식물병연구
Res. Plant Dis. 23(1): 82-87 (2017)
https://doi.org/10.5423/RPD.2017.23.1.82
Note Open Access

Incidence of Viral Diseases and Occurrence of Three Unreported Viruses in Yams in Korea

Joong-Hwan Lee[1†], Chung Youl Park[2†], Ha-Jeong Cho[2], Jonghee Oh[2], Bong-Sub Kim[3], Eun Hey Park[4], Chang-Gi Son[1], and Su-Heon Lee[2,5]*

[1]*Institute for Bioresources Research, Gyeongsangbuk-do Agricultural Research & Extension Services, Andong 36614, Korea*
[2]*School of Applied Biosciences, Kyungpook National University, Daegu 41566, Korea*
[3]*Functional Crop Resource Development Division, Department of Southern Area Crop Science, National Institute of Crop Science, Rural Development Administration, Miryang 50426, Korea*
[4]*Incheon International Airport Regional Office, Animal and Plant Quarantine Agency, Incheon 22382, Korea*
[5]*Institute of Plant Medicine, Kyungpook National University, Daegu 41566, Korea*

***Corresponding author**
Tel: +82-53-950-5763
Fax: +82-53-950-6758
E-mail: suheon@knu.ac.kr
†These authors contributed equally to this work as first authors.

Received November 10, 2016
Revised January 5, 2017
Accepted January 6, 2017

During 2012 to 2014, a survey for the presence of viral diseases in yam plants was carried out in a field of the Institute for Bioresources Research in Gyeongsangbuk-do, Korea. A total of 88 leaf samples were collected and tested by reverse transcription polymerase chain reaction using specific primer sets. Eighty-one samples were positive for *Broad bean wilt virus 2* (BBWV2), *Chinese yam necrotic mosaic virus* (ChYNMV), *Cucumber mosaic virus* (CMV), *Japanese yam mosaic virus* (JYMV), and *Yam mild mosaic virus* (YMMV), whereas *Yam mosaic virus* (YMV) was not detected. Additionally, seven samples were negative for all viruses. Several samples exhibited mixed (double and triple) infections. Three viruses (CMV, JYMV, and YMMV) were detected for the first time in yam plants in Korea. A BLAST search showed that three viruses shared nucleotide identities with CMV-Ca (98%), JYMV-O2 (91%), and YMMV-TG_NH_1 (86%). Thus, our findings confirmed that yam plants cultivated in Korea were infected with multiple viruses with three of these viruses reported for the first time in Korea.

Keywords: *Cucumber mosaic virus*, *Japanese yam mosaic virus*, Yam, *Yam mild mosaic virus*

According to the Food and Agriculture Organization of the United Nations (FAO), yams (*Dioscorea* species, *Dioscoreaceae*) are the most important food tuber crop, a class of foods including potatoes, sweet potatoes, and cassava (FAO, 2012). Approximately 600 species of yams are distributed throughout the world, and only 10 of these species are used as edible crops (Mambole et al., 2014). Yams are an important traditional crop and staple food and provide income to small farmers in West Africa (Asiedu and Sartie, 2010). Moreover, yams are commonly used as medicines and vegetables (Kwon et al., 2016a), and more than 4,000 tons are produced in Korea each year.

Yams are propagated through seeds or tubers, resulting in accumulation of various pathogens, such as viruses, fungi, and bacteria. Of the many pathogens that affect these plants, viral infections in particular, can cause significant reductions in the yield and quality of yam crops (Mantell and Haque, 1978). Approximately eight viruses (*Chinese yam necrotic mosaic virus* [ChYNMV], *Dioscorea alata badnavirus* [DaBV], *D. alata virus* [DAV], *Dioscorea dumetorum virus* [DDV], *Dioscorea esculenta virus* [DEV], *Yam mosaic virus* [YMV], *Yam mild mosaic virus* [YMMV], and *Japanese yam mosaic virus* [JYMV]) have been reported in Asia (Kenyon et al., 2001; Wang et al., 2015), whereas only two viruses (*Broad bean wilt virus 2* [BBWV2] and ChYNMV)

Research in Plant Disease
pISSN 1598-2262, eISSN 2233-9191
www.online-rpd.org

have been reported to occur in *Dioscorea opposita* cv. Jang-Ma in Korea (Kang et al., 2003; Kwon et al., 2016b; Lee et al., 2016). In recent years, the number of novel virus species identified from yams has been increasing in other countries (Lan et al., 2015; Mambole et al., 2014; Menzel et al., 2014). As international trade gradually increases, there will be a high possibility that virus-infected yams are imported to Korea. Despite the increasing demand for domestic consumption, investigation of viral diseases closely related to yam production yield has not been carried out. Therefore, we conducted a virus survey of yams in Andong during 3 years from 2012 to 2014 and present data on infection rates and identification of unreported viruses in Korea.

From 2012 to 2014, 88 leaf samples (2012: n=36, 2013: n=13, 2014: n=39) from yams were collected during the growing seasons both from symptomatic and asymptomatic plants in a field at the Institute for Bioresources Research of Gyeongsangbuk-do, Korea. A total of 82 samples collected from symptomatic leaves showed typical virus-like symptoms, while six samples were symptomless.

The various virial symptoms, including mosaic, chlorotic spots, vein chlorosis, vein banding, vein clearing, line patterns, and malformation, were observed in the field (Fig. 1). Mosaic symptom was one of the most frequently observed, whereas line pattern symptom was the least frequently observed in our samples.

In order to identify the causal agent of these symptoms, total RNA was isolated from all leaf samples using an Easy Spin (DNA-free) Total RNA Extraction Kit (iNtRON, Daejeon, Korea) following the manufacturer's protocol. First-strand cDNA was

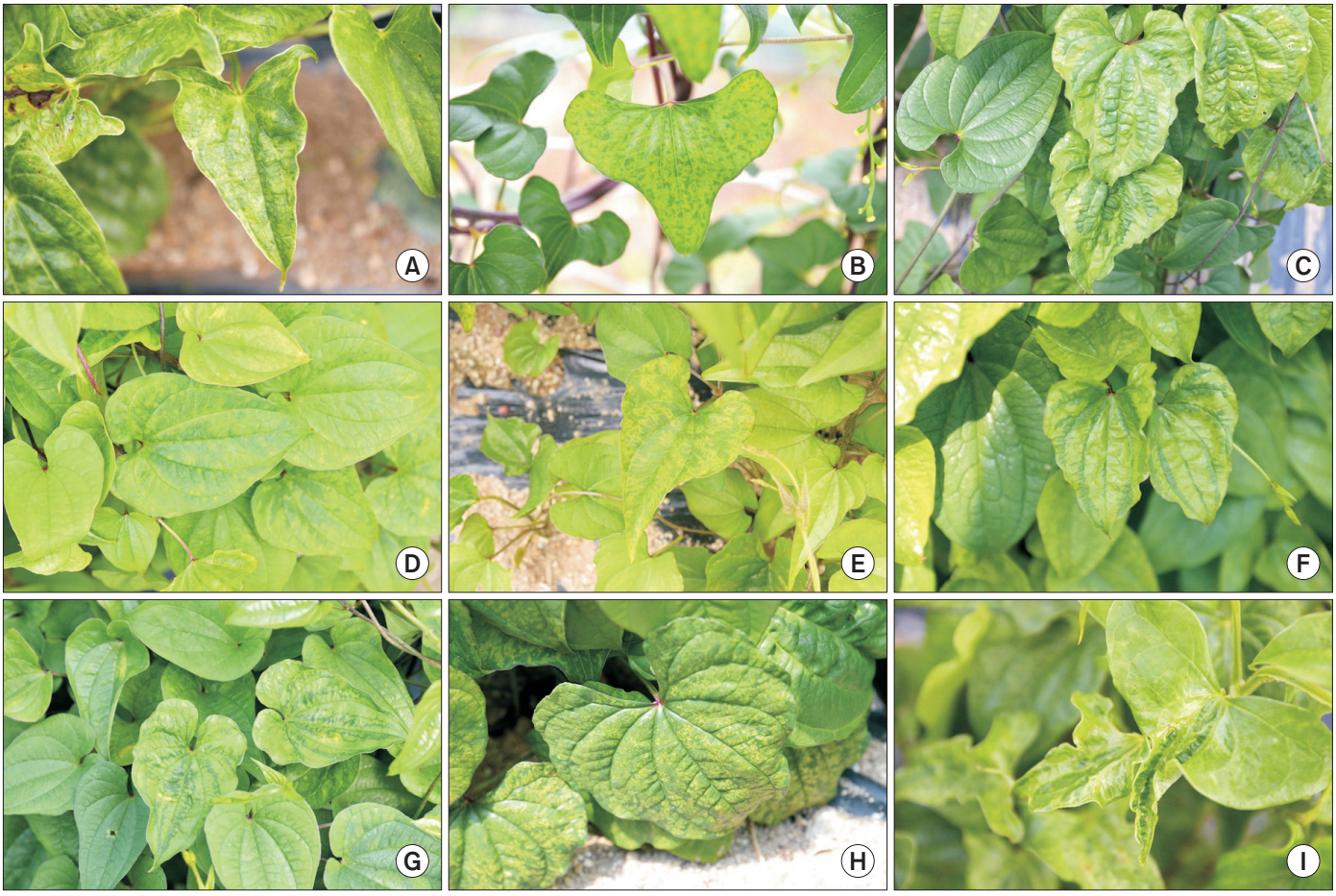

Fig. 1. The various virus symptoms of naturally infected yam plants in Korea. (A) Mosaic (CMV+JYMV+YMMV), (B) mosaic (ChYNMV), (C) mosaic (CMV+JYMV), (D) chlorotic spots and mosaic (JYMV), (E) vein chlorosis and chlorotic ring spots (BBWV2), (F) vein banding (CMV+JYMV), (G) dark green line pattern (JYMV), (H) vein clearing (BBWV2+ ChYNMV), (I) malformation (JYMV+YMMV). CMV, *Cucumber mosaic virus*; JYMV, *Japanese yam mosaic virus*; YMMV, *Yam mild mosaic virus*; ChYNMV, *Chinese yam necrotic mosaic virus*; BBWV2, *Broad bean wilt virus 2*.

synthesized from total RNA using TOPscript Reverse Transcriptase (Enzynomics, Daejeon, Korea) and RN25 random primers according to the standard manual. Polymerase chain reaction (PCR) was performed using four specific primers for BBWV2, *Cucumber mosaic virus* (CMV), YMV, and YMMV (Gioria et al., 2002; Lee et al., 2004; Mumford and Seal, 1997; Wang et al., 2015), and primers specific for two viruses (ChYNMV and JYMV) were designed based on published sequences from the National Center for Biotechnology Information (NCBI) (Table 1). PCR conditions were adapted from previous reports, and conditions for detection of the two viruses with the newly developed primer sets were as follows: initial denaturation at 95°C for 10 min; 35 cycles at 95°C for 10 min, 55°C for 30 s, and 72°C for 1 min; and a final extension at 72°C for 5 min. The sizes of the PCR products were confirmed with 1.2% agarose gel electrophoresis, and some of the positive samples were purified using an Expin Combo GP fragment DNA purification kit (GeneAll, Seoul, Korea). Purified amplicons were cloned into a TA cloning vector (RBC Bioscience, Xindian City, Taiwan), and nucleotide (nt) sequence analysis was carried out by Solgent (Daejeon, Korea). The nt sequences were edited using DNAMAN software ver. 7.0 (Lynnon Biosoft, Quebec, QC, Canada) and analyzed by NCBI BLAST.

Five viruses (BBWV2, ChYNMV, CMV, JYMV, and YMMV) were detected in the 88 leaf samples but YMV was not detected. Thus, three new primer pairs were designed to detect YMV, and all samples were tested by PCR with the new primer sets. All samples were negative for YMV, whereas the positive control (total RNA from an YMV-infected leaf sample) yielded a product of the expected size (data not shown). For all analyses,

Table 1. Primer sets used for detection of virus infection in yam plants

Virus	Primer name	Sequence (5′ to 3′)	Expected size (bp)	Reference
BBWV2	BBWV2-C40	GCTAGGTCCAGGCAAATTGTA	579	Lee et al., 2004
	BBWV2-N30	GTTGGTGCGATGTCAGG		
ChYNMV	ChYNMV-F	TACATTGCCAGATTCAAGCCAGG	730	In this study
	ChYNMV-R	CGTCCTGGTCATTATCCGTGT		
CMV	CMV-Ma-F	GCCGTAAGCTGGATGGACAA	488	Gioria et al., 2002
	CMV-Ma-R	TATGATAAGAAGCTTGTTTCGCG		
JYMV	JYMV-F	TTGGCTAACACAAGGGCTAC	370	In this study
	JYMV-R	AAATCAAACGCATAGCGAGC		
YMV	YMV-F	ATCCGGGATGTGGACAATGA	586	Mumford and Seal, 1997
	YMV-R	TGGTCCTCCGCCACATCAAA		
YMMV	YMMV_DetF1	TTCCTCGCATCAAAGCACCAATGAG	539	Wang et al., 2015
	YMMV_DetR1	TGCTGCYTTCATYTGCATGTG		

BBWV2, *Broad bean wilt virus* 2; ChYNMV, *Chinese yam necrotic mosaic virus*; CMV, *Cucumber mosaic virus*; JYMV, *Japanese yam mosaic virus*; YMV, *Yam mosaic virus*; YMMV, *Yam mild mosaic virus*.

Table 2. Virus infection rates (%) of yam plants collected in the field located at the Institute for Bioresources Research in Gyeongsangbuk-do, Korea

Year	No. of samples	BBWV2	ChYNMV	CMV	JYMV	YMV	YMMV	None detected
2012	36	16	17	8	5	-	-	6
2013	13	6	2	1	6	-	-	-
2014	39	5	7	7	30	-	14	1
Total	88	27 (30.7)	26 (29.5)	16 (18.2)	41 (46.6)	0 (0)	14 (15.9)	7 (8.0)

Values are presented as number only or number (virus infection rates [%]).
BBWV2, *Broad bean wilt virus* 2; ChYNMV, *Chinese yam necrotic mosaic virus*; CMV, *Cucumber mosaic virus*; JYMV, *Japanese yam mosaic virus*; YMV, *Yam mosaic virus*; YMMV, *Yam mild mosaic virus*; -, no virus detected.

30.7% (27/88), 29.5% (26/88), 18.2% (16/88), 46.6% (41/88), 0% (0/88), 15.9% (14/88), and 8.0% (7/88) of samples were positive for BBWV2, ChYNMV, CMV, JYMV, YMV, and YMMV, respectively (Table 2). In samples collected during the year 2012, ChYNMV (47.2%, 17/36) and BBWV2 (44.4%, 16/36) showed higher infection rates than CMV (22.2%, 8/36) and JYMV (13.8%, 5/36), whereas YMV and YMMV were not detected. CMV and JYMV were detected for the first time in Korea. Additionally, out of the 36 samples collected in 2012, six samples were found to have no viral infections. In 2013, BBWV2 (46.2%, 6/13) and JYMV (46.2%, 6/13) were the two most frequent viruses. Unlike the samples collected in 2012, the samples collected in 2013 showed low infection rates for ChYNMV (15.4%, 2/13) and CMV (7.7%, 1/13). In samples collected in 2014, JYMV showed the highest most infection rate (76.9%, 30/39), and YMMV was detected (35.9%, 14/39) for the first time. BBWV2 (12.8%, 5/39), ChYNMV (17.9%, 7/39), and CMV (17.9%, 7/39) showed relatively low infection rates, and only one sample showed no viral infection. YMMV was detected for the first time in Korea in 2014. Analysis of the single infection rates confirmed that BBWV2 (12.5%, 11/88), ChYNMV (17.0%, 15/88), CMV (0%, 0/88), JYMV (19.3%, 17/88), YMV (0%, 0/88), and YMMV (1.1%, 1/88) were detected (Table 3). Many collected samples were infected by two or three different viruses. The double infections rates were as follows: BBWV2+ChYNMV (10.2%, 9/88), BBWV2+CMV (2.3%, 2/88), BBWV2+JYMV (2.3%, 2/88), ChYNMV+CMV (1.1%, 1/88), CMV+JYMV (9.1%, 8/88), and JYMV+YMMV (10.2%, 9/88), and the triple infections rates were as follows: BBWV2+ChYNMV+CMV (1.1%, 1/88), BBWV2+CMV+JYMV (1.1%, 1/88), BBWV2+JYMV+YMMV (1.1%, 1/88), and CMV+JYMV+YMMV (3.4%, 3/88). BBWV2+ChYNMV and JYMV+YMMV double infections were the most frequent among double infections, whereas CMV+JYMV+YMMV showed the highest infection rate for triple infections. The eight symptomless yam samples out of 88 leaf samples were infected with BBWV2, and seven samples showed no viral in-

Table 3. Single and multiple virus infections rates (%) in samples collected in the field located at the Institute for Bioresources Research in Gyeongsangbuk-do, Korea from 2012 to 2014

	Virus	Survey year			Total
		2012	2013	2014	
Single infection	BBWV2	7/36*	4/13	-	11/88 (12.5)
	ChYNMV	8/36	1/13	6/39	15/88 (17.0)
	CMV	-	-	-	0/88 (0)
	JYMV	-	6/13	11/39	17/88 (19.3)
	YMV	-	-	-	0/88 (0)
	YMMV	-	-	1/39	1/88 (1.1)
Multiple infection	BBWV2+ChYNMV	7/36	1/13	1/39	9/88 (10.2)
	BBWV2+CMV	1/36	1/13	-	2/88 (2.3)
	BBWV2+JYMV	-	-	2/39	2/88 (2.3)
	ChYNMV+CMV	1/36	-	-	1/88 (1.1)
	CMV+JYMV	5/36	-	3/39	8/88 (9.1)
	JYMV+YMMV	-	-	9/39	9/88 (10.2)
	BBWV2+ChYNMV+CMV	1/36	-	-	1/88 (1.1)
	BBWV2+CMV+JYMV	-	-	1/39	1/88 (1.1)
	BBWV2+JYMV+YMMV	-	-	1/39	1/88 (1.1)
	CMV+JYMV+YMMV	-	-	3/39	3/88 (3.4)

Values are presented as number only or number (virus infection rates [%]).
BBWV2, *Broad bean wilt virus 2*; ChYNMV, *Chinese yam necrotic mosaic virus*; CMV, *Cucumber mosaic virus*; JYMV, *Japanese yam mosaic virus*; YMV, *Yam mosaic virus*; YMMV, *Yam mild mosaic virus*; -, no virus detected.
*Number of samples infected/number of samples collected.

fection despite the presence of typical virus symptoms. Thus, these seven samples should be tested using other diagnostic methods. Our survey results showed that most of yam plants were infected with different viruses (infection rate: 92.0%, 81/88), and various co-infections were also confirmed. Additionally, the infection rate of JYMV showed a gradual increase, whereas those of BBWV2 and ChYNMV decreased in the analysis of our survey data. These results implied that the patterns of viral diseases in yam plants were changing in the field at the Institute for Bioresources Research.

The NCBI BLAST search results of partial nt sequences confirmed from positive samples showed that each virus had high nt identity with previously reported isolates. The JYMV Andong isolate shared 91% nt identity with the O2 isolate (NCBI GenBank accession no. AB029507) reported in Japan. The CMV Andong isolate showed the highest nt identity (98%) with the Ca isolate (AY429432) reported in *Arachis hypogaea* in China. YMMV shared the highest nt identity (86%) with the TG_NH_1 isolate (KJ125475) from *Dioscorea* sp. in China. These virus nt sequences were deposited in NCBI GenBank as accession numbers LC191988 (CMV Andong isolate), LC191987 (JYMV Andong isolate), and KX156847 (YMMV Andong isolate). Notably, the main cultivated yam plants were thought to have originated from Japan and China (Kang et al., 2003), and two viruses (JYMV and YMMV) were not listed as quarantine viruses. Thus, yam plants infected with viruses have been introduced in domestic fields and have settled in Korea. Until now, the biological properties of infected yams have not been reported in Korea; therefore, further studies are needed. To the best of our knowledge, this is the first report describing three viruses (CMV, JYMV, and YMMV) infecting yams in Korea.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgement

This research was carried out with the support of Cooperative Research Program for Agricultural Science and Technology Development (Project No. PJ0112522016) and National Institute of Crop Science (PJ0111282016), Rural Development Administration (RDA), Republic of Korea.

References

Asiedu, R. and Sartie, A. 2010. Crops that feed the world 1. Yams. *Food Sec*. 2: 305-315.

[FAO] Food and Agriculture Organization of the United Nations (2012 onwards). Value of Agricultural Production. URL http://www.fao.org/faostat/en/#data/QV [1 March 2016].

Gioria, R., Espinha, L. M., Rezende, J. A. M., Gaspar, J. O. and Kitajima, E. W. 2002. Limited movement of *Cucumber mosaic virus* (CMV) in yellow passion flower in Brazil. *Plant Pathol*. 51: 127-133.

Kang, D. K., Kondo, T., Shin, J. H., Shin, H. Y., Sung, J. H., Kang, S. G. and Chang, M. U. 2003. *Chinese yam necrotic mosaic virus* isolated from Chinese yam in Korea. *Res. Plant Dis*. 9: 107-115. (In Korean)

Kenyon, L., Shoyinka, S. A., Hughes, J. A. and Odu, B. O. 2001. An overview of viruses infecting *Dioscorea* yams in sub-Saharan Africa. In: Plant Vology in Sub-Saharan Africa: Proceeding of a Conference Organized by IITA, eds. by J. A. Hughes and B. O. Odu, pp. 432-493. 4-8 June, 2001, International Institute of Tropical Agriculture, Ibadan, Nigeria.

Kwon, S. J., Cho, I. S., Choi, S. K., Yoon, J. Y. and Choi, G. S. 2016b. Complete sequence analysis of a Korean isolate of Chinese yam necrotic mosaic virus and generation of the virus specific primers for molecular detection. *Res. Plant Dis*. 22: 194-197.

Kwon, S. J., Cho, I. S., Yoon, J. Y., Choi, S. K. and Choi, G. S. 2016a. First report of *Broad bean wilt virus 2* in *Dioscorea opposita* Thunb. in Korea. *Plant Dis*. 100: 538.

Lan, P., Li, F., Wang, M. and Li, R. 2015. Complete genome sequence of a divergent strain of *Japanese yam mosaic virus* from China. *Arch. Virol*. 160: 573-576.

Lee, J. H., Son, C. G., Kwon, J. B., Nam, H. H., Kim, Y., Lee, S. H., Zhao, F. and Moon, J. S. 2016. Complete genome sequence of Chinese yam necrotic mosaic virus from *Dioscorea opposita* in the Republic of Korea. *Genome Announc*. 4: e00778-16.

Lee, S. H., Lee, J. B., Kim, S. M., Choi, H. S., Park, J. W., Lee, J. S., Lee, K. W. and Moon, J. S. 2004. The incidence and distribution of viral diseases in pepper by cultivation types. *Res. Plant Dis*. 10: 231-240. (In Korean)

Mambole, I. A., Bonheur, L., Dumas, L. S., Filloux, D., Gomez, R. M., Faure, C., Lange, D., Anzala, F., Pavis, C., Marais, A., Roumagnac, P., Candresse, T. and Teycheney, P. Y. 2014. Molecular characterization of yam virus X, a new potexvirus infecting yams (*Dioscorea* spp) and evidence for the existence of at least three distinct potexviruses infecting yams. *Arch. Virol*. 159: 3421-3426.

Mantell, S. H. and Haque, S. Q. 1978. Incidence of internal brown spot disease in White Lisbon yams (*Dioscorea alata*) during storage. *Exp. Agric*. 14: 167-172.

Menzel, W., Thottappilly, G. and Winter, S. 2014. Characterization of an isometric virus isolated from yam (*Dioscorea rotundata*)

in Nigeria suggests that it belongs to a new species in the genus *Aureusvirus*. *Arch. Virol*. 159: 603-606.

Mumford, R. A. and Seal, S. E. 1997. Rapid single-tube immunocapture RT-PCR for the detection of two yam potyviruses. *J. Virol. Methods* 69: 73-79.

Wang, M., Li, F., Zhou, G., Lan, P., Xu, D. and Li, R. 2015. Molecular detection and characterization of *Chinese yam mild mosaic virus* isolates. *J. Phytopathol*. 163: 1036-1040.