deformed leaves which were in agreement with symptoms of CGMMV in cucurbits reported in other countries (Antignus *et al*., 2001; Kim *et al*., 2003; Nematollahi *et al*., 2013).

CGMMV was not detected by DAS-ELISA in the sap of ground whole cucumber seeds, endosperm without peel, seed coats, cotyledons or leaves (10 dap.) in the seed lot tested. In 2006, Shim *et al*. 2006 reported that CGMMV seed transmission rate for cucumber were usually 8% up to one month after seed harvesting; this rate decreased to 0.1% in 5 months. The infection rates for the seeds could have been much lower than was detectable by our methods. The rate of CGMMV contamination of seed in mechanically inoculated plants was similarly high in watermelon (100%) and melon (93.85%), when ELISA was used to detect the presence of CGMMV in single seeds (Wu *et al.*, 2011). Seed transmission of CGMMV in cucumber was detected at low rates (3.0%) despite 17% of seed contamination (Liu *et al*., 2013). In current study, CGMMV was detected in 30% of seedlings from Huai Luek where the crop was grown in the greenhouse, but not detected in the cucumber grown at Mae Tha Nuea at the seedling stage. This phenomenon should be based on the low rate of CGMMV seed contamination and the long storage of almost 2 years in the cool room (8 ^{o}C) before planting that leaded too the decreasing of CGMMV in the contaminated seeds as reported by Shim *et al*. (2006) that the CGMMV seed transmission rate for cucumber was high after seed harvesting and decreased after 5 months of storage. Liu *et al*. (2013) reported that the rate of transmission from seed to seedling in cucumber was up to 76.7%. In our study, CGMMV was consistently detected in disease samples that were collected from the main cucumber growing area at flowering through the end of growing stage. Thonmo and Thummabenjapone (2008) found CGMMV in 25% of cucurbits samples collected from cucurbit seed production fields in Northeastern Thailand. CGMMV causes one of the most common diseases in commercial cucumber production throughout the world (Slovokhotova *et al*., 2007; Yoon *et al*., 2008). The relatively low percentage of positive results for CGMMV in the symptomatic samples might have been due to the fact that the cucumber plants were infected with different viruses such as CMV, ZYMV, PRSV-W, WMV-2, and SqMV as well as by CGMMV (Noda *et al*., 1993; Notajak *et al*., 2012; Siriyan *et al*., 2006; Shabanian *et al*., 2007). Mixed infection of cucumber plants by CGMMV and other viruses and abiotic causes (nutrient deficiencies, herbicide, etc.) of the symptoms also cannot be ruled out. Yield of cucumber from the main cucumber growing areas was lower than from the area where cucumber was a new crop. CGMMV is a worldwide problem in cucumber production areas like the Europe, United stated and Asia (Varveri *et al*., 2002;

Moradi and Jafarpour, 2011; Yoon *et al*., 2008; Zhou *et al*., 2008). The cucumber fruit infected with this virus appear to be distorted and reduced in size, resulting in poor quality and marketability. Many of the fruit in this study exhibited symptoms of CGMMV infection, being dark green, with a blistered appearance (Liu *et al*., 2013). Yield losses caused by CGMMV have been estimated at up to 15% (Mazereeuw, 2010; Varveri *et al*., 2002). So, control of CGMMV and other virus diseases in the cucumber production involves preventive measures designed to reduce sources of infection from inside and outside the crop and to minimize the effect of infection on yield. Use of virus-free seed for cucumber production and avoidance of infecting transplants during production is essential to ensure that only uninfected transplants are set in the field. During the growing seasons, control measures include rouging and sanitation (Budzanivska *et al*., 2006; Choi, 2001).

Conclusion

CGMMV was not detected by DAS-ELISA in 1600 seeds of Japanese cucumber from a single seed lot. The virus was also not detected in any of the growth stages of cucumber at Thung Roeng where the crop had never been grown before. However, CGMMV was detected in each of the three stages at the other two sites, where the crop had previously been grown except in the seedling stage at Mae Tha Nuea, at incidences ranging from 7.3 to 30%. Total marketable cucumber yield was significantly higher at Thung Roeng (21.25 kg) than at Huai Luek (16.75 kg) and Mae Tha Nuea (15.87 kg). These results suggest that soil transmission of CGMMV was likely and that the virus played a role in the reduction of cucumber yields.

Acknowledgements

We would like to sincerely thank the Research Division, Royal Project Foundation for supporting the research fund and our research assistants and extension staffs in the studied areas are indebted for their good collaboration.

References

Adkins, S., Kamenova, I., Achor, D. and Lewandowski, D.J. (2003). Biological and molecular characterization of a novel Tobamovirus with a unique host range. Plant Disease 87:1190-1196.

Ainsworth, G.C. (1935). Mosaic disease of cucumber. Annual Application Biology 22:55-67.

Antignus, Y., Wang, Y., Pearlsman, M., Lachman, Lavi, O.N. and Gal-On, A. (2001). Biological and molecular characterization of a new cucurbit-infecting tobamovirus. Phytopathology 91:565-571.

Bananej, K. and Vahdat, A. (2008). Identification, distribution and incidence of viruses in field-grown cucurbit crops of Iran. Phytopathologia Mediterranea 47:247-257.

Budzanivska, I.G., Rudneva, T.O., Shvchenko, T.P., Boubriak, I. and Polischuk, V.P. (2006). Investigation of Ukrainian isolates of Cucumber green mottle mosaic virus. Archives of Phytopathology and Plant Protection 40:376-380.

Choi, G.S. (2001). Occurrence of two tobamovirus diseases in cucurbits and control measures in Korea. Plant Pathology Journal 17(5):243-248.

Coolhapitagtam, M. and Hongprayoon, R. (2004). Variation in symptomatology caused by cucumber mosaic virus in Thailand. Agricultural Science Journal 35(5-6):207-214.

Gumus, M. and Paylan, I.C. (2013). Detection of viruses in seeds of some vegetables by reverse transcriptase polymerase chain reaction (RT-PCR). African Journal of Biotechnology 12(25):3891-3897.

Hossain, M., Asghar, S., Akbar, H.P. and Mehdi, S. (2007). Occurrence distribution and relative incidence of seven viruses infecting green house-grown cucurbits in Iran. Plant Diseases 91:159-163.

International Seed Testing Association (ISTA). (2010). Detection of squash mosaic virus, cucumber green mottle mosaic virus and melon necrotic spot virus in cucurbit. International rules for seed testing, Annex to chapter 7: Seed health testing methods. pp.1-6.

Kim, D.H. and Lee, J.M. (2000). Seed treatment for cucumber green mottle mosaic virus (CGMMV) in gourd (*Lagenaria siceraria*) seeds and its detection. Journal Korean Society Horticultural Science 41:1-6.

Kim, S.M., Lee, J.M., Yim, K.O., Oh, M.H., Park, J.W. and Kim, K.H. (2003). Nucleotide sequences of two Korean isolates of cucumber green mottle mosaic virus. Molecular Cells Biology 16:407-412.

Ko, S.J., Lee, Y.H., Cho, M.S., Park, J.W., Choi, H.S., Lim, G.C. and Kim, K.H. (2007). The incidence of virus diseases on melon in Jeonnam province during 2000-2002. Plant Pathology Journal 23(3):215-218.

Li, J., Wei, Q., Liu, Tan, Y. X., Zhang, W. and Wu, J. (2013). One-step reverse transcription loop-mediated isothermal amplification for the rapid detection of cucumber green mottle mosaic virus. Journal of Virological Methods 193:583-588.

Liu, Y., Wang, Y., Wang, X. and Zhou, G. (2009). Molecular characterization and distribution of cucumber green mottle mosaic virus in China. Journal of Phytopathology 157:393-399.

Mandal, S., Mandal, B., Mohd, Q., Haq, R. and Varma, A. (2008). Properties, diagnosis and management of cucumber green mottle mosaic virus. Plant Virus 2(1):25-34.

Mazereeuw, J., Kampen, B.V., Faber, N. and Wilterdink, R. (2010). Marker genetically linked to tobamovirus resistance in cucumber and the use thereof. Patent Application Publication, United States, pp. 4.

Moradi, Z. and Jafarpour, B. (2011). First report of coat protein sequence of cucumber green mottle mosaic virus in cucumber isolated from Khorasan in Iran. International of Virology 7(1):1-12.

Nematollahi, S., Haghtaghi, E., Koolivand, D.L. and Hajizadeh, M. (2013). Molecular detection of cucumber green mottle mosaic virus variants from cucurbits fields in Iran. Archives of Phtopathology and Plant Protection. p. 8.

Noda, C., Kittipakorn, K., Inchan, P., Wanapee, L. and Deema, N. (1993). Distribution of cucurbits viruses and reactions of some cucurbits species to certain viruses. Proceedings of the 31th Kasetsart University Annual Conference: Plants, Bangkok, Thailand: 341-347.

Nontajak, S., Jonglaekha, N. and Smitamana, P. Incidence and distribution viruses infecting cucurbits in the Royal Project's areas, Poster O-III-09. The International Conference Tropical and Sub-tropical Plant Diseases 2012, Plant Diseases in Agriculture and Food Security, Feb 7-10, 2012. The Empress Hotel, Chiang Mai, Thailand.

Qin, B., Cai, J., Liu, Z., Chen, Y., Zhu, G. and Huang, F. (2005). Preliminary identification of a cucumber green mottle mosaic virus infecting pumpkin. Plant Quarantine 4:198-200.

Sevik, M.A. and Arli-SŐkmen, M. (2003). Viruses infecting cucurbits in Samsun, Turkey. Plant Diseases 87: 341-344.

Shabanian, M., Masomi, H., Hoseinipour, A., Heidarnejad, J. and Azami, Z. (2007). Identification and distribution of cucumber-infecting viruses in the Jiroft greenhouses and partial characterization of Zucchini yellow mosaic virus collected from this region. Journal of Science Technology Agricultural National Resource 11:393-406.

Shang, H., Xie, Y., Zhou, X., Qian, Y. and Wu, J. (2011). Monoclonal antibody-based serological methods for detection of cucumber green mottle mosaic virus. Virology Journal 8:228-235.

Shim, C.K., Lee, J.H., Hong, S.M., Han, K.H. and Kim, H.K. (2006). Construction of antibodies of detection and diagnosis of cucumber green mottle mosaic virus from watermelon plants. Plant Pathology Journal 22(1):21-27.

Siriyan, R., Thummabenjapone, P., Lertrat, K. and Sirithorn, P. (2006). Standard protocol for screening cucumber resistant to CGMMV. Agricultural Science Journal (6):211-214.

Slovokhotova, A.A., Andreeva, E.N., Shiian, A.N., Odintsova, T.I. and Pukhalskii, V.A. (2007). Specifics of the coat protein gene in Russian strains of the Cucmber green mottle mosaic virus. Genetika 43:1461-1467.

Tan, S.H., M. Nishiguchi, M. Murata and F. Motoyoshi (2000). The genome structure of kyuri green mottle mosaic tobamovirus and its comparison with that of cucumber green mottle mosaic tobamovirus. Archives of Virology 145:1067-1079.

Thonmo, Y. and Thummabenjapone, P. (2008). Virus diseases in cucurbit seed production fields in northeast Thailand. KKU Agricultural Science Seminar 2008.

Varveri, V., Vassilakos, N. and Bem, F. (2002). Characterization and detection of Cucumber green mottle mosaic virus in Greece. Phytoparasitica 30:493-501.

Vitenskapskomiteen for Mattrygghet (VKM). (2008). Pest risk assessment of the cucumber green mottle mosaic virus in Norway. In opinion of the panel on plant health of the Norwegian scientific committee for food safety. pp. 28.

Yoon, J.Y., Choi, G.S., Choi, S.K., Hong, J.S., Choi, J.K., Kim W., Lee, G.P. and Ryu, K.H. (2008). Molecular and biological diversities of cucumber green mottle mosaic virus from cucurbitaceous crops in Korea. Phytopathology 156:408-412.

Yuki, V.A., Ditajima, E.W. and Kuniyuki, H. (2000). Occurrence, distribution and relative of five viruses infecting cucurbits in the state of Sao Paulo, Brazil. Plant Disease 84(5):516-520.

Zhou, L., Wu, Y., Zhao, X., Li, L., Cai, M., Wang, L. and Wang, W. (2008). The biological characteristics of cucumber green mottle mosaic virus and its effects on yield and quality of watermelon. Journal of Shenyang Agricultural University 39:417-422.

(Received 10 November 2013; 12 January 2014)