

## Symptom Variances in Mixed Infections of Six *Turnip mosaic virus* and One *Ribgrass mosaic virus* Isolates in Crucifers

Jeom-Deog Cho, Hong-Soo Choi<sup>1</sup>, Jeong-Soo Kim<sup>2\*</sup>, Kook-Hyung Kim and Kyung-Soo Kim<sup>3</sup>

College of Agriculture and Life Sciences, Seoul National University, Suwon 441-744, Korea

<sup>1</sup>National Institute of Agricultural Science and Technology, Rural Development Administration (RDA), Suwon 441-707, Korea

<sup>2</sup>National Horticultural Research Institute, RDA, Suwon 441-707, Korea

<sup>3</sup>Department of Plant Pathology, University of Arkansas, USA

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**Turnip mosaic Potyvirus (TuMV) and Ribgrass mosaic Tobamovirus (RMV) are major viruses infecting crucifer crops in Korea. RMV-FG22 was isolated from oriental cabbage. TuMV isolates were TuMV-CA7 from oriental cabbage, TuMV-TU and TuMV-TU2 from turnip, TuMV-RA from rape, TuMV-ST from stock, and TuMV-R9 from radish. The six isolates of TuMV were classified by symptom expression in inbred lines of crucifers. TuMV-CA7 and TuMV-TU isolates infected mostly oriental cabbages; TuMV-ST, TuMV-TU2, and TuMV-R9 infected radishes; and TuMV-RA infected both oriental cabbages and radishes. Crops used in six combinations of mixed infections were 'Tambok' cultivar resistant to TuMV, 'SSD63' susceptible inbred line of oriental cabbage, pure line of leaf mustard, and 'Daeburyungyeorum' cultivar of radish. External symptoms in 'Tambok' and radish by each of the six single infections of TuMV showed similar results by bioassay. Synergistic response of necrotic death occurred within 1 week after inoculation in all combinations mixed with TuMV and RMV-FG22 on leaf mustard. In oriental cabbage 'SSD63', synergism of necrosis occurred in four TuMV isolates, but not in TuMV-ST and TuMV-R9. In oriental cabbage 'Tambok', synergism was expressed only in two combinations of RMV-FG22+TuMV-CA7 and RMV-FG22+TuMV-TU, but other combinations had the same symptoms produced by RMV-FG22. In radish 'Daeburyungyeorum', only mild mosaic symptoms were induced by combinations of RMV-FG22+TuMV-CA7, RMV-FG22+TuMV-TU, RMV-FG22+TuMV-RA, and RMV-FG22+TuMV-R9. Mosaic and severe mosaic were induced in combinations of RMV-FG22+TuMV-TU2 and RMV-FG22+TuMV-ST, respectively.**

**Keywords :** crucifer, mixed infection, *Potyvirus*, RMV, synergism, *Tobamovirus*, TuMV.

Oriental cabbage, a major ingredient of 'kimchi' which is a very important traditional Korean food, was cultivated in 46,000 ha, or 46% of total leaf vegetables in Korea (Ministry of Agriculture and Forestry, 2000). Virus diseases have been generally caused by single or by mixed infection of two or more viruses. In Korea, *Cucumber mosaic virus* (CMV) (Lee, 1981), *Turnip mosaic virus* (TuMV), and *Ribgrass mosaic virus* (RMV) have been infecting oriental cabbage. Natural hosts were studied in order to improve oriental cabbage's quality and yield, and to overcome seasonal production in the country. More than 60% (63.4%) of mixed infection with RMV and TuMV was observed in oriental cabbage cultivated in summer and autumn seasons (Kim et al., 1993). The rate of disease outbreak by single infection of the TuMV, RMV, and by their mixed infection was 13.9%, 4.5%, and 31.9%, respectively (Yoon et al., 1995).

In mixed infection, symptom severity was greatly increased by high viral titer and enhanced movement of viruses in plant. TuMV produced severe veinal necrosis by the mixed infection with *Potato virus X* and *Potato virus Y*, as compared with mild mottle or vein banding by single infection (Matthew, 1991). The cowpea stunt disease by double infection of CMV and *Bean common mosaic virus* (BCMV) which occurred in mid-eastern USA caused yield loss of 84%, while CMV reduced yield by 14.2% and BCMV by 2.5% (Pio-Ribeiro et al., 1978). In Korea, economical damage by virus diseases caused by mixed infection was reported on oriental cabbage showing necrotic stunt by TuMV and RMV in the mid-eastern alpine area of Pyungchang (Kim et al., 1993; Kim et al., 2001), and on watermelon showing necrosis by mixed infection of *Cucumber green mottle mosaic virus* (CGMMV) and *Watermelon mosaic virus* (WMV) in Jinju and Haman areas (Cho, 1998; Kim et al., 2000).

This study investigated the varying symptoms in crucifer crops by mixed infection of an isolate of RMV and six isolates of TuMV showing different biological characteristics.

\*Corresponding author.

Phone) +82-31-290-6220, FAX) +82-31-295-9548

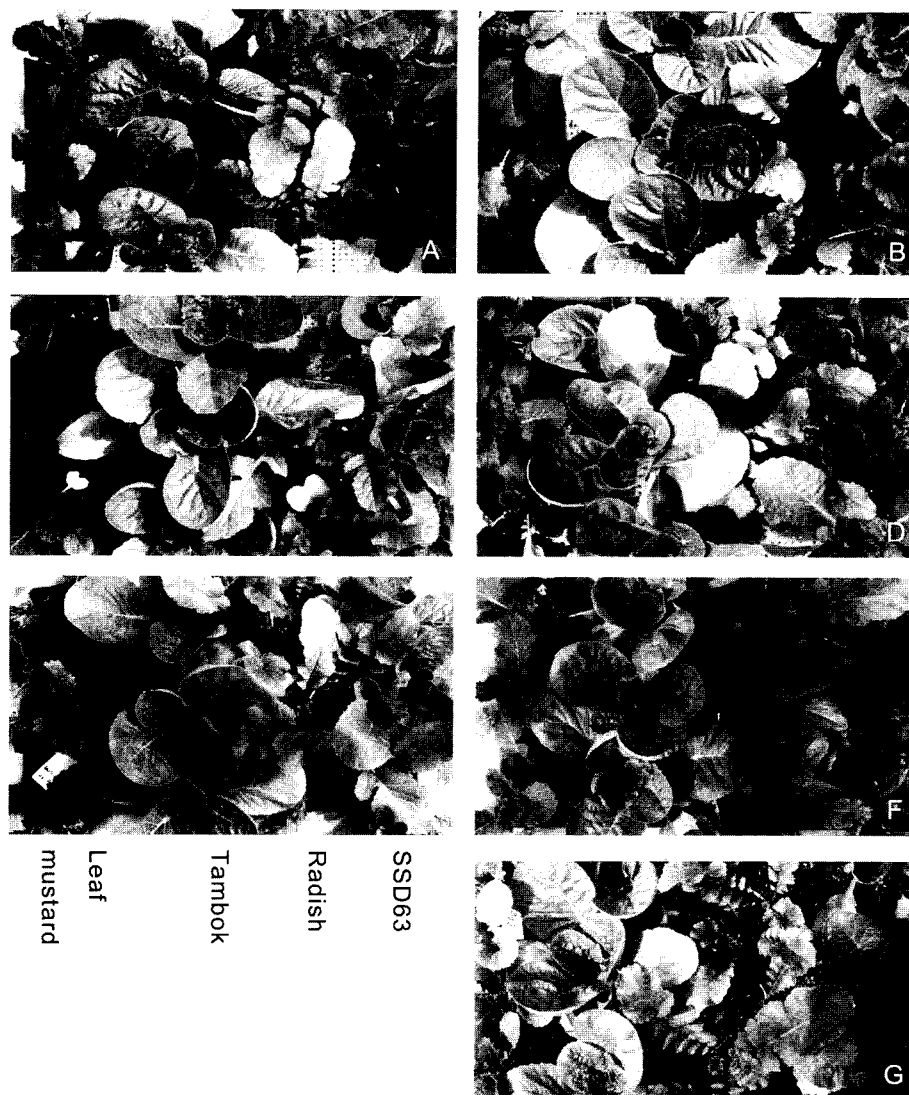
E-mail) kimjsoo@rda.go.kr

## Materials and Methods

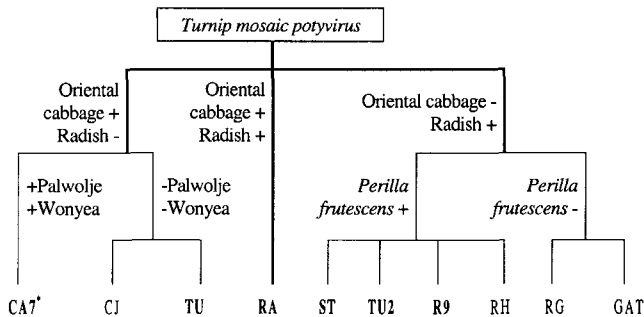
RMV-FG22 was isolated in Pyungchang, alpine area (Kim et al., 1993). Six TuMV isolates were chosen from 10 isolates classified by bioassay using differential plants by Choi et al. in 1998 (Fig. 1). TuMV-CA7 and TuMV-TU had virulence on oriental cabbage but not on radish. TuMV-Rape infected both oriental cabbage and radish, but TuMV-ST, TuMV-TU2 and TuMV-R9 infected only radish (Kim et al., 1994).

Two oriental cabbages, *Brassica campestris* ssp. *pekinensis* 'Tambok', a commercial cultivar known to be resistant to TuMV, and 'SSD63' inbred line having elite-horticultural characteristics

which is susceptible to TuMV, were used. RMV-FG22 and six TuMV isolates namely, TuMV-CA7, TuMV-TU, TuMV-RA, TuMV-ST, TuMV-TU2 and TuMV-R9 were inoculated singly and mixedly on 'Tambok' and 'SSD63', leaf mustard (*B. juncea*) of a pure line of 'Ganghwa' and radish (*Raphanus sativus*) 'Dae-buryungyeorum' cultivar. Leaves of leaf mustard showing mosaic symptoms by mechanical inoculation of each virus isolate were used as the virus source. Same mass of mustard leaves infected with each virus was homogenized in 4 vol. of 0.01 M Na-phosphate buffer, pH 7.0, with mortar and pestle. The mixed virions were then mechanically inoculated onto 3-4 leaf stage of crucifers dusted with carborundum (600 mesh).



**Fig. 1.** External symptoms on crucifer by single infections of RMV-FG22 and six TuMV isolates namely, TuMV-CA7 (A), TuMV-ST (B), TuMV-TU (C), TuMV-TU2 (D), TuMV-RA (E) and TuMV-R9 (F). In 'Tambok', TuMV-CA7 and TuMV-TU isolates did not infect systemically. Necrotic spots were induced by TuMV-RA, TuMV-ST, TuMV-TU2, and TuMV-R9 isolates on inoculated leaves. In 'SSD63', most TuMV isolates infected systemically except TuMV-ST. In leaf mustard, all TuMV isolates expressed systemic symptoms of severe mosaic and blisters. In radish, TuMV-ST, TuMV-TU2, and TuMV-R9 isolates produced systemic symptoms of mild mosaic, severe mosaic, and mosaic respectively. RMV-FG22 isolate induced vein clearing in oriental cabbages 'Tambok' and 'SSD63', severe mosaic in leaf mustard and no symptoms in radish (G).



**Fig. 2.** Classification of TuMV by bioassay using differential crops. Isolates in bold letters were used. + = systemic infection; - = no infection.

\*CA7 and CJ, TuMV isolated from oriental cabbages; TU and TU2, TuMV isolated from turnips; RA, TuMV isolated from rape; ST, TuMV isolated from stock; R9, RH, and RG, TuMV isolated from radishes; GAT, TuMV isolated from leaf mustard.

## Results

**Single infection.** On oriental cabbages 'Tambok', TuMV-CA7 showed large necrotic spots on inoculated and developed leaves; TuMV-TU had no symptom on inoculated leaves but showed mosaic on the upper leaf; and TuMV-RA caused necrotic spots on inoculated leaves and no symptom on the upper leaves. However, TuMV-ST, TuMV-TU2, and TuMV-R9 showed only large necrotic spots on inoculated leaf. In susceptible oriental cabbage 'SSD63', severe mosaic by TuMV-CA7, mosaic by TuMV-TU and vein clearing by TuMV-RA were observed, but systemic symptoms of large chlorotic spots and chlorotic spots were produced by TuMV-TU2 and TuMV-R9, respectively, and no symptom by TuMV-ST. All six isolates of TuMV caused systemic severe mosaic and rugose in leaf mustard. In radish, TuMV-ST, TuMV-TU2 and TuMV-R9 caused systemic symptoms of vein clearing,

**Table 1.** Symptoms on crucifer induced singly by six isolates of TuMV<sup>a</sup> and RMV-FG22<sup>b</sup>

Virus isolate	Symptoms <sup>a</sup> produced on			
	'Tambok'	'SSD 63' <sup>d</sup>	Leaf mustard	Radish
TuMV-CA7	LNS/LNS <sup>e</sup>	BNS/SM	-/SM	-/-
TuMV-TU	-/M	-/M	-/SM, B	-/-
TuMV-RA	NS/-	-/VC,MM	-/SM, B	-/-
TuMV-ST	LNS/-	-/-	-/SM, B	-/VC, MM
TuMV-TU2	LNS/-	LCS/LCS	-/SM, B	-/SM
TuMV-R9	LNS/-	NS/CS	-/SM, B	-/M
RMV-FG22	-/VC, MAL	-/VC, MAL	-/SM	-/-

<sup>a</sup>TuMV: *Turnip mosaic virus, Potyviridae*.

<sup>b</sup>RMV: *Ribgrass mosaic virus, Tobamovirus*.

<sup>c</sup>'Tambok': Commercial cultivar of oriental cabbage.

<sup>d</sup>'SSD 63': Inbred line of oriental cabbage.

<sup>e</sup>Symptom development was observed at 10-14 days post inoculation (DPI). A total of five plants were inoculated. Symbols indicate as follows: CS = chlorotic spots; LCS = large chlorotic spots; LNS = large necrotic spots; MAL = malformation; M = mosaic; MM = mild mosaic; NS = necrotic spots; B = blister; SM = severe mosaic; VC = vein clearing; - = negative reaction and inoculation on leaf/upper leaf.

severe mosaic and mosaic, respectively. When RMV-FG22 isolate was infected singly, vein clearing was observed in oriental cabbage 'Tambok' and 'SSD63', severe mosaic in leaf mustard and no symptom in radish (Table 1; Fig. 1). **Mixed infection.** Mixed virions of TuMV-TU+RMV-FG22 showed strong synergistic symptoms of vein necrosis, stunt, and malformation on 'Tambok'. Mixed virions of TuMV-CA7+RMV-FG22 produced similar symptoms but weak synergism than that of TuMV-TU+RMV-FG22. On 'Tambok' cultivar, other combinations of TuMV-RA+RMV-FG22, TuMV-ST+RMV-FG22, TuMV-TU2+RMV-FG22 and TuMV-R9+RMV-FG22 developed symptoms similar to single infection of RMV-FG22 such as systemic vein

**Table 2.** Symptoms on crucifer induced by mixed infection of RMV-FG22<sup>a</sup> and 6 isolates of TuMV<sup>b</sup>

Mixed infection	Symptoms produced on			
	'Tambok' <sup>c</sup>	'SSD 63' <sup>d</sup>	Leaf mustard	Radish
RMV + TuMV-CA7	NS/VN, ST, MAL <sup>e</sup>	-/D	-/D	-/MM
RMV + TuMV-TU	LCS/VN, ST, MAL	-/D	-/D	-/MM
RMV + TuMV-RA	NS/VC, MAL	-/D	-/D	-/MM
RMV + TuMV-ST	NS/VC, MAL	-/Y, MAL	-/D	-/M
RMV + TuMV-TU2	LNS/VC	CS/D	-/D	-/SM
RMV + TuMV-R9	-/VC, MAL	-/Y, MAL	-/D	-/MM

<sup>a</sup>RMV: *Ribgrass mosaic virus, Tobamovirus*.

<sup>b</sup>TuMV: *Turnip mosaic virus, Potyviridae*.

<sup>c</sup>'Tambok': Commercial cultivar of oriental cabbage.

<sup>d</sup>'SSD 63': Inbred line of oriental cabbage.

<sup>e</sup>Symptom development was observed for 10-14 DPI. A total of five plants were inoculated. Symbols indicate as follows: CS = chlorotic spots; D = plant death; LCS = large chlorotic spots; LNS = large necrotic spots; MAL = malformation; M = Mosaic; MM = mild mosaic; NS = necrotic spots; SM = severe mosaic; ST = stunt; VC = vein clearing; VN = vein necrosis; Y = yellow; and - = negative reaction.

**Table 3.** Synergism induced by combinations of RMV-FG22<sup>a</sup> and six isolates of TuMV<sup>b</sup> on crucifer crops

Crop <sup>a</sup>	T-CA7+R	T-TU+R	T-RA+R	T-ST+R	T-TU2+R	T-R9+R
'Tambok' <sup>c</sup>	+ <sup>e</sup>	++	-	-	-	-
'SSD63' <sup>d</sup>	++	+++	++	-	+	-
Radish	-	-	-	-	-	-
Leaf mustard	+++	++	++	++	++	++

<sup>a</sup>RMV: *Ribgrass mosaic virus, Tobamovirus*.

<sup>b</sup>TuMV: *Turnip mosaic virus, Potyviridae*.

<sup>c</sup>'Tambok': Commercial cultivar of oriental cabbage.

<sup>d</sup>'SSD 63': Inbred line of oriental cabbage.

<sup>e</sup>+ = weak synergism; ++ = medium synergism; +++ = strong synergism; and - = no synergism.

clearing and malformation. In susceptible oriental cabbage 'SSD63' cultivar, very strong synergism causing death occurred by the combinations of TuMV-TU+RMV-FG22, TuMV-CA7+RMV-FG22, TuMV-RA+RMV-FG22 and TuMV-TU2+RMV-FG22, in that order. In combinations of

TuMV-R9+RMV-FG22 and TuMV-ST+RMV-FG22, severe systemic symptoms of yellowing and malformation than those of single infection by TuMV isolates were observed. Leaf mustards died in about 1 week after inoculation because of extremely strong synergism by the six combi-



**Fig. 2.** External symptoms by mixed infections of TuMV-CA7+RMV-FG22 (A), TuMV-ST+RMV-FG22 (B), TuMV-TU+RMV-FG22 (C), TuMV-TU2+RMV-FG22 (D), TuMV-RA+RMV-FG22 (E), and TuMV-R9+RMV-FG22 (F). In resistant oriental cabbage 'Tambok', combinations of TuMV-CA+RMV-FG22 and TuMV-TU+RMV-FG22 showed synergism such as vein necrosis, stunt and malformation on the upper leaves. In susceptible oriental cabbage 'SSD63', four combinations, except TuMV-ST+RMV-FG22 and TuMV-R9+RMV-FG22, expressed synergistic symptoms of plant death. In leaf mustard, all of six combinations caused death within 1 week after mechanical inoculation. In radish, synergistic symptoms did not occur and weak systemic symptoms emerged in all combinations A to F. Plants left to right in photos A to F were oriental cabbage Tambok, inbred line SSD63, leaf mustard, and radish.

nations. The combinations of TuMV-CA7+RMV-FG22 and TuMV-TU+RMV-FG22 showed synergistic symptoms on the two oriental cabbage cultivars, with TuMV-TU+RMV-FG22 being more. However, it was most severe in leaf mustard, with TuMV-CA7+RMV-FG22. In radish, however, synergism did not occur by any combination because the RMV-FG22 could not infect 'Daeburyungyeorum' cultivar (Table 2, 3, Fig. 2).

## Discussion

Synergism induced by mixed infections of 'unrelated' viruses, tobamovirus and potyvirus, varied depending on the virus combinations and the host species. In crucifer, the different external symptom expression is suggested to depend not only on characteristics of hosts but also on the characteristic interactions between the co-infecting viruses. In oriental cabbage, patterns of external symptoms by the mixed infections were similar to the results by bioassay of the six TuMV isolates. However, radish was extremely resistant to RMV-FG22 and leaf mustard was very susceptible to all the isolates of TuMV and RMV-FG22, which showed little difference from the bioassay results.

*Potyruses* have been reported to be the major factor in the synergistic interactions involved in serious virus diseases, and using molecular biology, it has been determined that the genome of potyvirus is the origin of the synergism (Pruss et al., 1997; Vance et al., 1995). Synergism induced by mixed infection of potyvirus and its 'unrelated' partner virus, therefore, is suggested to be a result of complicated interactions among the two different viruses and their hosts, with the potyvirus genome being the major cause of the synergism. However, it should be noted that external symptom severity seemed to depend not only on the potyvirus infection but also on the tobamovirus partner, as well as the susceptible host species.

In previous study on mixed infection of TuMV and RMV (Cho et al., 2002), the host showing synergistic symptom had specific ultrastructures in its cells. Consequently, in crucifer crops of oriental cabbage and leaf mustard infected mixedly with some combinations of TuMV isolates and RMV-FG22, synergistic symptoms were expressed, which suggests that the cells in the hosts showing synergism could have specific ultrastructures. However, in case of mixed infection with CGMMV and WMV or ZYMV, specific ultrastructures were made different by the virus isolates and hosts (Cho, 2002), which suggests that the cells in the hosts that expressed synergistic symptoms by mixed infection of the six TuMV and RMV-FG22 could have some specific ultrastructures.

TuMV-resistant 'Tambok' cultivar became susceptible when some TuMV isolates were mixedly infected with

RMV-FG22 on the host, such as in mixed infection of TuMV-AC18 or -C5 and RMV-CA1 (Cho et al., 2002). This suggests that mixed infection and single infection tests are essential in breeding for resistance to prevent serious virus diseases. Identification of various virus isolates in the fields is likewise very important and will be continued because viruses can mutate and change diversely often resulting in serious outbreaks of virus diseases by mixed infection.

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