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Occurrence and Distribution of Viruses Infecting Pepper in Korea

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We conducted a survey on pepper virus diseases in 31 regions in Korea from November 2001 to December 2004. Using electron microscopy, test plant reaction, rapid immuno-filter paper assay (RIPA), reverse transcription-polymerase chain reaction (RT-PCR) and/or analysis of viral nucleotide sequences, we found a number of viruses from 1,056 samples that we collected. These included *Cucumber mosaic virus* (CMV), *Pepper mottle virus* (PepMoV), *Pepper mild mottle virus* (PMMoV), *Broad bean wilt virus 2* (BBWV2), *Tobacco mild green mosaic virus* (TMGMV), and *Tomato spotted wilt virus* (TSWV). Of the samples analyzed, 343 (32.5%) were infected with CMV, 209 (19.8%) with PepMoV, 141 (13.4%) with PMMoV, 12 (1.1%) with BBWV2, 40 (3.8%) with TMGMV, 5 (0.5%) with TSWV, 153 (14.5%) with CMV and PepMoV, 54 (5.1%) with CMV and PMMoV, 31 (2.9%) with PepMoV and PMMoV, 3 (0.3%) with CMV and BBWV2, 1 (0.1%) with CMV, PepMoV and BBWV2, 8 (0.8%) with CMV, PepMoV and PMMoV, and 30 (2.8%) samples were infected with viruses which were not identified. CMV was the most predominant virus in all inspected fields and the number of the samples infected with PMMoV was relatively low as compared PepMoV infection level in pepper. TMGMV was only found in the southern part of Korea, while TSWV was isolated in Anyang and Yesan. However, we did not encounter in this survey the *Alfalfa mosaic virus* (AMV), *Potato virus Y* (PVY), *Tobacco mosaic virus* (TMV), and Pepper vein chlorosis virus (PVCV).

Keywords : *Capsicum annuum*, detection, distribution, pepper, RIPA, survey, virus

Pepper (*Capsicum* spp.) is an important vegetable crop worldwide. In 2003, Korean farmers cultivated this crop in 63,150 ha and produced 350,174 tons (www.naqs.go.kr). Pepper contains a rich flavor, capsicum, vitamins A and C. The unripe pepper can be used in salads and the powder from dried ripe fruits is used as seasoning for Korean food, especially "Kimchi", one of the traditional foods in Korea.

During growing season, farmers encounter many disease problems. Pepper plants are easily infected with fungi, bacteria, and virus which cause severe damage. Among these pathogens, plant viruses are practically difficult to control and are estimated to cause economic losses of about 15 billion US dollars per annum worldwide (van Fanbing, 1999). Approximately 50 pepper viral diseases have been reported in the world (Green and Kim, 1991).

Among the reported pepper viruses, eight viruses and tentative one virus were recorded in the *List of Plant Diseases in Korea* (2004): *Alfalfa mosaic virus* (AlMV), *Broad bean wilt virus 2* (BBWV2), *Cucumber mosaic virus* (CMV), *Pepper mottle virus* (PepMoV), *Pepper mild mottle virus* (PMMoV), *Potato virus Y* (PVY), *Tobacco mild green mosaic virus* (TMGMV), and *Tobacco mosaic virus* (TMV), and Pepper vein chlorosis virus (PVCV). Surveys were carried out to about 15 years ago to identify viruses infecting pepper in Korea (Im et al., 1991; Kim et al., 1990). However, researchers suspect that there are still other pepper viruses endemic in Korea only or viruses recently introduced pepper field in Korea. Here we report the field survey results of pepper infecting viruses from 2001 to 2004 in Korea.

Materials and Methods

Areas surveyed and samples collected. We collected a total of 1,056 leaves and fruits showing virus-like symptoms in 31 pepper-growing areas of Korea from November 2001 to December 2004 (Fig. 2). Each sample was placed in a plastic bag, symptom types were recorded and brought into the laboratory for virus analysis. Positive controls for the viruses were obtained from the Plant Virus GenBank and stock culture collections of the National Horticultural Research Institute in this study.

Detection of viruses. The DAS-ELISA (Clark and Adams, 1977), rapid immunofilter paper assay (RIPA) (Choi et al., 2001), RT-PCR (Choi et al., 1998), immunocapture (IC)/ RT-PCR (Wetzel et al., 1992), electron microscopy, and/or indicator plant tests were applied to confirm the virus from

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Table 1. RT-PCR primers for detection of the viruses infecting pepper

Virus	Prime[a]	Sequences (5'-3')	Size (bp)
PMMoV	PMCP-S PMCP-R	GTGTACTTCTGCGTTAGG TTAAGGAGTTGTAGCCCACG	395
TMGMV	CPTMG-S CPTMG-R	TCGAGTACGTTTTAATCAAT ATTTTAGGAAATCTCACAAC	524
PepMoV	PepCP-S PePCP-R	AGCAGCTCAAGATCAGACAC TGTTCGTCTCCATCCATCAT	437
BBWV1	BBCP1-S BBCP1-R	GTAAGAGCACAAGAAGCTTGAGAT CATTGGAGCTCAAACTATTCTCAT	870
BBWV2	BBCP2-S BBCP2-R	AATGAAGTGGTGCTCAACTACACA TTTTGGAGCATTCAACCATTTGGA	654
TSWV	TSN-S TSN-R	ATGTCTAAGGTTAAGCTCAC TCAAGCAAGTTCTGCGAGTT	777
CMV	CPTALL-5 CPTALL-3	YASYTTTDRGGTTCAATTCC GACTGACCATTTTAGCCG	940

[a]The primers of CMV and TMGMV were modified from Choi et al. (1999) and Choi et al. (2002), respectively.

the collected samples, The polyclonal antisera used in the experiment were prepared against purified CMV, PepMoV, PMMoV, PVY, TMGMV, TMV, and ToMV that has been provided as positive control from the Plant Virus GenBank. The antisera were produced in National Horticulture Research Institute of Rural Development Admission in Korea.

DAS-ELISA and RIPA was carried out as described in previous studies (Choi et al., 1998, 2002). Primers used for RT-PCR assay are listed in Table 1. Synthesis of 1st strand cDNA was performed at 42°C for 45 min, and then the amplification process was carried out for 35 cycles in a Gene AMPr condition: 94°C for 30 sec, 50°C for 30 sec and 72°C for 1 min. The last cycle was followed by a prolonged extension for 20 min at 72°C. RT-PCR products were analyzed on 1.2% agarose gel. The leaf extracts of the sample with or without symptoms and collected in the field were also examined with electron microscope (Carl Zaiss LEO 906E). Common mechanical inoculation on indicator plants was done only with those samples that were negative in all of above assays.

Results and Discussions

Kinds of viruses infecting pepper. In this study, we have collected 1,056 pepper samples from 31 areas in Korea, and identified viruses from these samples using DAS-ELISA, RIPA, RT-PCR, IC RT-PCR, electron microscopy, and indicator plant assay. About 1,026 (97.2%) of these samples were identified to have one- or mix-infected viruses. Of the samples analyzed, 343 (32.5%) were infected with CMV, 209 (19.8%) with PepMoV, 141 (13.4%) with PMMoV, 12 (1.1%) with BBWV2, 40 (3.8%) with TMGMV, 5 (0.5%) with TSWV, 153 (14.5%) with CMV and PepMoV, 54(5.1%) with CMV and PMMoV, 31 (2.9%) with PepMoV and PMMoV, 3 (0.3%) with CMV and BBWV2, 1 (0.1%) with CMV, PepMoV and BBWV2, 8 (0.8%) with CMV, PepMoV and PMMoV, and 30 (2.8%) samples were infected with viruses which were not identified (Fig. 1).

However, we did not detect PVY, PVX, TMV, ToMV, AlMV and ChiVMoV from the samples. The major viruses detected in pepper were CMV, PepMoV and PMMoV. The single and mixture infection rates of these viruses were 71.1% and 26.1%, respectively. No virus was identified

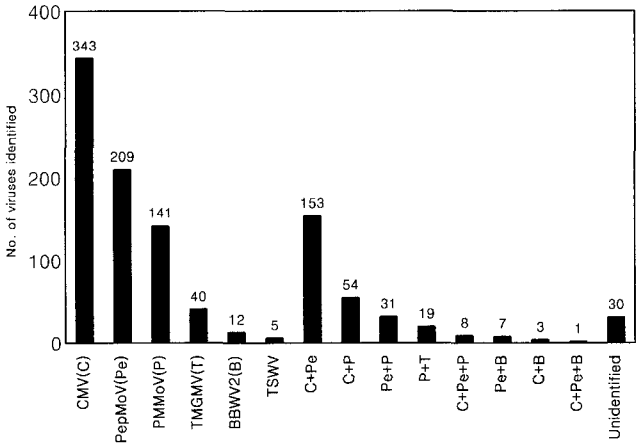

Fig. 1. Frequency of the viruses isolated from pepper in Korea, November 2001 to September 2004. The samples were collected from 31 areas in Korea and were detected by DAS-ELISA, RIPA, RT-PCR, IC/RT-PCR, electron microscopy and/or indicator plant assay.

Fig. 2. Map of Korea showing the areas surveyed and the distribution of pepper viruses [PMMoV (●), PepMoV (■), CMV (▲), BBWV2 (○), TMGMV (□), and TSWV (△)] identified in each area, from November 2001 to December 2004.

from 30 samples showing viral disease-like symptoms and these symptomes were probably caused by pesticide damage, physiological disorder, or unidentified virus. During 1998 pepper growing season, TMV was the most prevalent virus, with 89% infection rate on pepper (Kim et al., 1990). Im et al. (1991) reported that the majority of infection was either single or mixed infection of TMV, CMV AlMV and TSWV. About 94% of all viruses were detected by using ELISA tests in the same year.

We detected PMMoV and TMGMV of tobamoviruses in this survey, but not TMV. Choi et al. (1989) discussed that a pepper strain of TMV (TMV-P) was different from both TMV-OM and TMV-T on the basis of serological relationships and reactions of indicator plants. We think that the TMV isolate, identified from pepper by previous researchers, might be PMMoV because PMMoV was newly classified from TMV by differences of nucleotide sequences of the two virus isolates (Alonso et al., 1991).

PepMoV have many characteristics which are common to PVY and TEV, including symptom expression and aphid transmission (Ladera et al., 1982). PepMoV was first identified in Korea in 2002.

Lee et al. (1993) reported that BBWV was isolated in red pepper showing yellow mosaic. However BBWV was recently reclassified with two species, BBWV1 and BBWV2, based on the differences of nucleotide sequence identities, host plant reactions, and serology (Goldbach et al., 1995). We identified BBWV2, but not BBWV1 in the survey.

TSWV, transmitted mainly by thrips, was reported in paprika growing under glasshouse in Korea (Kim et al., 2004). Also, the virus was detected in the leaves of the red pepper showing chlorotic rings on the leaves of the crop growing in the open fields of Anyang in 2004.

Comparison of the viruses identified in this survey with those observed 15 years shows that aphid transmitted CMV and PepMoV are most frequently. Tobamovirus, which was most abundant in pepper 15 years ago, is now the third ranked virus. These might be reasons which prompt researchers to introduce L gene for resistance of pepper cultivars to tobamoviruses and to produce virus-free seeds. Consequently, management practices which maintain the health status of pepper culture must include protection of these plants from CMV and PepMoV infection.

Geographical distribution of pepper viruses in Korea. We identified the following viruses in our survey: CMV, PepMoV, PMMoV, BBWV2, TMGMV, and TSWV.

However, we detected TMGMV only in Gyeongsangnam province where green pepper was mainly cultivated during winter season. Green pepper cultivated using a hydroponic system in a plastic house showed 100% infection at the mid growing stage. The casual viruses were two tobamoviruses, TMGMV and PMMoV.

In Yesan in 2003, we detected TSWV in a paprika fruit showing necrotic spot, and in Anyang, 2004, we found malformation fromi red pepper. However, we did not detect TSWV in other areas in Korea. TSWV has a very broad host range estimated to be up to 900 species within 80 families. It has a worldwide distribution (Goldbach and Peters, 1994). TSWV might be occurring in other areas and infecting other crops because the virus-transmissible vector, *Frankliniella* spp., is widely spread in Korea.

In conclusion, we conclude that CMV, PepMoV, and PMMoV are the most frequent viruses infecting pepper in Korea. TSWV might be able to cause severe damage in the future if researchers do not come up with preventive management practices.

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