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Three Different Viruses Isolated from Typical Weed Plants that Grown Adjacent to Common Crop Fields

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Weeds are widely grown in the field and are infected by many viruses. A survey was conducted to identify viruses infecting weeds in Korea. Virus-infected weed samples including *Rorippa indica* (L.) Hiern, *R. islandica* (Oed.) Bord, *Crepidiastrum denticulatum* (Houtt.) Pak &Kawanno, *Achyranthes japonica* (Miq.) Nakai, and *Chrysanthemum boreale* (Makino) Makino were collected in Kyonggi Province. These weeds were grown in the greenhouse and were inoculated on 10 test plants. Several virus isolates were isolated from infected tissues and were further studied by host range assay, serological test, electron microscopy (EM), reverse transcription-polymerase chain reaction (RT-PCR) and sequencing. Each isolated virus strain was mechanically transmitted to weeds and various hosts including *Nicotiana* spp., *Brassica* spp., *Vigna unguiculata*, *Capsicum annuum*, and *Cucumis sativus* and showed systemic mosaic, vein clearing, necrosis, mottle, malformation, chlorosis, and/or death of host plants in some cases. Each virus was then purified using infected leaves and observed by EM. From these results three viruses were isolated and identified as *Turnip mosaic virus* (TuMV), *Broad bean wilt virus* (BBWV), and *Cucumber mosaic virus* (CMV). RT-PCR using virus-specific oligonucleotide primers and the cloning were conducted to determine the nucleotide sequences of coat proteins of the three viruses their amino acid sequence were deduced. The amino acid sequence homologies were about 92.7 to 99.7%, 96.2 to 97.7%, and 93.9 to 98.6% to other reported TuMV, BBWV, and CMV strains, respectively. These results suggest that many weeds may serve as primary inoculum source of diseases caused by TuMV, BBWV, CMV and that the management of these viral diseases can be achieved through weed control.

Keywords : Weed viruses, TuMV, BBWV, CMV, RT-PCR, CP gene, sequence homology.

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The pattern of virus spread in the crop and rate and extent of spread is affected by many factors including (i) the source of the inoculum, i.e. whether it comes from outside the crop, from diseased individuals within the crop arising from seed transmission or through vegetative propagation, from weed or other plants within the crop, or from crop debris; (ii) the amount of potential inoculum available; (iii) the nature and habitats of the vectors; for example, with aphids, whether they are winged transient or colonizers; (iv) whether virus is nonpersistent, semipersistent, or persistent in the vector; (v) the time at which vectors become active in relation to the lifetime of the crop; and (vi) whether conditions (Gibson, 1989).

Weeds are susceptible to a number of viruses that occur in worldwide and serve as a primary inoculum source for many viral diseases and as habitant of insects (Agrios, 1997). Living hosts as sources of infection may include (i) perennial weed hosts, annual weed hosts in which the virus is seed transmitted, or annual weed hosts that have several overlapping generations throughout the year; (ii) perennial ornamental plants that often harbor infection in a mild form; (iii) unrelated crops; (iv) plants of the same species remaining from a previous crop; these may be groundkeepers as with potatoes or seedling volunteers; and (v) seed crops of biennial plants that may be approaching maturity about the time the annual crop is emerging. Therefore, weed was important source for virus transmission, overwintering, and oversummering of many economically important viruses. A diversity of weed hosts gives a virus much greater opportunities to maintain itself and to spread viruses widely that have perennial weed hosts. Those viruses have become distributed extensively around the world. Some important examples are (i) *Peanut stunt virus* and *Alfalfa mosaic virus* carried in clover; (ii) *Cucumber mosaic virus* (CMV) carried in grass and pigweed. In addition to weeds, wild plants, hedgrows, ornamental trees, and shrubs may also act as virus reservoirs (Pederson, 1994).

Rorippa indica (L.) Hiern, *R. islandica* (Oed.) Bord, *Crepidiastrum denticulatum* (Houtt.) Pak & Kawanno, *Achyranthes japonica* (Miq.) Nakai, and *Chrysanthemum boreale* (Makino) Makino are distributed on field in Korea.

R. indica (L.) Hiern is known as perennial herbaceous plant which belongs to Brassicaceae family. *R. islandica* (Oed.) Bord, and *Cre. denticulatum* (Houtt.) Pak & Kawanno are a biennial herbaceous plants and a member of Brassicaceae and Compositae family, respectively (Choi et al., 1994 and Rist et al., 1989). These weeds were found in the field adjacent to commercial crops in Korea. *Ach. japonica* (Miq.) Nakai was known as a perennial herbaceous plant which is belong to Amarantaceae family (Misra, 1991). *Achyranthes* spp. is a erect herb (0.3-0.9 m in height) distributed as a weed throughout Korea but no virus was reported yet. *Chrysanthemum* spp. is an important cut flower in Korea. Six viruses and two viroids were reported as infectious pathogenic agents on chrysanthemum worldwide including *Tomato aspermy virus*, *Chrysanthemum virus B*, *Tomato spotted wilt virus*, CMV, and *Impatients necrotic virus* (Bouwen, 1995).

In Korea, Choi et al. (1992) showed that two weed species, *R. indica* (L.) Hiern and *R. islandica* (Oed.) Bord, serve as overwintering hosts for *Turnip mosaic virus* (TuMV), and therefore, an important source of primary inoculum. In addition, the potential of managing the virus disease through removal of weed hosts of viruses growing in proximity to fields has been suggested previously but also has been considered impractical because of the numerous host species involved and the topography of the fields. No studies have been done in Korea on the development and incidence of virus disease in weeds under natural field conditions. The objectives of this study were to determine the identity and incidence of viruses infecting weeds in the fields in Korea. Three different viruses, i.e. TuMV, *Broad bean wilt virus* (BBWV), and CMV were isolated and their characteristics were determined by host range studies, observation of electron microscope (EM), serological test, and reverse transcription-polymerase chain reaction (RT-PCR).

Materials and Methods

Weed collection and Virus isolation. Virus-infected weeds were collected from March to June in Kyonggido. Several weed plants showing typical viral symptoms were identified as *R. indica* (L.) Hiern, *R. islandica* (Oed.) Bord, *Cre. denticulatum* (Houtt.) Pak & Kawanno, *Ach. japonica* (Miq.) Nakai, and *Ch. boreale* (Makino)

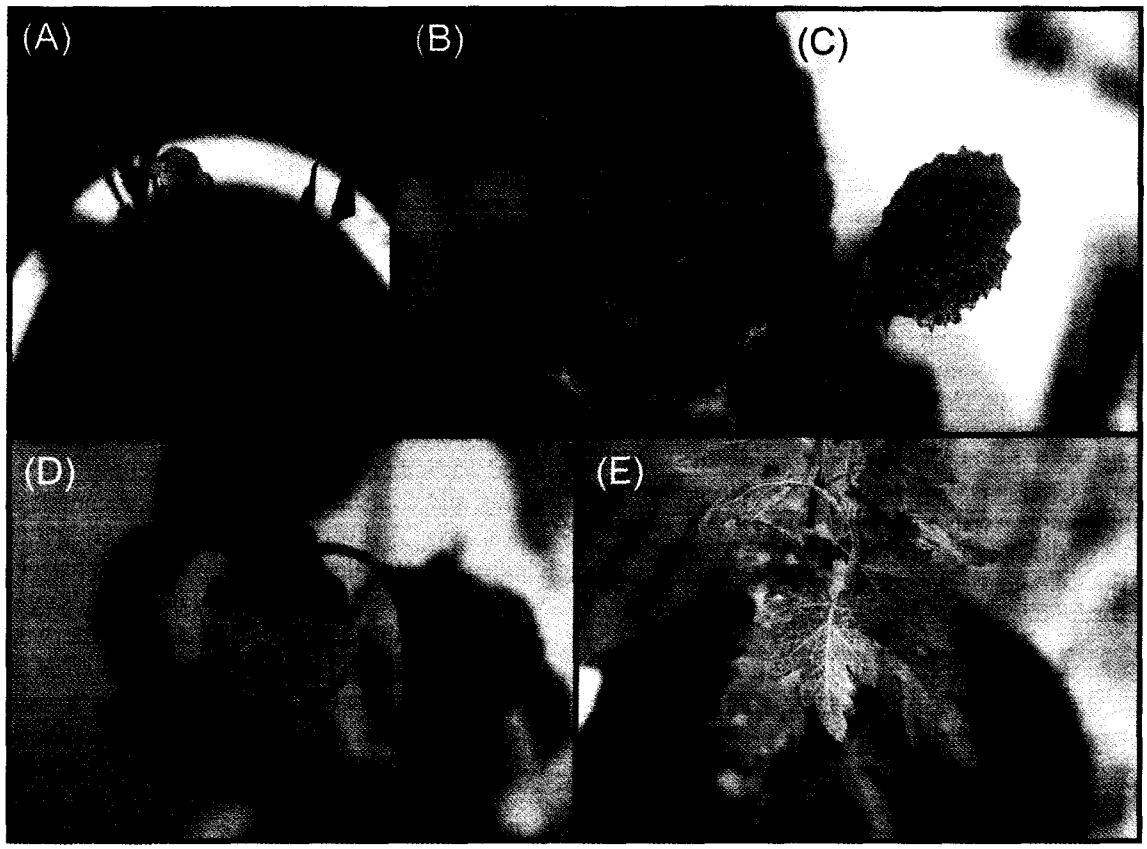

Fig. 1. Viral symptoms naturally occurring in weeds. TuMV caused severe mottle and dwarf in *Rorippa indica* (A), mosaic in *R. islandica* (B), and severe mosaic and chlorosis in *Crepidiastrum dentidulatum* (C). BBWV and CMV infection in *Achyranthes japonica* (D) and *Chrysanthemum boreale* (E) caused malformation and mottle and vein clearing, respectively.

Makino (Fig. 1). Crude sap inocula were prepared from the collected weeds by grinding leaves in solution of 0.01 M Tris-HCl (pH 7.6) with carborundum added as an abrasive. Extracts from weeds were mechanically inoculated onto leaves of indicator plants, i.e. *Capsicum annuum*, *Chenopodium amaranticolor*, *C. quinoa*, *Cucumis sativus*, *Datura stramonium*, *Lycopersicon esculatum*, *Nicotiana benthamiana*, *N. glutinosa*, *Physalis floridana*, and *Tetragonia expansa*. Inoculated plants were kept at 20 to 25°C for 14 days and symptom developments were checked occasionally. Virus isolates showing distinct symptoms were inoculated onto local lesion indicator plants and passaged three time to get single isolate.

Host range assay. Sap inocula were obtained from infected indicator plant tissues as described above and were mechanically inoculated by using expressed sap from 1 g of leaves macerated in 2 ml of inoculation buffer. The inoculum was rubbed with absorbent cotton swab to the healthy plants after lightly dusting with carborundum. The inoculated plants were washed with tap water immediately after mechanical inoculation. A total of 35 species were inoculated including *Brassica juncea*, *B. rapa*, *Cap. annuum* cv. Geosung, *Cap. annuum* cv. Seokwang, *C. amaranticolor*, *C. quinoa*, *C. sativus*, *Cucurbita moschata*, *D. stramonium*, *Garland chrysanthemum*, *Gomphrena globosa*, *N. benthamiana*, *N. clevelandii*, *N. glutinosa*, *N. occidentalis*, *N. sylvestris*, *N. tabacum* Bright yellow, *N. tabacum* KY57, *N. tabacum* Turkish, *N. tabacum* Xanthi-nc, *Perilla frutenscens*, *Petunia hybrida*, *Phaseolus angularis*, *Ph. vulgaris*, *P. floridana*, *Pisum sativum*, *Raphanus sativus*, *Spinacia oleracea*, *Sesamum indicum*, *T. expansa*, *Vicia faba*, *Vigna sinensis*, and *Zinnia elegans*. Inoculated plants were grown at 20 to 25°C in insect-proof glasshouse.

Electron microscopy. TuMV particles were observed using dip methods by sap dip preparations (Noordam, 1973) and were observed using Hitachi H-800 EM. After negative staining with 2% sodium phosphotungstate (pH 7.0), the size of TuMV particles were determined from a total of about 100 different particles at x15,000.

BBWV and CMV particles were observed using purified virus preparations. Isolated virus was propagated on *N. glutinosa* and *C. amaranticolor* for 30 days for CMV and BBWV, respectively. Leaves showing symptoms were harvested and purified using Murant's methods (Noordam, 1973). Briefly, tissue was ground in cold 0.5 M phosphate buffer (pH 7.5) containing 0.1% thioglycollic acid, filtered through cheesecloth, stirred while adding equal volume of ether at 4°C. The resulting mixture was centrifuged at 7,000 g for 5 min and the supernatant was ultracentrifuged at 30,000 rpm for 2 hrs. The pellets were resuspended at 4°C overnight in 1 ml cold 0.06 M phosphate buffer (pH 7.5), and were layered onto 10 to 40% linear-log sucrose gradients and centrifuged at 28,000 rpm for 2 hrs. Fraction containing virus was collected. The quality of the purified viruses was checked using 12.5% SDS-PAGE analysis (Sambrook et al., 1989). Each purified virus was negatively stained using 4% uranil acetate (UA) and was observed on EM. The size BBWV and CMV particles were measured from the EM at × 30,000.

Agar gel double diffusion test. Ouchterlony double-diffusion tests were performed in 100 × 15 mm plastic petri dishes loaded with 15 ml 0.6% agar in 0.1M Tris-HCl buffer (pH 8.0) containing 0.2% sodium dodecyl sulfate, 0.7% NaCl and 0.1% NaN_3. Virus antigens for gel diffusion were obtained by grinding infected tissue in distilled water (1 : 2, w/v). Purified viral antigens of selected isolates resuspended in water with an A_{260} of 1.0 reading were also tested. Three polyclonal antiserum used to test were kindly provided by National Alpine Agricultural Experiment Station.

Total RNA extraction and RT-PCR. Total RNAs were extracted from virus inoculated test plants using Trizol reagents (Gibco BRL, USA). The purity and relative quality of extracted total RNAs were confirmed by separating RNAs in 1.2% agarose gel. First strand cDNA synthesis was carried out in a 20 μl reaction volume using cDNA synthesis kit for RT-PCR (Omniscript™, QIAGEN, USA) according to the manufacture's instructions with 2 μg total RNAs extracted from infected-leaves as well as healthy plant as a template using each specific primer complementary to each viral RNA (TuMV 3pr; 5'-CTAGCATACAACTTCATAAC-3'; nucleotides 9615 to 9634 of TuMV strain Tu-1 from data in GenBank accession D10927, BBWV 3pr; 5'-GACCCTCGCCTTTTATT-3'; nucleotides 3450 to 3467 of BBWV RNA 2 isolate IA from data in GenBank accession AB032403, and CMV 3pr; 5'-TGGAATCAGACTGGGAGCA-3'; nucleotides 1900 to 1917 of CMV RNA3 strain Fny from data in GenBank accession D10538). PCR reactions contained template cDNA (from cDNA synthesis reactions above), 25 pmol of each amplification primers (TuMV 5pr; 5'-TGTGTTATCACCAGGCAG-3': nucleotides 8740 to 8758 of TuMV strain Tu-1 from data in GenBank accession D10927, BBWV 5pr; 5'-AGGTCACTTGATCCTGA-3'; nucleotides 2773 to 2789 of BBWV RNA2 isolate IA from data in GenBank accession AB032403, CMV 5pr; 5'-TACAATTGAGTCGAGTCATG-3'; nucleotides 1240 to 1259 of CMV RNA3 strain Fny from data in GenBank accession D10538, and three 3pr primers mentioned above), 200 μM each dNTP, 1.5 mM $MgCl_2$, 50 mM KCl and 10 mM Tris-HCl (pH 8.3) in a 50 μl reaction volume. Template DNA denatured at 94°C for 30 sec and then 2.5 units of *Taq* DNA polymerase (TaKaRa, Japan) was added. All PCR amplifications consisted of 35 cycles, of 80 sec at 93.5°C for denaturation, 80 sec at 55°C for annealing, and 80 sec at 72°C for synthesis, followed by 10 min at 72°C.

Cloning and sequencing of PCR products. DNA fragments generated by PCR amplification were separated by electrophoresis in 1% agarose gels and purified according to the DNA extraction procedure using phenol and chloroform extractions before further manipulation of amplified DNA. The RT-PCR generated cDNAs were then ligated into TOPO vector (Invitrogen, USA) and transformed into *Escherichia coli* DH5 10F' (Invitrogen, USA). Recombinants were analyzed by digestion with *Eco*RI. The plasmid that contained cDNA inserts of the correct sizes (890 bp for TuMV, 690 bp for BBWV, and 670 bp for CMV) was selected and sequenced using the ABI Prism TM Terminator Cycle Sequencing Ready Reaction Kit and ABI 377 Genetic Analyzer (Perkin Elmer, USA) according to the manufacture's instructions. Sequence analysis was carried out using the Clustal W algorism of LaserGene TM program (DNASTAR Inc., USA).

Table 1. Reactions of indicator plants to mechanical inoculation of the TuMV isolated from *Rorippa indica*, *R. islandica*, and *Crepidiastrum denticulatum*

Host Plants[a]	Symptoms[b]		
	R. indica	*R. islandica*	*Cre. denticulatum*
Brassica juncea	–/VC, SM[c]	–/VC, M	–/SM, Mal
B. rapa	–/VC, SM, Mal	–/SM, Mal	–/SM, Mal
Chenopodium amaranticolor	CL/–	CL/–	NL/SM, Mal
C. quinoa	NR, VN/–	CL/–	NL/VN
Garland chrysanthemum	CL/CL	CL/CL	–/ SM, f
Nicotiana benthamiana	NL/VC, VN, D	NL/SM, Mal	NL/SM, Mal
N. clevelandii	NL/NL, D	NL/CL	CL/CL, M
N. occidentalis	NR/CL, VC	NR/CL, VC	NR/M
Petunia hybrida	NL, VL/NL, VN	CL/CL	–/MR
Physalis floridana	CL/CL	–/M, Mal	– / –
Raphanus sativus	–/VB, SM	CL/VB	–/VB
Sesamum indicum	CL/VC, VN	CL/VC, VN	NL/M, R
Spinacia olercea	CL/CL	CL/CL, M	CL/CL
Tetragonia expansa	CR/–	CR/–	CR/–
Zinnia elegans	–/M	–/M, Mal	–/M

[a] Test plants were mechanically inoculated with TuMV isolated from *R. indica*, *R. islandica*, and *Cre. denticulatum*.
[b] NL; necrotic local lesion, VC; vein clearing, SM; severe mosaic, Mal; malformation, CL; chlorotic local lesion, VB; vein banding, NR; necrotic ringspot, f; fetal, M; mosaic, VN; vein necrosis, D; death, –; no visible symptom.
[c] Symptoms on inoculated leaves/upper of test plants.

Results

Host range and symptomatology. On the basis of key host reactions, isolated viruses used in this study were classified into three distinct groups. The first was comprised primarily of TuMV isolated from *R. indica* (L) Hiern, *R. islandica* (Oed.) Bord, and *Cre. denticulatum* (Houtt.) Pak & Kawanno (Table 1). They caused systemic mosaic and malformation in *B. rapa*, *B. juncea*, and *Raphanus sativus* and local lesions in *C. amaranticolor* and *N. benthamiana* which soon thereafter became necrotic local lesion with further enlarging.

Member of the second group isolated from *Ach. japonica* (Miq.) Nakai possessed properties of BBWV showing mosaic, chlorosis and malformation in *N. benthamiana* and *N. clevelandii*, local lesion in *P. vulgaris*, *C. quinoa*, *C. amaranticolor*, and *V. faba*, and vein clearing and chlorosis in *N. occidentalis*, and *V. sinensis* (Table 2).

The third virus isolated from *Ch. boreale* (Makino) Makino showed characteristics of CMV (Table 3). It generally did not infect spinach, radish, turnip, pea and bean, but infected susceptible tobaccos and cucumber causing mild mosaic or stronger systemic mosaic but no necrosis or death. It also caused necrotic local lesion and vein clearing in *C. amaranticolor* and *C. quinoa*.

Electron microscopy. EM observation of the virus isolate that showing TuMV characteristics on host range and symptom assay revealed filamentous virus particles. In virus inoculated leaf sap of *Brassica* spp., TuMV particles occurred in great numbers and evenly distributed throughout the preparations. The normal length was 720 nm (Fig. 2A).

Table 2. Reactions of indicator plants to mechanical inoculation of the BBWV isolated from *Achyranthes japonica*

Host Plants[a]	Symptoms[b]
	Ach. japonica
Capsicum annuum	CL/CL, VC[c]
Chenopodium amaranticolor	CL/SM, D
C. quinoa	CL/SM, D
Gomphrena globosa	NL/–
N. benthamiana	–/SM, D
N. clevelandii	CR/CL, M
N. occidentalis	CL/CL, VC
Petunia hybrida	CL/CL, VC
Physalis floridana	–/M, R
Phaseolus vulgaris	– / –
Pisum sativum	– / –
Sesamum indicum	– / –
Spinacia olercea	NL/CL
Tetragonia expansa	CR/–
Vicia faba	– / –
Vigna sinensis	CR/VC, CR

[a] Test plants were mechanically inoculated with BBWV isolated from *Ach. japonica*.
[b] NL; necrotic local lesion, VC; vein clearing, SM; severe mosaic, CL; chlorotic local lesion, M; mosaic, D; death, –; no visible symptom.

Purified preparations of both BBWV and CMV obtained by the 10 to 40% sucrose density ultracentrifugation. Analysis of purified virus by SDS-PAGE showed two protein (MW of 42 kDa and 26 kDa for BBWV) (data not shown) and only one protein (MW of 24 kDa for CMV; Fig. 3A),

Table 3. Reactions of indicator plants to mechanical inoculation of the CMV isolated from *Chrysanthemum boreale*

Host Plant[a]	Symptoms[b]
	Ch. boreale
Capsicum annuum cv. Geosung	CL/CL, R, M[c]
Cap. annuum cv. Seokwang	–/VC
Chenopodium amaranticolor	NL, VN/–
C. quinoa	NL, VC/–
Cucumis sativus	–/VC, M
N. benthamiana	–/SM, Mal
N. glutinosa	–/YM, D
N. tabacum Bright yellow	–/M
N. tabacum KY57	–/SM, Mal
N. tabacum Turkish	–/SM, Mal
N. tabacum Xanthi-nc	–/SM, Mal
Perilla frutescens	–/SM, D
Petunia hybrida	–/SM, f, D
Phaseolus angularis	–/VC, M
Physalis floridana	–/M, f
Spinacia olercea	NL/M, P
Tetragonia expansa	CL/CL, SM
Vicia faba	NL/–

[a] Test plants were mechanically inoculated with CMV isolated from *Ch. boreale*.
[b] M; mosaic, SM; severe mosaic, Mal; malformation, YM; yellow mosaic, NL; necrotic local lesion, VC; vein clearing, CL; chlorotic local lesion, f; fetal, D; death, –: no visible symptom.
[c] Symptoms on inoculated leaves/upper of test plants.

presumed to be the capsid protein (CP). From purified suspension, the isometric virus particles with 25 nm and 29 nm diameter were aggregated which is typical of BBWV and CMV, respectively (Fig. 2B and 2C, respectively).

Agar gel double diffusion test. In Ouchterlony double diffusion test, sap extracted from infected *R. indica* (L) Hiern, *R. islandica* (Oed.) Bord, and *Cre. denticulatum* (Houtt.) Pak & Kawanno gave sharp precipitin lines with TuMV antiserum (data not shown). Sap extracted from infected *Ach. japonica* (Miq.) Nakai strongly reacted with BBWV antiserum (data not shown). Sap extracted from *Ch. boreale* (Makino) Makino positively reacted with antiserum to CMV (Fig. 3B). These results were in agreement with the results obtained from host range data and EM observation.

RT-PCR and sequence analysis. To confirm each isolated virus and to compare their similarity to other reported viruses, nucleotide and deduced amino acid sequences were performed by using RT-PCR and subsequent cloning and sequencing of amplified DNA fragment. DNA fragment of 890 bp corresponding to TuMV CP gene was amplified

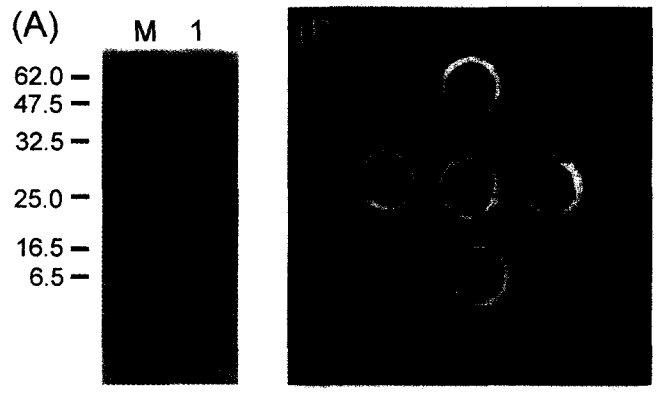

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and agar gel double diffusion test of CMV-*Ch. boreale*. (A) Purified virus separated on 12.5% SDS-PAGE and was stained with Comassie blue. Lane M, protein molecular weight markers; lane 1, purified CMV- *Ch. boreale*. (B) Serological reaction in agar gel double diffusion test. The central well (AS) contains CMV antiserum. The peripheral wells contain sap from *Ch. boreale* (1), 10^{-1} dilution sap (2), 10^{-2} dilution sap (3), healthy plant for negative control (4), respectively.

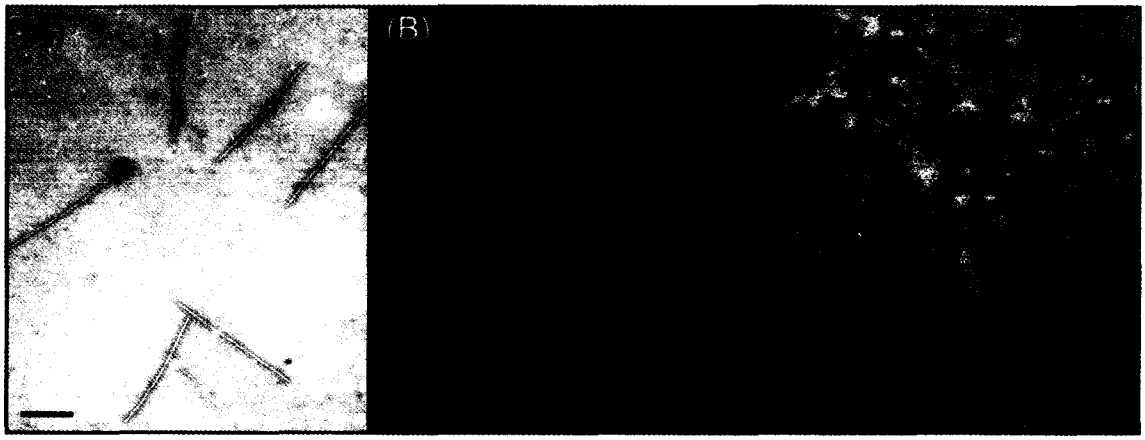

Fig. 2. Electron micrograph of TuMV, BBWV, and CMV. (A) Virus particles from *R. indica*, *R. islandica*, and *Cre. denticulatum* infected with TuMV stained with 2% phosphotungstate. Bar represents 200 nm. (B & C) Virus particles purified from *Ach. japonica* infected BBWV and *Ch. boreale* infected CMV, respectively, stained with with 4% uranyl acetate. Bar represents 30 nm.

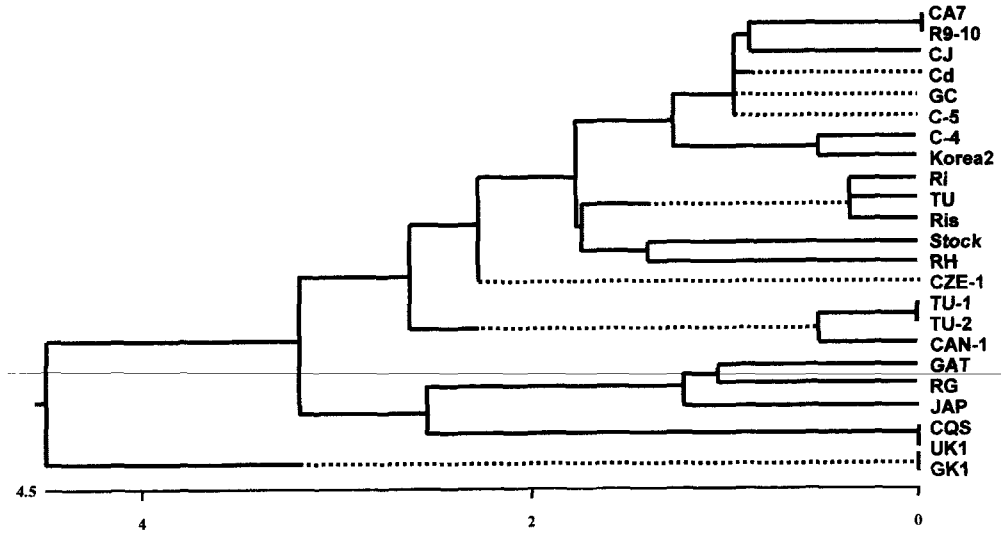

Fig. 4. Relationships of CP gene amino acid sequences between isolated TuMV strains and the other TuMV strains. The degree of relatedness was indicated by horizontal distance. References for CP sequences of the TuMV strains are as follows; CA7 (AF- 103786), R9-10 (AF103785), CJ (AF103788), GC (AF233888), C-5 (AF233890), C-4 (AF233889), Korea2 (L12396), Tu (AF- 103791), Stock (AF103792), RH (AF103790), CZE-1, GK1 (Lehmann et al., 1997), Tu-1 (Nicolas et al., 1992), Tu-2 (Tremblay et al., 1990), CAN-1 (X81140), GAT (AF103787), RG (AF103789), JAP (Sano et al., 1992), CQS (Choi and Choi, 1993), and UK1 (X65978). The dendrogram was constructed from aligned CP gene sequences using ClustalW method (DNASTAR).

using specific primer sets and total RNAs from infected plant of *R. indica*, *R. islandica* and *Cre. denticulatum*. Similarly 690 and 670 bp DNA fragments of BBWV polyprotein and CMV CP gene were amplified using specific primer sets and total RNAs isolated from infected plant of *Ach. japonica* and *Ch. boreale*, respectively.

The nucleotide sequences of the DNA clones from five isolates of three viruses were obtained deposited to GenBank, and compared to the other reported sequences. The CP genes of three TuMV isolates obtained from *R. indica*, *R. islandica* and *Cre. denticulatum* showed 88.3 to 99.7% nucleotide sequence identities and 92.7 to 99.7% deduced amino acid sequence identities with many strains. TuMV-*R. indica* (TuMV-Ri; accession number AF316359) isolate and TuMV-*R. islandica* (TuMV-Ris; accession number AF316360) isolate were almost identical in both nucleotide (99.7%) and deduced amino acid sequences (99.0%), while the TuMV-*Cre. denticulatum* (TuMV-Cd; accession number AF316361) shares 99.1% nucleotide and 98.6% deduced amino acid sequence identity with both TuMV-Ri and TuMV-Ris, respectively. The phylogenetic tree analysis of TuMV CP amino acid sequences showed that TuMV-Ri and TuMV-Ris belong to same group as TuMV-Tu (accession number AF 103791), whereas TuMV-Cd is closely related with TuMV-GC (accession number AF 233888) and TuMV-C-5 (accession number AF 233890, Fig. 4). The nucleotide sequence of the BBWV clone isolated from *Ach. japonica* (BBWV-Aj; accession number

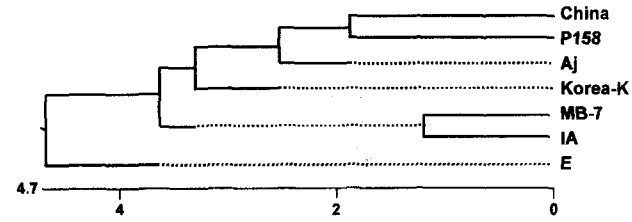

Fig. 5. Relationships of polyprotein amino acid sequences corresponding to CP gene between isolated BBWV strain and the other BBWV strains. The degree of relatedness was indicated by horizontal distance. References for CP sequences of the BBWV strains are as follow; China (Qi et al., 2000), P158 (AF228423), Korea-K (AF104335), MB-7 (Nakamura et al., 1998), IA (Kuroda et al., 2000), and E (Kobayashi et al., 1999). The dendrogram was constructed from aligned polyprotein gene sequences using ClustalW method (DNASTAR).

AF 316363) was identified BBWV RNA-2 polyprotein. Amino acid sequence of the RNA-2 polyprotein comparison revealed sequence identities of 96.2 to 97.7% to the other strains (Fig. 5). The sequence of the amplified CP gene segment of CMV clone isolated from *Ch. boreale* (CMV-Cb; accession number AF 316362) was confirmed 657 nucleotides and 218 amino acids. Compared to the other CMV strains, CP nucleotide sequence was distantly related to the subgroups II strains (70.3 to 73.2%) but it was closely related to subgroup I (93.9 to 98.6%). A relationship dendrogram derived from subgroup I amino acid sequences comparison is shown Fig. 6.

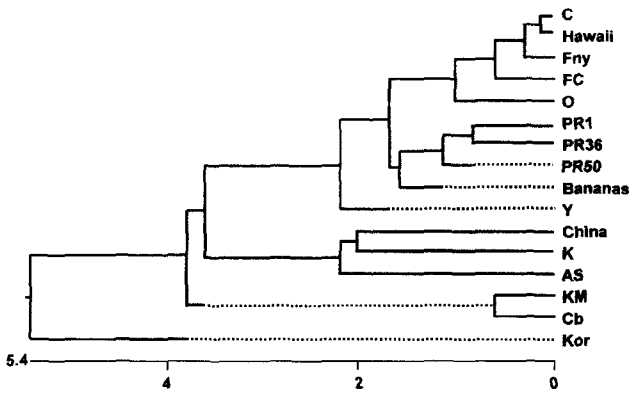

Fig. 6. Relationships of CP gene sequences between isolated CMV strain and other CMV strains. The degree of relatedness was indicated by horizontal distance. References for CP sequences of the CMV strains are as follows; C (Quemada et al., 1989), Hawaii (Hu, 1995), Fny (Owen et al., 1990), FC (Shintaku, 1991), O (Hayakawa et al., 1989), PR1 (M98499), PR36 (M98500), PR50 (M98501), Bananas (Gafny et al., 1996), Y (Nitta et al., 1988), China (Hu et al., 1989), K (Roossinck et al., 1999), AS (X77855), and KM (AB004780). The dendrogram was constructed from aligned CP gene sequences using ClustalW method (DNASTAR).

Discussion

The analyses of host range, EM observation, and serology have been used as diagnostic tools for many viruses. Recently, RT-PCR and subsequent sequencing of amplified nucleotide using virus specific primers have been used for detection and exact identification of viruses (for a review, see Kim, 1999). Using these approaches, three different viruses were isolated from naturally infected weeds and were identified as TuMV, BBWV, and CMV.

Agar gel diffusion test probed to be a very useful and generally reliable means for screening large numbers of samples representing many different plant species. Crude sap from each infected weed reacted with each antiserum in agar gel double diffusion test. As the results, serological relationship between each virus and each antiserum were reacted strongly, but no reaction was occurred in crude extracts from healthy plants. In EM observation, TuMV was observed by dip-method of infected leave samples, but BBWV and CMV not were observed. Therefore, they were purified by Murant's methods (Noordam, 1973) and showed isometric particle that is typical of Favavirus and Cucumovirus, respectively.

Though most potyviruses have narrow and restricted host ranges TuMV, a member of potyvirus group, infect a large array of economically important crop plants particulary the *Brassica* genus (Word and Shukla, 1991). As previously reported TuMV-Ri and TuMV-Ris infected *Brassica* species showing necrotic local symptoms on *Nicotiana* spp. They caused local chlorotic spots on inoculated leaves on *C. amaranticolor* but did not show a systemic symptom (Choi et al., 1992) whereas TuMV-Cd developed systemic mosaic symptom on *C. amaranticolor*. In addition, symptom on *Garland chrysanthemum* and *Physalis floridana* plants inoculated with TuMV-Ri, TuMV-Ris, and TuMV-Cd showed some differences. TuMV-Ri and TuMV-Ris caused chlorotic local symptom on inoculated leaves and upper leaves on *G. chrysanthemum* while TuMV-Cd caused severe mosaic and fetal symptom only on upper leaves. However these isolates did not show any symptom differences on *P. floridana*. Nucleotide and deduced CP amino acid sequence analyses revealed that there is a clear difference between TuMV-Cd and TuMV-Ri and TuMV-Ris. Comparison of deduced CP amino acid sequences showed that it contains 288 amino acids CP as a portion of a large polyprotein (Dougherty and Carrington, 1988) and that there are two amino acid differences at position 20 and 33 between TuMV-Ri and TuMV-Ris. Interestingly, N and E at position 43 and 50 in TuMV-Ri and TuMV-Ris were substituted with D and K in TuMV-Cd, respectively. Though disease symptoms are the results of complex interactions between the pathogen and the host (Matthews, 1991), CP gene plays an important role in viral pathogenicity such as efficient accumulation, virus spread and symptom development (Neeleman et al., 1991). Thus, these amino acid substitution between TuMV-Cd and TuMV-Ri and TuMV-Ris could be responsible for symptom differences.

BBWV was isolated from *Ach. japonica* and CMV was isolated from *Ch. boreale*. Symptoms induced by BBWV and CMV in certain naturally infected weeds sometimes can be used as diagnostic tool for identification of these viruses. However, symptoms are not a reliable mean for routine virus identification because of inconsistent symptom expression in many hosts and existence of symptomless host species (Rist et al., 1989). During the present study, BBWV isolate caused chlorotic local lesion and chlorotic ringspot, following by various systemic symptoms of whole test plants. Though isolated CMV did not show any symptom on inoculated leaves, it caused chlorotic or necrotic lesion, green systemic mosaic, usually without necrosis on upper leaves in many test plants. They showed major differences in systemic symptom on upper leaves of *C. amaranticolor* and *C. quinoa*. BBWV-Aj caused death after severe mosaic, while CMV-Cb not developed systemically. RNA-2 CP (213 amino acids) sequence analysis of the BBWV isolated from *Ach. japonica* revealed that it has high sequence homology with Korea-K strain (90.0%), but relatively low homology with China strain (83.7%). Deduced amino acid sequences showed that BBWV-Aj and Korea-K (accession number

AF104335) revealed sequence identities of 97.7% and codon changes at position 33, 93, 127, 196, and 207. Strains of CMV are characterized into two subgroups on the basis of serological relationships, host range, peptide mapping of viral CP, and nucleic acid hybridization (Edward and Gonsalves, 1983 and Palukaitis et al., 1992). In addition, sequence comparison results show that CMV strains within each subgroup share 91% to 99% amino acid and nucleotide sequence identity, whereas strains between subgroups share 76% to 84% sequence identity (Owen et al., 1990; Rizos et al., 1992). Some of the best characterized CMV strains belonging to subgroup I include strains -C, -Fny, -T and -Chi (Kearney et al., 1990). Fewer strains have been identified as CMV subgroup II and the two well-characterized strains are -Q and WL. Based symptoms of test plants and on nucleotide and amino acid sequence comparison of the complete CP sequence, CMV-Cb was group as CMV subgroup I. Particularly, it showed 100% amino acid sequence homology with CMV-KM (accession number AB 004780) though there are few nucleotide sequence differences.

The present study is the first report of natural infection of *Cre. denticulatum* by TuMV, *Ach. japonica* by BBWV and *Ch. boreale* by CMV. Although the virus infection on other weeds that are not used in this study need to be further studied, the listed species certainly include the majority of Korean weeds that serve as host for virus infection. Weeds are known as reservoirs for many viruses. Two species, *R. indica* and *R. islandica*, are especially important for TuMV and CMV (Choi et al., 1994 and Rist & Lorbeer, 1989) because of their abundance, relatively high incidences of infection, and capacity to serve as overwintering hosts of the virus. So, these weed species are primary sources of the viruses that damage crops annually. Studies are underway further to determine if direct correlations exist between the presence of these viruses in weed plant and diseases in cultivated crops growing adjacent to these weeds.

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