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Virulence Differentiation of Eight *Turnip mosaic virus* Isolates Infecting Cruciferous Crops

Hong-Soo Choi[1]*, Seong-Han Sohn[2], Moo-Kyoung Yoon[3], Jeong-Uk Cheon[4], Jeong-Soo Kim[5], Hassan Karakacha Were[6], Jang-Kyung Choi[7], Kook-Hyung Kim[8] and Yoichi Takanami[6]

[1]*Department of Plant Pathology, National Institute of Agricultural Science and Technology, Suwon 441-857, Korea*
[2]*Division of Research Planning & Coordination, National Institute of Agricultural Biotechnology, Suwon 441-857, Korea*
[3]*Division of Vegetable Research, National Horticultural Research Institute, Suwon 441-440, Korea*
[4]*Division of Crop Research, National Institute of Alpine Agriculture, Pyeongchang 232-955, Korea*
[5]*Division of Horticultural Environment, National Horticultural Research Institute, Suwon 441-440, Korea*
[6]*Department of Applied Genetics and Pest Management, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan*
[7]*Department of Agricultural Biology, Kangwon National University, Chunchon 200-701, Korea*
[8]*School of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea*

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***Turnip mosaic virus* (TuMV) is an infectious viral pathogen on the cruciferous crops, predominantly Chinese cabbage (*Brassica campestris* subsp. *pekinensis*) and radish (*Raphanus sativus*). On the basis of the symptom development in selective differential hosts from indicator host species, Chinese cabbage and Korean radish inbred lines, the representative eight isolates of TuMV were divided into two major groups/or six types. Group I includes Tu 1, Ca-ad7, and Cj-ca2-1 isolates, while group II includes the other isolates (rg-pf1, r 9-10, Rh-cq1-2, Stock and Mustard). According to the molecular phylogenetic analysis, these isolates, however, divided into two groups and two independent isolates. Phylogenetic analysis indicated that four isolates (Tu 1, r 9-10, Stock and Rh-cq1-2) formed a distinct phylogenetic group, and the other two isolates (Ca-ad7 and Cj-ca2-1) also formed another group. Mustard and rg-pf1 isolates did not seem to have any relationship with these two groups. Taken together, these results indicated that virulence differentiation on host plants, molecular phylogenetic analysis of the nucleotide and the deduced amino acid of TuMV coat proteins did not show any relationship. The multi-resistant lines, Wonyae 20026 and BP058 in Chinese cabbage represent valuable genetic materials that can be used for crucifer breeding programs on TuMV resistance, but not in Korean radish.**

Keywords : cruciferous crops, resistance, *Turnip mosaic virus*, virulence differentiation

Turnip mosaic virus (TuMV) is one of the most important viruses of cruciferous crops particularly of *Brassica* and *Raphanus* genus (Tomlinson, 1970). TuMV has a very wide host range, infecting 318 species in 156 genera of 43 plant families including *Brassica* crops, ornamentals, and weed plants belonging to 14 different families. TuMV is transmitted in the non-persistent manner by aphids (40-50 species) and naturally infects *Brassica* crops, ornamentals, and weed plants belonging to 14 different families (Shattuck, 1992). Early researchers became aware of TuMV strains from host range and symptomatology of infected plants including *Brassica* and *Nicotiana* species (Green and Deng, 1985; Pound and Walker, 1945; Provvidenti, 1980; Yoshii, 1963).

The differential host system has been widely used for Chinese cabbage, but two commercial hybrids of the differential limit its usefulness. Walsh (1989) used oilseed rape and rutabaga differentials to separate eight European and Canadian isolates into four groups. Group four in Walsh's classification is identical to TuMV-C3. The Chinese strain identification system of Liu et al. (1990), based on cumulative data on the quantitative interaction between TuMV and six cruciferous hosts, identified seven strains, Tu 1-7. Fujisawa (1990) used various cabbage, Chinese cabbage, and Japanese radish (*R. sativus* L.) cultivars to differentiate 47 TuMV isolates from the major cruciferous vegetable growing areas in Japan into 9 TuMV strains. Walsh and Jenner (1995) described strains as pathotypes defined on *B. napus* differentials. These pathotypes are discriminated by three different phenotypes when TuMV isolates are mechanically inoculated on four differential hosts: no symptoms (possible immunity), systemic infection (susceptible), and local infection with no systemic spread (resistance). Jenner et al. (1996) classified TuMV isolates into 12 groups, or pathotypes, according to the pattern of the phenotypes on the four *B. napus* lines. Most of the virus isolates were classified as pathotypes 1, 3 or 4.

*Corresponding author.
Phone) +82-31-290-0401, FAX) +82-31-290-0434
E-mail) hschoi@rda.go.kr

Pathotype 1 was the most common.

Serotypic variation in TuMV has also been described. Three main serotypes, namely Predominant, BEL 1, and JPN 1 have been defined using a panel of monoclonal antibodies on a large collection of isolates differing in their host and geographical origin. However, despite all the evidence indicating the presence of wide phenotypic variability in TuMV, little is known about the genetic structure of the virus. The coat protein (CP) gene is a variable region in *Potyviruses* genomes and has been widely accepted for classification of isolates in the genus. CP gene restricto-typing has been used in different potyviruses in addition to PVY as an approach to study. In most cases, viral CP has been known to be deeply related to viral infection, symptom development, movement in host plant, and transmission. For instances, CP seemed to be required for systemic infection and long-distance movement in *Alfalfa mosaic virus* (AIMV; Spitsin et al., 1999) and in *Tobacco etch virus* (TEV; Dolja et al., 1994), for aphid transmission in *Maize dwarf mosaiv virus* (MDMV; Salomon and Bernardi, 1995). Moreover, CP was also reported in involvement of replication of viral RNA (Reusken et al., 1995), virus particle assembly (Sit et al., 1995) and cell-to-cell movement (Schmitz and Rao, 1998). Considering the various functions of the CP, this study adopted CP as a representative gene for investigation on evolutionary relationship between classical classification and molecular phylogenetic analysis.

In Korea, a survey of TuMV suggested that the virus was widespread throughout the vegetative-growing areas. Chinese cabbage was common host infected by a mixture of strains and TuMV-C4 was the most prevalent among samples infected with a single strain (Suh, 1993). Choi et al. (1980) differentiated eight ordinary strains of TuMV isolates into three groups based on symptoms on *B. rapa* and attempted to correlate these differences with symptoms on indicator plants and serological and electrophoretic mobility. In this study, the representative eight isolates of TuMV selected from different areas and crops in Korea were correlated with their relationships based on the pathogenicity and phylogenetic analysis of CP genes. Additionally, we investigated and classified these eight isolates of TuMV using the selective differential hosts from indicator host species, Chinese cabbage and Korean radish inbred lines. The information generated from this study is hoped to be of value in breeding of TuMV-resistant varieties of cruciferous vegetables.

Materials and Methods

TuMV isolates and host rang studies. Eight isolates out of fifty-four TuMV-isolates were representatively used depending on indicator host species described previously (Choi et al., 2004, unpublished data). Eight TuMV isolates include two isolates (cj-ca2-1 and ca-ad7) from Chinese cabbage, three isolates (r 9-10, rh-cq1-2 and Rg-pf1) from Korean radish and each one isolate (Tu1) from turnip, stock and mustard in Korea (Table 1). The representative eight isolates were maintained in *N. bentamiana* plants throughout the study. To determinate the virulence of virus isolates and the symptoms on tested plants, 5-10 seedlings from each of the 11 species, *Chenopodium amaranticolor, C. quinoa, Perilla frutescens, Impatiens balsamina,* L., *Zinnia elegans* Jacq., *Physalis floridana, Vicia faba, Raphanus sativus* L., *Brassica campestris, B. rapa* L., *Chrysanthemum coronarium,* at the 3-5 leaf stage were inoculated by sap extracted in 0.1 M phosphate buffer, pH 7.0. Fifty Chinese cabbage and Korean radish inbred lines and two Chinese cabbage hybrids, Tropical Delight and Crusader, were used for TuMV strain classification (Tables 3, 4 and 5). Three plants of each line and cultivar were inoculated in 3 replications. The tested plants were placed in an insect-free glasshouse maintained at 20-25°C with 12-15 h light period. Disease symptoms were recorded twice a week for 30 days after inoculation. Both symptomatic and non-symptomatic plants were verified for TuMV infection by enzyme linked immunosorbent assay (ELISA) and electron microscopy (EM).

Table 1. TuMV isolates used in this work

Isolate	Location of collected samples	Host plant
Rh-cq1-2	Suwon	Radish (*Raphanus sativus*)
Rg-pf1	Geochang	Radish (*Raphanus sativus*)
R 9-10	Andong	Radish (*Raphanus sativus*)
Stock	Pyeongchang	Stock (*Matthiola incana*)
Ca-ad7	Andong	Chinese cabbage (*Brassica campestris* subsp. *pekinensis*)
Cj-ca2-1	Jeju	Chinese cabbage (*Brassica campestris* subsp. *pekinensis*)
Tu-1	Ganghwa	Turnip (*Brassica rapa* subsp. *rapa*)
Mustard	Jeju	Mustard (*Brassica campestris* subsp. *napus*)

Indirect-ELISA. Indirect-antibody coated enzyme linked immunosorbent assay (indirect-ELISA) was conducted essentially as described by Clark and Bar-Joseph (1984) using monoclonal antibodies (MAb) from Agdia (Indiana, USA). The MAbs and conjugate were both diluted 1:200 and all incubations were carried out at 37°C for 1 h except for the MAbs which were incubated for 2 h. Quantitative measurements of generated p-nitrophenol were made by determining absorbance at 405 nm (A405) in a Thermo Max microplate reader 4.0 (Molecular Devices, USA). Twice the mean absorbance readings of non-infected negative control values were used as the positive/negative thresholds.

Electron microscopy. Dip preparations were prepared by grinding a small piece of infected *Chenopodium amaranticolor* in 2-3 drops of 2% phosphotungstic acid, pH 7.0. Ultrathin sections were conducted as described by Choi et al. (2004). For interpretation of results, the sections were viewed under electron microscope LEO 912AB (Carl Zeiss, Germany) at 80 kV.

Sequence and molecular phylogenetic analyses. Total RNAs were extracted from infected leaf samples essentially as described by Prescott and Martin (1987). In order to clone CP gene from each isolates, total RNAs were purified from virus-infected plant leaves. With the RNA, PCR was carried out according to the procedures by Sohn et al. (1995). The primer set (Tu5', 5'-AAGATGGTGC(C/T)TG-AAG(T/C)(G/T)TGTGT(T/C)TATCACCAG-3'; Tu3', 5'-ACTAACGAGTAAACTTAA(C/A)TACTTATAGTCT-AC-3') for CP gene was designed and synthesized to contain full-length of it, based on the previously reported sequences of TuMV available in GenBank (NCBI, USA). cDNA sequencing was also carried out by using OmniBase DNA Cycle Sequencing System (USA, Promega). The identity matrixes for each nucleotide and amino acid sequence were calculated by the program of BioEdit (Hall, 1999). TuMV sequences obtained were phylogenetically compared to those of the other TuMV (GenBank and EMBL) using the multiple sequence alignment application of DNAMAN version 4.0 (Lynnon Biosoft, Quebec, Canada). Full optimal sequence alignments and neighbor-joining method options of Saitou and Nei (1987) with 1000 bootstrap (Felstein, 1985) replications (Altschul et al., 1998) were conducted. Percent nucleotide (nt) and open reading frame (ORF) amino acid (aa) sequence identities between virus isolates were calculated using the distance between all pairs of sequences in the multiple alignments.

Results

Host range. All the plants tested were susceptible to the selected six TuMV isolates (Table 2). It is not surprising that three isolates (rh-cq1-2, rg-pf1, and stock) were caused systemic symptoms in the all cultivars of *R. sativus*, whereas no symptoms in the cultivars of *B. campestris* sp. *pekinensis*. However, all the 2 cultivars of *B. campestris* sp. *pekinensis* were found to be equally susceptible to the other

Table 2. Symptoms developed on indicator plants inoculated with six Korean TuMV isolates[a]

Indicator plant \ Isolate	Radish		Stock	Turnip (Tu-1)	Chinese cabbage	
	rh-cq1-2	Rg-pf1			ca-ad7	cj-ca2-1
Brassica rapa L.	–/sm,mal	–/sm,mal	–/sm,mal	–/sm,mal	–/sm,mal	–/sm,mal
Impatiens balsamina L	–/vc	–/–	–/vc	–/nl,vn,d	–/vc	–/vc
Zinnia elegans Jacq.	–/m	–/m	–/m	–/m	–/m	–/m
Perilla frutescens var. *japonica*	–/vc	–/vc	–/vc,m	–/vc	–/vc,m	–/vc,m
Chrysanthemum coronarium	cl/cl,m	cl/cl	cl/cl	cl/cl	cl/cl	cl/cl
Chenopodium amaranticolor	nl/nl	nl/nl	nl/nl	nl/sm,mal	nl/nl	nl/nl
C. quinoa	nl/nl	nl/nl	nl/nl	nl/sm,nl	cl/sm,mal	cl/sm,mal
Physalis floridana	–/sm,mal	–/ym	nl/sm,mal	nl/sm,mal	nl/sm,mal	nl/sm,mal
Vicia faba	nl/–	nl/–	nl/–	nl/–	nl/cl,m	nl/nl
Raphanus sativus 'Baikun'	–/m	–/m	–/m	–/–	–/–	–/–
Raphanus sativus 'Baigok'	–/m	–/sm	–/m	–/–	–/–	–/–
Raphanus sativus 'Samyang'	–/vc	–/m	–/m	–/–	–/–	–/–
Raphanus sativus 'Jinmi'	–/m	–/m	–/m	–/–	–/–	–/–
Raphanus sativus 'Seoho'	–/m	–/sm	–/m	–/–	–/–	–/–
Raphanus sativus 'Yebbun'	–/m	–/m	–/m	–/–	–/–	–/–
Brassica campestris sp. *pekinensis* 'Jangmi'	–/–	–/–	–/–	–/sm,mal	–/sm,mal	–/m,mal
Brassica campestris sp. *pekinensis* 'Saianorang'	–/–	–/–	–/–	–/sm,mal	–/sm,mal	–/sm,mal

[a]m, mosaic; sm, severe mosaic; vc, vein clearing; cl, chlorotic local lesion; nl, necrotic local lesion; –, no symptom; Inoculated leaf /Upper leaf.

Table 3. Effects of six Korean TuMV isolates on Provvidenti's *B. campestris* subsp. *pekinensis* strain differentials

Cultivar \ Isolate	Reaction[a]						
	Radish			Stock	Tu 1	Chinese cabbage	
	Rg-pf1	Rh cq1-2	R 9-10			ca-ad7	cj-ca2-1
Tropical Delight (F1)	R	R	R	R	S	S	S
Crusader (F1)	R	R	R	R	S	S	S
PI419105	R	R	S	S	S	S	S
PI418957	–[b]	–	–	–	S	R	S

[a] S: susceptible, R: resistant; [b] – not test.

isolates (tu1, ca-ad7, and cj-ca2-1) whereas no symptoms were observed in all the 6 cultivars of *R. sativus* (Table 2). Especially, only rg-pf1 isolate did not induce symptoms in *I. balsamiana,* whereas both ca-ad7 and cj-ca2-1 isolates produced systemic symptoms in *Vicia faba.*

Classification of TuMV isolates based on Provvidenti's strain differentials. These six isolates could be classified 2 groups according to the pattern of the phenotypes on the four *B. campestris* differential hosts with Provvidenti's strain differentials (Table 3). As same as indicator host species, three isolates (tu1, ca-ad7, and cj-ca2-1) were classified as strains C4 and/or C5. However, the other 4 isolates (rh-cq1-2, rg-pf1, r 9-10, and stock) were less aggressive than the other strains and could not be classified by Provvidenti's strain differentials.

Classification of TuMV isolates based on reaction to Chinese cabbage inbred lines. These isolates were divided into 7 groups according to their different reactions to four inbred lines of Chinese cabbage (Table 4). Two inbred lines, BP058 and Wonyae 20026, had resistant to all these isolates, whereas 31 out of 48 inbred lines showed susceptible responses. Also, 0-2 inbred line was susceptible to TuMV-ad 7 and cj-ca2-1 isolated from Chinese cabbage, whereas Wonyae 20031 line was susceptible to only TuMV-ad 7 isolate. Nine inbred lines including Sambo, Palweolje, Wonyae 20027, Pyunggangshin No.1, Chinese michihili, Hasanchunse, SSD139, 90-21(3)-2-1-1, and 94CC422 showed the same resistance response as the cultivars of Chinese cabbage. Interestingly, two inbred lines, Chungbang and SSD63, showed resistance response to only the rg-pf1 isolate, whereas AVRDC and T.F.F.L inbred lines were resistant to only the stock isolate.

Classification of TuMV isolates based on reaction to Korean radish inbred lines. In case of fifty inbred lines of Korean radish, these lines were divided into 11 groups based upon resistance and/or susceptible responses against tested TuMV isolates. This is more diverged than Chinese

Table 4. Reactions of 48 lines of *Brassica campestris* sp. *pekinensis* by six Korean TuMV isolates[a]

Inbred line	Isolate					
	radish		Stock	Tu 1	Chinese cabbage	
	Rg-pf1	Rh cq1-2			Ad 7	Cj-ca2-1
BP058	0[b]	0	0	0	0	0
Wonyae 20026	0	0	0	0	0	0
Wonyae 20031	0	0	0	0	5	0
0-2	0	0	0	0	4	2
Pyunggangshin No.1	0	0	0	4	3	4
Chinese michihili	0	0	0	2	1	3
Hasanchunse	0	0	0	6	7	5
SSD139	0	0	0	6	5	9
90-21(3)-2-1-1	0	0	0	9	9	9
Wonyae 20027	0	0	0	6	6	6
94CC422	0	0	0	9	9	9
Sambo	0	0	0	3	9	6
Palweolje	0	0	0	8	8	7
Chungbang	0	1	4	7	7	7
SSD63	0	1	1	9	9	7
AVRDC	3	4	0	4	5	5
T.F.F.L	3	4	0	1	7	4
Gikyae	8	8	7	8	9	7
50 days	*	6	8	6	8	8
Shiisang	2	3	3	9	9	9
Naebyungjagryg No.2	7	2	6	9	7	9
Chosangkyungsam	3	7	7	9	8	7
Haekbaechu	2	3	1	8	8	6
Pyungchong No.1	2	1	0	9	9	9
Chunpayaki	2	9	9	6	8	7
YC 30	8	6	9	6	6	6
60 days	5	1	3	7	8	7
Naebyung 60days	8	5	1	7	7	8
Seoulbaechu	7	6	5	5	2	6
Kyungdo No.2	3	2	2	7	8	7
Songdo No.2	4	6	5	8	7	7

Table 4. Continued

Inbred line	Isolate					
	radish		Stock	Tu 1	Chinese cabbage	
	Rg-pf1	Rh cq1-2			Ad 7	Cj-ca2-1
Yaki No.2	7	6	7	6	9	8
Chosangchungbang	4	4	2	7	7	7
Aurdcacc	8	7	3	5	7	5
Miho No.1	5	4	4	4	5	6
Jeongu	7	3	2	8	8	5
Daehyunggarag	1	3	3	9	*	9
Kyungdo No.3	1	2	4	4	8	7
Nongnim No.1	4	4	6	8	9	8
Chihili	8	9	8	8	6	8
Michihili	6	8	8	6	6	7
BP079	4	6	3	4	5	4
SSD31	9	8	7	8	8	8
An111	3	4	3	9	9	6
A(HS-6)	2	6	4	7	4	8
((SSD31*02)[3]*(SSD31))[4]	5	5	2	3	2	2
Wonyae 20021	5	4	4	5	4	6
Wonyae 20022	3	4	3	4	2	4

[a] Each group of *Brassica campestris* sp. *pekinensis* inbred lines was separated by solid lines based upon responses against each TuMV isolate.
[b] Resistance and/or susceptible responses were scored as previously reported (Choi et al. 2004, unpublished data)

cabbage. However, 5 (LT 032576, 032535, 032539, 032540, and 032571) out of 50 inbred lines showed susceptible response to all TuMV isolates, whereas no fifty inbred lines of Korean radish showed resistant response to both rg-pf1 and rh-cq1-2 isolates. Twenty-one inbred lines including LT032551, LT032558, LT032558 and others showed resistance response to Chinese cabbage isolate of TuMV including Tu1, Ad7, and Cj-ca2-1, while showing susceptible response against radish isolates including Rg-pf1, Rh cq1-2, and stock. Three inbred lines, LT032528, LT032530 and LT032532, showed susceptible response to only both rg-pf1 and rh-cq1-2 TuMV isolates. The other inbred lines had resistance to one or two TuMV isolates.

Classification of TuMV isolates. For TuMV isolates classification, *P. frutescens var. japonica*, *P. floridana*, *B. campestris* Sambo, Palweolje, Wonyae31, SSD63, and AVRDC, *R. sativus* LT 032528, LT 032538, and LT 032554 out of indicator host species, forty-eight inbred lines and two hybrids of Korean Chinese cabbage and fifty inbred lines of Korean radish were selected by the virulence differentiation to the representative six isolates of TuMV.

Table 5. Reactions of 50 lines of *Raphanus sativus* by six Korean TuMV isolates[a]

Inbred line	Isolate					
	radish		Stock	Tu 1	Chinese cabbage	
	Rg-pf1	Rh cq1-2			Ad 7	Cj-ca2-1
LT 032528	9[b]	3	0	0	0	0
LT 032530	9	2	0	0	0	0
LT 032532	9	4	0	0	0	0
LT 032557	7	5	0	2	0	0
LT 032558	6	7	6	0	0	0
LT 032559	5	7	8	0	0	0
LT 032560	7	6	6	0	0	0
LT 032561	7	6	8	0	0	0
LT 032562	5	4	2	0	0	0
LT 032563	6	5	6	0	0	0
LT 032564	9	8	4	0	0	0
LT 032566	9	3	5	0	0	0
LT 032567	5	3	7	0	0	0
LT 032569	8	7	8	0	0	0
LT 032527	9	7	9	0	0	0
LT 032529	2	4	7	0	0	0
LT 032531	9	8	8	0	0	0
LT 032544	8	9	8	0	0	0
LT 032545	9	8	8	0	0	0
LT 032546	4	1	6	0	0	0
LT 032548	4	7	7	0	0	0
LT 032551	8	1	5	0	0	0
LT 032554	6	5	5	0	0	0
LT 032555	8	7	7	0	0	0
LT 032556	6	8	5	0	0	0
LT 032538	0	7	8	5	0	6
LT 032553	5	6	7	0	0	2
LT 032537	5	7	7	0	0	2
LT 032573	5	9	6	0	8	0
LT 032547	5	5	5	0	4	0
LT 032541	8	8	8	3	0	0
LT 032549	7	8	7	4	0	0
LT 032570	8	3	8	9	0	0
LT 032574	9	8	5	0	7	2
LT 032575	9	8	9	0	8	1
LT 032533	9	7	9	0	6	7
LT 032543	6	6	9	0	1	4
LT 032550	7	6	7	0	3	3
LT 032565	3	8	8	0	3	3
LT 032568	5	7	3	0	2	4
LT 032552	6	8	3	2	0	3
LT 032542	6	8	7	5	8	0
LT 032534	8	8	7	8	7	0
LT 032536	5	7	7	5	5	0
LT 032572	7	8	9	9	8	0
LT 032576	9	7	9	7	6	9
LT 032535	9	5	8	2	2	2
LT 032539	9	9	9	8	9	5
LT 032540	8	9	6	9	9	5
LT 032571	9	7	8	8	8	8

[a] Each group of *Raphanus sativus* inbred lines was separated by solid lines based upon responses against each TuMV isolate.
[b] Resistance and/or susceptible responses were scored as previously reported (Choi et al. 2004, unpublished data)

Table 6. Comparison of the nucleotide and the amino acid identity with eight Korean TuMV isolates

Isolaes	Stock	Tu 1	Rh cq1-2	Cj-ca2-1	Ca-ad 7	R 9-10	Rg-pf1	
Stock		98.2	98.4	97.7	97.9	98.1	89.5	90.4
Tu 1	97.2[a]		98.3	98.1	98.6	98.7	89.4	90.6
Rh cq1-2	96.8	97.5		97.6	97.9	98.2	89.2	90.1
Cj-ca2-1	95.4	97.5	95.8		98.1	97.7	89.1	90.2
Ca-ad 7	96.1	98.2	96.5	98.2		98.2	89.4	90.6
R 9-10	97.2	98.2	97.2	96.5	97.2		89.6	90.4
Rg-pf1	93.0	93.4	92.3	91.6	92.3	94.0		98.2
Mustard	94.4	95.4	93.7	93.7	94.4	95.1	97.9	

Nucleotide sequence identity

[a]Numbers represent amino acid identity between each isolate.

Eight isolates including to the representative six isolates were used to divide the group by check the virulence differentiation. Three isolates, such as ca-ad7, cj-ca2-1 and tu-1 belong to a group (Group I), whereas rg-pf1, r9-10, rh-cq1-2, stock and mustard isolates also showed almost similar pattern of the virulence differentiation (Group II). Although group I showed similar infectivities to *B. campestris* Sambo and Palweolje. Moreover, three isolates of Group I (ca-ad7, cj-ca2-1 and tu-1) can be divided into 2 subgroups, subgroup 1 (cj-ca2-1 and tu-1) showed negative reaction to *B. campestris* Wonyae31 and *R. sativus* LT 032554, whereas subgroup 2 (ca-ad7) infected both tested plants.

Group II can be divided to 4 subgroups; interestingly, the division of these subgroups was similar to the difference of host isolated. First of all, three isolates (rg-pf1, r9-10 and rh-cq1-2) isolated from Korean radish were divided into 2 subgroups based upon infectivity on *B. campestris* SSD63 and *R. sativus* LT 032538. And the difference between stock and three isolates from Korean radish was revealed by the reaction on *B. campestris* AVRDC and *R. sativus* LT 032528. Mustard and the other isolates showed different infectivity on *P. frutescens* and *P. floridana.*

EM and ELISA. EM examinations of all the eight isolates revealed the same flexuous rod-shaped particles with an average length of 720 nm long and the typical cytoplasmic inclusions bodies like pinwheels, scrolls and laminated aggregates in cells of *N. benthamiana* were observed (data not shown). All the eight isolates were easily detected by ELISA and no false positive or negative were found with this method.

CP sequence analysis. In sequence analysis, all the cloned cDNAs for CP gene contained 944 bp DNA fragment in lengh, which contain the full-length CP gene including border sequences at both-sides. The actual CP gene consists of 864 bp and 288 amino acids. Each nucleotide sequences of eight TuMV isolates (data not shown) are registered to GenBank as accession No. AF103785 to AF103792. In aspect of sequence identity (Table 6), eight isolates appeared to be divided into two groups (Group consists of Tu-1, rh-cq1-2, Stock, r 9-10, cj-ca2-1 and ca-ad7 and Group containing rg-pf1 and mustard isolates). Their identity indexes of intragroup and intergroups showed 98% and 90%, respectively. Even in aspect of amino acid similarity, the same tendency also appeared even though the difference of identity indexes slightly decreased.

Discussion

In a preliminary field survey it was found that TuMV seriously infected in cruciferous vegetables in Korea. There was no specific difference of TuMV isolates depending on growing provinces. Strain specificity is an important factor to be considered in developing resistant cultivars to TuMV, since their performance will depend upon the presence and distribution of these strains in a given locality. The purposes of this differentiation were to determine the presence and prevalence of TuMV strains infecting in Korea, and to make those strains available for the development of stable yield and disease-resistant cruciferous varieties in the breeding program. Provvidenti (1981) classified TuMV isolates on the basis of differential host reactions to a select group of *B. campestris* subsp. *pekinensis* cultivars, which included two hybrid commercial varieties, and divided the isolates into four strains (TuMV C1-C4). As indicated in our study, TuMV isolates can be differentiated into 2 groups by different reactions on *B. campestris* subsp. *pekinensis* cultivars with Provvidenti's strain differentials. In this study, all isolates from Chinese cabbage and turnip were classified as strain C4 or C5, whereas all isolates from radish and stock plants cannot be differentiated with Provvidenti's *B. campestris* subsp. *pekinensis* strain differentials. Even thought TuMV-C4 strain was the most common strain in Taiwan and Korea (Green and Deng,

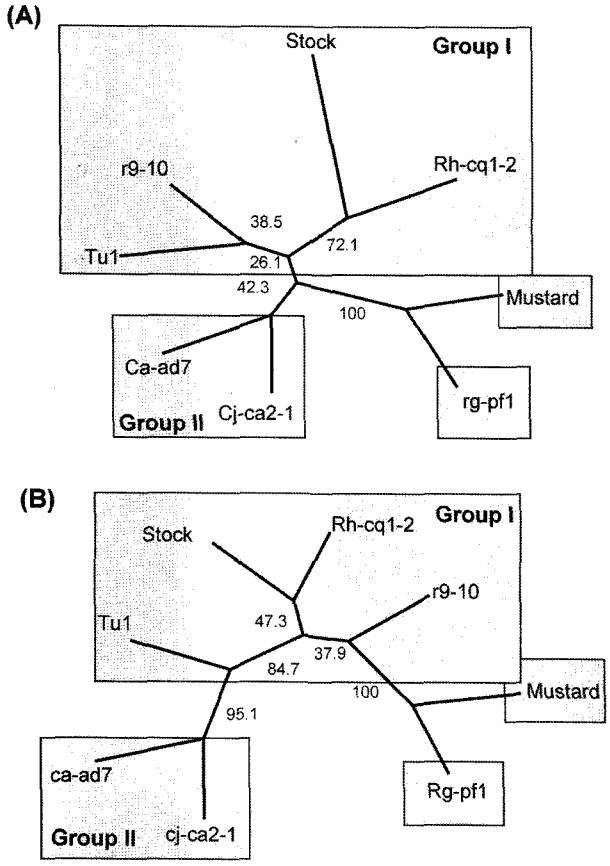

Fig. 1. The virulence differentiation of the representative eight isolates by the indicator host species, forty-eight inbred lines and two hybrids of Korean Chinese cabbage and fifty inbred lines of Korean radish were used for isolate differentiation.

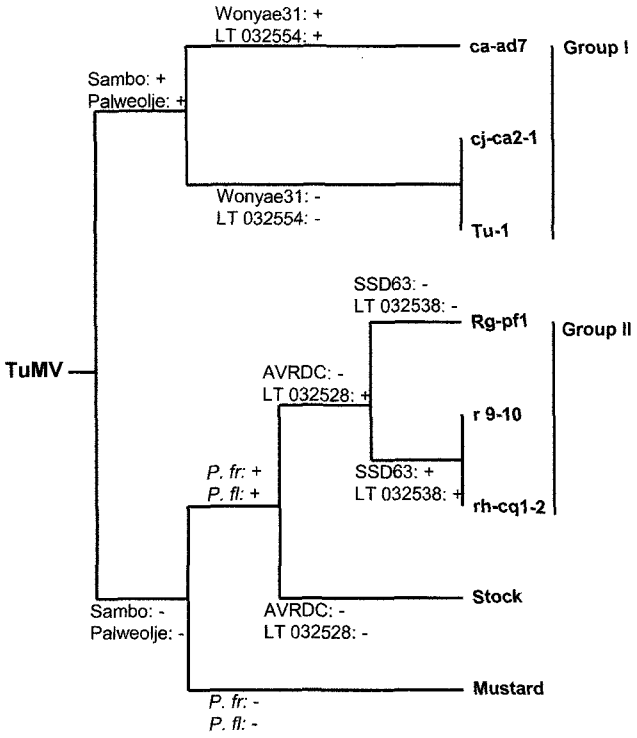

Fig. 2. Phylogenetic tree constructed from the analysis of the nucleotide and the deduced amino acid of coat protein nucleotide sequence alignments of the CP fragments.

1985; Suh, 1993) and TuMV-C4 and TuMV-C5 in China (Feng et al., 1990; Lin et al., 1990), these results suggested that the isolates from Chinese cabbage and turnip could be classified, but not Korean radish and stock.

Additionally, the set of *B. campestris* subsp. *pekinensis* proposed by Provvidenti (1980) included commercial cultivars and F1 hybrids which were sometimes found to be genetically impure responses with respecting TuMV infection, and which show clear symptoms only in the cool season, making strain detection quite difficult (AVRDC, 1986). Therefore, five Chinese cabbage inbred lines (Palweolje, Sambo, Wonyae 20031, AVRDC, and SSD63), 3 Korean radish lines (LT 032528, LT 032538, and LT 032554) and 2 indicator host species (*P. frutescens var. japonica* and *P. floridana*) were used for more specific strain differentiation. This differential series has proved very useful in this context, and represents an important component for future integrated control strategies where resistance genes will play a major role in TuMV control. As further sources of resistance are identified, the pathotyping system will evolve, and resistances based on additional genes will be developed and will hopefully result in more robust growth of plants in the field. The multi-resistant lines Wonyae 20026 and BP058 of Chinese cabbage represent valuable genetic materials that can be used for crucifer breeding programs on TuMV resistance, whereas not in Korean radish.

Regarding comparison of nucleotide and amino acid sequences of TuMV CP gene, six isolates (Group I, Tu-1, rh-cq1-2, Stock, r 9-10, cj-ca2-1, and ca-ad7) are different from these of the other two isolates (rg-pf1 and mustard; Group II). As indicated above, CP was reported to be required for systemic infection (Spitsin et al., 1999), aphid transmission (Salomon and Bernardi, 1995), viral RNA replication (Reusken et al., 1995), and movement (Schmitz and Rao, 1998). Because CP could reflect host specificity in many ways, the molecular phylogenetic analysis by means of CP gene could give a way to classify plant virus and to confirm the virulence differentiation. However, based on the molecular phylogenetic trees, TuMV isolates divided into two groups and two independent isolates. Two phylogenetic trees indicated that four isolates (Tu 1, r 9-10, Stock, and Rh-cq1-2) belonged to a group (Group I in Fig. 2), and the other two isolates (Ca-ad7 and Cj-ca2-1) to another group (Group II in Fig. 2). However, Mustard and rg-pf1 isolates do not have relationship with any groups.

These grouping of eight TuMV isolates revealed the different pattern as the classification as compared to the pathogenecity (Fig. 2). Therefore, based on analysis of the nucleotide and the deduced amino acid of the CP, it seems that TuMV isolates do not have any relationship between the virulence differentiation and the molecular phylogenesis.

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