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Vegetative Compatibility Group of *Fusarium oxysporum* f. sp. *radicis-lycopersici* in Korea

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Vegetative compatibility groups (VCGs) of *Fusarium oxysporum* f. sp. *radicis-lycopersici* isolates collected from tomatoes in Korea were analyzed to determine the genetic characteristics and compared to those of foreign isolates. In comparison of VCG specificity with foreign VCG subgroup testers, Korean isolates were revealed to be VCG 0094 and to be similar to those of Israel and Florida, USA having a "Universal" property. Results of this study will contribute the effective control of disease through precise estimation of fungal damage, the prediction of new pathogenic isolates appearance, and the movement of foreign pathogens.

Keywords **:** *Fusarium oxysporum* f. sp. *radicis-lycopersici*, subgroup, tomato, universal, VCG 0094

Many economical damages from the most tomato cultivating countries were reported throughout the world due to *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Caron et al., 1986, Katan and Katan, 1999). In Korea, *F. oxysporum* f. sp. *lycopersici* was the main pathogen of tomato in the past, but recently damage due to *F. oxysporum* f. sp. *radicis-lycopersici* was getting severe at the forced culture fields in greenhouses and the harvesting was almost impossible at the severely damaged fields (Yang et al., 2000a).

Typical classification between *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici* is impossible in morphology. The symptom on tomato caused by *F. oxysporum* f. sp. *radicis-lycopersici*, however, was different from that of *F. oxysporum* f. sp. *lycopersici* and infected part of soil surface less than 25 cm, browned the vasicular bundle, caused the crown and root rot. Also, the *F. oxysporum* f. sp. *radicis-lycopersici* is classified as psychrophilic fungus since the optimum temperature of disease outbreak of *F. oxysporum* f. sp. *radicis-lycopersici* is 18°C, different from the optimum temperature of disease outbreak of the *F. oxysporum* f. sp. *lycopersici* of 27°C. Meanwhile, these are often coexisting at the same plant, and pathogenic classification of these fungi is very difficult since these are appeared differently according to inoculation method of pathogen, inoculation concentration, and development status of a plant.

The crown and root rot of tomato damaged cultivation of tomato in Korea, and the damage has been increased continuously. After the first report of the crown and root rot of tomato at the nutriculture regions by Lee et al. (1994), Kim (2000) defined the pathogenicity, physiological and biological characteristics of this fungus. Also, interests about this fungus are increasing with the research of outbreak according to soil environments (Yang, 2000b).

The vegetative compatibility has been used to identify the forma specialis, race, and pathogenic group of fungi such as *Neurospora, Aspergillus,* and *Podospora* spp., and applied as an important basic data for research of genetic diversity of fungi so far. Generally, mutants of *Nit* gene related to the nitrogen source utilization are mostly used, and induce mutants using chlorate, and classify isolate forming heterokaryon among *nit*-mutant that contains complemental multilocus when opposite cultured these mutants as the same group.

Rosewich et al. (1999) investigated vegetative compatibility group (VCG) among *F. oxysporum* f. sp. *radicis-lycopersici* distributed at Europe and America, analyzed the DNA polymorphism within the same group and traced the relatedness among VCG 0090~0099 as well as dissemination path of pathogen from America to Europe based on the analyzed DNA polymorphism, then recently verified the changing procedure of major VCG group from 0094 to 0098 in pathogen complex in Florida.

Researches on mutual interrelation between the VCG of *F. oxysporum* f. sp. *radicis-lycopersici* and pathogenicity test were carried out and known to be that characteristics of pathogens -such as the size of colony, capability of creation of antibiotics, pathogenicity, and isozyme pattern- belong to

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the same VCG are the same. Kistler et al. (1998) stated that *F. oxysporum* contained over 131 VCG in 22 formae speciales, and VCG of *F. oxysporum* f. sp. *radicis-lycopersici* contained 10 groups from 0090 through 0099 and two to five subgroups. Meanwhile, Katan et al. (1999) and Rosewich et al. (1999) reported that the VCG 0094 is dominant group in worldwide currently. In Japan, which exports the most varieties of tomato to Korea, the symptom of the crown and root rot was reported at 1969 (Sato and Araki, 1974) and Yamamoto et al. (1974) denominated as new race "J3" which was different as the *F. oxysporum* f. sp. *lycopersici*.

In Korea, however, VCG group of *F. oxysporum* f. sp. *radicis-lycopersici* was not yet defined, and there were little genetic and microbiological researches on the fungus. Thus, this research carried out to offer fundamental data for establishment of disease control plan by comparing the VCG of Korean isolates, which damaged tomato in Korea, with foreign isolates.

Table 1. Isolates of Korean *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *lycopersici* used in this study

Isolate	Forma specialis	Origin	VCGs
TF - 447	FORL	Dalsong, Daegu	0094
TF - 411	FORL	Dalsong, Daegu	0094
TF - 409	FORL	Dalsong, Daegu	0094
TF - 404	FORL	Dalsong, Daegu	0094
TF - 355	FORL	Dalsong, Daegu	0094
TF - 459	FORL	Angang, Kyongbuk	0094
TF - 456	FORL	Angang, Kyongbuk	0094
TF - 352	FORL	Angang, Kyongbuk	0094
TF - 417	FORL	Angang, Kyongbuk	0094
TF - 415	FORL	Angang, Kyongbuk	0094
TF - 510	FORL	Damyang, Chonnam	0094
DY - 03	FORL	Damyang, Chonnam	0094
TF - 391	FORL	Damyang, Chonnam	0094
TF - 472	FORL	Damyang, Chonnam	– [a]
TF - 471	FOL	Damyang, Chonnam	–
TF - 388	FORL	Bosung, Chonnam	–
TF - 551	FORL	Jangsong, Chonnam	0094
TF - 495	FORL	Iksan, Chonbuk	0094
TF - 397	FORL	Iksan, Chonbuk	0094
TF - 394	FORL	Iksan, Chonbuk	0094
IS - 11	FORL	Iksan, Chonbuk	0094
PY - 01	FORL	Puyo, Chungnam	0094
TF - 346	FORL	Puyo, Chungnam	0094
PY - 07	FORL	Puyo, Chungnam	0094
TF - 444	FORL	Chongwan, Chungbuk	0094
TF - 445	FORL	Chongwan, Chungbuk	0094
TF - 438	FORL	Chongju, Chungbuk	0094

[a] not belonged to VCG 0094.

Materials and Methods

Isolates. Isolates of *Fusarium oxysporum* f. sp. *lycopersici*, which was preserved at plant pathology lab., Chungnam National University were used as Korean isolates and some of them were obtained from Higeshi Nippon Gakuen University in Japan and University of Florida in USA. Also, isolates of *F. oxysporum* f. sp. *radicis-lycopersici* were prepared from Buyeo Tomato Experiment Station, Agricultural Research and Extention Services of Chungnam province as domestic isolates and standard isolates were prepared from Department of Plant Pathology, Institute of plant protection, Ministry of Agriculture in Israel (Table 1 and 2).

Pathogenicity test. The pathogenicity and race determination of Korean *Fusarium oxysporum* f. sp. *radicis-lycopersici* were carried out using differential tomato varieties such as Ponderosa, Okitsu 3, Walter and Zuiken (Yamamoto et al., 1974).

The inoculum was inoculated in PD broth of 100 ml at

Table 2. Vegetative compatibility group and phenotype of *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *lycopersici* isolates from foreign contries used in this study

Isolate	Formae speciales	Origin	VCGs	Phenotype
I - 1	FORL[a]	Belgium	0094-	WT
I - 2	FORL	Belgium	0094-	WT
I - 3	FORL	Belgium	0094-	*nit* M
I - 4	FORL	Belgium	0094-	*nit* M
I - 5	FORL	United Kingdom	0094-	WT
I - 6	FORL	United Kingdom	0094-	*nit* M
I - 7	FORL	United Kingdom	0094-	*nit* M
I - 8	FORL	United Kingdom	0094-	WT
I - 9	FORL	United Kingdom	0094-	*nit* M
I - 10	FORL	United Kingdom	0094-	*nit* M
I - 11	FORL	USA	0094-	WT
I - 12	FORL	USA	0094-	WT
I - 13	FORL	USA	0094-	*nit* M
I - 14	FORL	USA	0094-	*nit* M
I - 15	FORL	Israel	0094-Uni.	*nit* M
I - 16	FORL	Israel	0094-Uni.	*nit* M
AFL 548	FOL[b]	USA	0030	WT[c]
AFL 7400-1	FOL	USA	0030	WT[c]
IFo 31213	FOL	Japan	0031	WT[c]
Tomato V	FOL	Japan	0031	WT[c]
AFL 8174	FOL	USA	0032	WT[c]
JFL No. 1	FOL	Japan	0033	WT[c]

[a] *Fusarium oxysporum* f. sp. *radicis-lycopersici*.
[b] *Fusarium oxysporum* f. sp. *lycopersici*.
[c] reversed wild-type.

Table 3. Pathogenicity of *Fusarium oxysporum* isolates were obtained from wilted tomato plants in greenhouse on four differential tomato varieties

Isolate	Variety				Formae speciales
	Ponderosa	Okitsu 3	Walter	Zuiken	
TF447	3.9	4.0	3.9	0	FORL[a]
TF411	4.0	4.0	4.0	0.3	FORL
TF409	3.7	3.3	3.7	0.3	FORL
TF404	2.2	2.5	2.5	0.1	FORL
TF355	2.5	1.1	2.8	1.1	FORL-1
TF459	3.3	3.6	2.3	2.5	FORL-1
TF456	3.3	1.8	3.6	0	FORL
TF352	4.0	4.0	4.0	0	FORL
TF417	2.1	3.3	3.4	2.3	FORL-1
TF415	4.0	4.0	3.5	3.4	FORL-1
TF510	1.7	2.0	2.1	2.2	FORL-1
DY-03	–[c]	–	–	–	FORL
TF391	3.0	3.2	3.0	0	FORL
TF472	1.3	1.5	1.7	1.4	FORL-1
TF471	1.4	2.6	1.4	–	FOL-2[b]
TF388	1.1	1.0	1.5	0.1	FORL
TF551	–	–	–	–	FORL
TF495	3.9	2.6	2.7	0.8	FORL
TF397	4.0	3.5	2.7	0.5	FORL
TF394	2.9	2.4	2.8	0.2	FORL
IS-11	2.4	2.4	2.2	2.1	FORL-1
PY-01	2.2	2.5	2.4	0.3	FORL
TF346	2.2	2.2	2.4	0.5	FORL
PY-07	2.5	2.7	2.8	0.4	FORL
TF444	4.0	4.0	4.0	0	FORL
TF445	4.0	4.0	4.0	0	FORL
TF438	4.0	3.4	4.0	0	FORL

[a] *Fusarium oxysporum* f. sp. *radicis-lycopersici.*
[b] *Fusarium oxysporum* f. sp. *lycopersici* race 2.
[c] not tested.

the 300 ml triangular flask and, then carried out shaking incubation (120 rpm, 26°C, 72hr). Incubated media were filtered with double layers of gauze and centrifuged (5,000 g, 10 min) to obtain spores. The spores were washed with sterilized water and adjusted as 1×10^6 spores/cm. The control isolates used for this test were four isolates of FOL-1 (race-1), FOL-841D (race 2), FORL-C85A, and FORL-809P obtained from Israel and three isolates of #38 (race 1), #82 (FORL), and #153 (FORL) obtained from Canada. Seedlings of tomato were cultured for 30 days at vinyl pot (9×12 cm) filled with horticultural top soil, then used as inoculated seedlings. The roots of seeding was cleaned with water and soaked with conidial suspension (1×10^6 spores/ml) for one hour after cut upper part of roots. Then the seedlings were transplanted at the 9 cm of vinyl pot filled sterilized soil (peatmoss : perlite : vermiculite = 1 : 1 : 1, 120°C, 2hr). Sterilized water and PD broth were used for the control. Treated pots were stored at indoor with constant temperature and disease development was investigated after two weeks.

Vegetative compatibility. Selection of Nitrate nonutilizing (*nit*) mutant was followed as Correll et al. (1987) and Puhalla (1985) methods. Inducement of *nit* mutant was selected using chlorate minimal medium (CMM) containing 1.5-2.8% $KClO_3$ as a mutagen. The phenotype of *nit* mutant obtained from test isolates was determined by growing type of colony based on consumption rate of nitrogen on the cultural media which contained three different nitrogen nutrients [(1) nitrate medium = basic media [sucrose 30 g, KH_2PO_4 1g, $MgSO_4\cdot7H_2O$ 0.5 g, trace element solution (citric acid 5 g, $Fe(NH_4)_2(SO_4)\cdot6H_2O$ 0.25 g, $MnSO_4\cdot H_2O$ 0.05 g, $NaMoO_4\cdot2H_2O$ 0.05 g, distilled water 95 ml) 0.2 ml, agar 20 g, distilled water 1 l]+$NaNO_3$ 2 g/l (=minimal medium), (2) nitrite medium = basic cultural media +$NaNO_2$ 0.5 g/l, (3) hypoxanthine medium = basic cultural media +hypoxanthine 0.2 g/l§] such as the method of Correll et al. (1987).

The separated *nit*-mutants were opposite cultured for four to thirty days at 25°C. Complementary *nit*-mutants were formed aerial mycelia like a wild-type by forming the heterokaryon at contact parts of isolates. At this time, isolates which formed heterokaryon very well were selected and determined as a *nit* tester, and these were opposite cultured on the minimum cultural media for four to twenty days with a distance of 2-3 cm in each other. The vegetative compatibility was determined with presence of formulation of heterokaryon between testers.

Results and Discussion

***nit*-mutants acquisiton.** Vegetative compatibility of Korean *Fusarium oxysporum* f. sp. *radicis-lycopersici* isolates was analyzed and regional distribution of their VCG was defined, and genetic characteristics of these isolates were compared and analyzed with other isolates of VCG 0094 obtained from Europe, Israel, and Florida in the USA

$KClO_3$ showed toxic against *F. oxysporum* according to restoration by the nitrate reductase. At this time, the apex hyphae separated from the chlorate resistant sector which was created from chlorate cultural media could not form aerial mycelia after transfer the apex hyphae to the minimum culture medium. The *nit*-mutant was separated from these since this chlorate resistant strain could not restore the nitrite from nitrate. Korean isolate could not obtain thin growth mycelia since wild-type mycelia were grown in clusters at 1.5% CMM which was contrast to

Table 4. Phenotypes of *nit* mutants recoverd from *Fusarium oxysporum* f. sp. *radicis-lycopersici*

Number of isolates	Phenotypes of *nit* mutants			Total
	nit 1	*nit* 3	*nit* M	
27	692	53	349	1094

results of Katan who selected *nit*-mutant from CMM (chlorate minimal medium) added $KClO_3$ 1.5% (Katan et al., 1991). Thus, *nit*-mutants were selected by adjustment of the $KClO_3$ addition concentration as 1.5, 1.8, 2.0, 2.2, 2.5, and 2.8%. Results from that, the mycelial growth at 2.8% was severely almost restrained, and thin growth mycelium was obtained at 1.8-2.5% CMM. Also, when we selected the *nit*-mutants with different chlorate of more two concentrations, three types (*nit*-1, *nit*-3, *nit*-M) of phenotype of *nit*-mutants were easily obtained. Thus, for the selection of *nit*-mutants of *F. oxysporum* f. sp. *radicis-lycopersici*, the chlorate concentration of CMM would be more than two concentrations among 1.8-2.5% for the future studies.

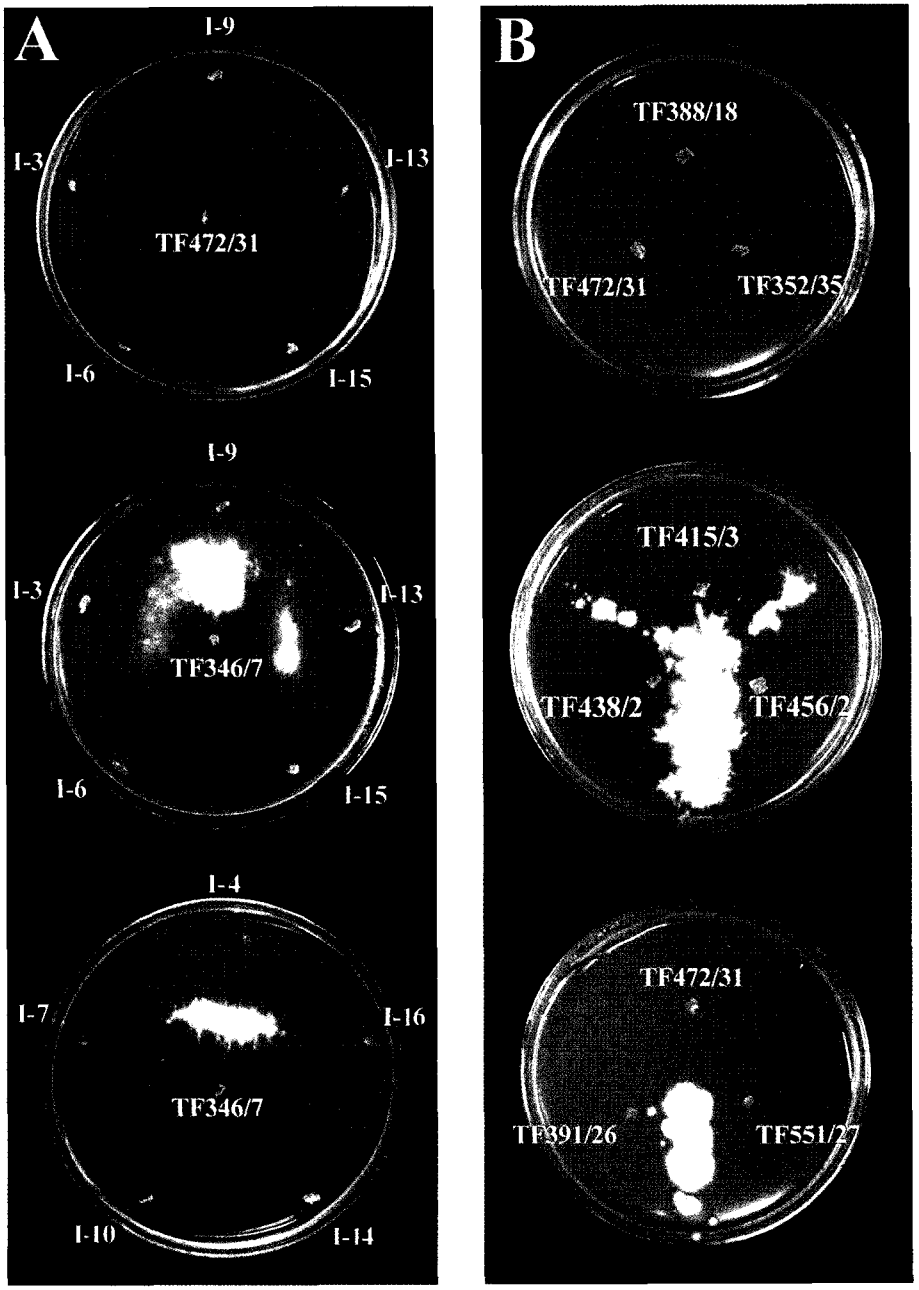

Fig. 1. Heterokaryon formation among *nit* mutants of *Fusarium oxysporum* f. sp. *radicis-lycopersici* on the minimal medium. A : between foreign VCG 0094 and Korean *nit* mutants, B : among Korean *nit* mutants.

Vegetative compatibility. The phenotype of selected *nit* mutants was determined based on the three different nitrogen utilization (Table 4 and Fig. 1). Based on the determined *nit* mutant, *nit* M and *nit* 1, which was known as the best phenotype forming heterokaryon, were selected as *nit* tester and investigated VCG in each isolate using the *nit* testers.

From 27 isolates of the tomato crown and root rot pathogen obtained from Buyeo Tomato Experiment Station, 1,094 *nit*-mutants were selected. Then, heterokaryon formation presence was investigated with each tester (Table 5).

Results from that the total of 24 isolates among 27 isolates belong to VCG 0094, and rest of the three isolates including one *F. oxysporum* f. sp. *lycopersici* (TF471) was non-compatible with VCG 0094 and incompatibility was investigated among these three isolates (Table 5). Katan et al. (1991) classified VCG 0094 isolates, which were different separation region as subgroup according to formation speed of heterokaryon and reported that there was weak compatibility or no compatibility among different subgroup. Also, he investigated bridge strains, which had compatibility on both VCG 0090 and 0092 group and reported that these groups were treated as the same subgroup.

And, even though the compatibility of TF472 and TF388, which were incompatible with VCG 0094 of *F. oxysporum* f. sp. *radicis-lycopersici*, their compatibility was not investigated since this research did not have different VCG

Table 5. Formation of complementary heterokaryons between *nit* mutants of foreign isolates of *Fusarium oxysporum* f. sp. *radicis-lycopersici* and Korean isolates

Isolate/ mutant No.		*nit* M testers									
		VCG 0094-I		VCG 0094-II		VCG 0094-III		VCG 0094-IV		VCG 0094-Uni.	
		I-3	I-4	I-6	I-7	I-9	I-10	I-13	I-14	I-15	I-16
TF-447	/ 13	++[b]	++	+[c]	+	++	+	+	++	+++[a]	+
TF-411	/ 2	+	+	+	+	+	+	++	+	+	+
TF-409	/ 9	++	++	+	+	+	++	+	++	+	+
TF-404	/ 8	+	++	+	+	+	+	+	+	+	+
TF-355	/ 9	+++	++	+	+	++	+	++	+	++	+
TF-459	/ 26	+++	++	+	+	+	+	++	++	+++	++
TF-456	/ 2	++	++	++	++	++	+	+	+	++	+
TF-352	/ 35	+++	++	+	+	+	+	++	+	++	+
TF-417	/ 3	+++	++	++	++	++	+	++	+	++	+
TF-415	/ 3	+	+	+	+	+++	+++	+	+	++	+
TF-510	/ 35	+++	++	+	+	+	+	++	+	++	+
DY-03	/ 2	+	+	+	+	+	+	++	+	++	+
TF-391	/ 26	++	+	+	+	+	+	++	+	++	+
TF-472	/ 31	−[d]	−	−	−	−	−	−	−	−	−
TF-471	/ 5	−	−	−	−	−	−	−	−	−	−
TF-388	/ 18	−	−	−	−	−	−	−	−	−	−
TF-551	/ 27	++	+++	+	+	+	+	+++	++	+++	++
TF-495	/ 49	+	+	+	+	+++	*	++	++	++	+
TF-397	/ 18	+	+	+	+	+	+	+	+++	+	++
TF-394	/ 4	+	+	++	++	+	+	++	++	+	+
IS-11	/ 12	+	+++	++	+++	++	+	+	++	+	++
PY-01	/ 9	++	+++	+	+	+	+	+++	++	+++	+
TF-346	/ 7	++	+	+	+	+++	++	++	+	+	+
PY- 07	/ 3	++	+	+	+++	++	++	+	+	+	+
TF-444	/ 11	+	+	+	+	+	+	+	++	++	+
TF-445	/ 24	+	+++	+	+	+	+	+	+	++	++
TF-438	/ 2	++	+++	+	++	++	+	+	++	+++	+

[a] wild-type growth after 4-7 days.
[b] weakly wild-type growth after 8-14 days.
[c] weak and slow wild-type growth after 14-24 days.
[d] no wild-type growth after 25 days.

tester. By considering the fact, however, that the tomato culture was vitalized from early 1990's and *F. oxysporum* f. sp. *cubense* contained the total of 21 VCG (Kistler et al., 1998), TF472 and TF388, different from new vegetative compatible groups or self incompatible isolates, were considered to be a single-member VCG, which had compatibility among *nit*-mutants originated from the same isolates but incompatible with other vegetative compatible groups.

Meanwhile, by viewing the distribution aspect, the subgroup of 0090 was mostly separated in Israel and subgroup of 0094 originated from Netherlands (Katan et al., 1991). From this fact that the subgroup was considered to be had genetic mutation, a new isolate different from the original vegetative compatible group may be appeared, by the dissemination as well as adaptation of one vegetative compatible group and went through a long settlement period to other different environment regions.

The Korean isolates, different from European isolates, had four subgroups of VCG 0094, and especially, isolate of TF551, IS-11, and PY-01 had "Universal" characteristics. The "Universal" isolates had compatibility with more than two subgroups and this kind characteristic appeared on isolate of Florida, USA and Israel. All Korean isolates showed compatibility after opposite culture with each late compatible VCG tester after more than two weeks. Thus, the Korean isolates showed similar genetic characteristics with Florida or Israel isolates rather than European isolates.

Consequently, VCG 0094 dominated in domestically distributed *F. oxysporum* f. sp. *radicis- lycopersici* in Korea, and Korean isolates had universal characteristics such as isolate in Florida, USA and Israel rather than isolates in Europe, and are considered that domestic isolates had close genetic relationship with those isolates.

By taking into considering the fact that most Korean tomato seeds were imported from Japan, the incoming period of *F. oxysporum* f. sp. *radicis-lycopersici* was considered to be the same as the outbreak period of disease in Japan. Mes et al. (1999) and Jurriaan et al. (1999a, b) selected a new variety, which had resistance against race 1 and 2 of *F. oxysporum* f. sp. *lycopersici* by injecting radioactivity and causing artificial mutation on *F. oxysporum* f. sp. *lycopersici*, and investigated the association of genetic mutation between plants and pathogen by inducing pathogens can infect resistant varieties of tomato. These results showed the possibility that the resistant plants against *F. oxysporum* f. sp. *radicis-lycopersici* could be changed to sensitivity. Also, results from the pathogenicity test of Korean isolates, showed that resistant variety, Seogeon was infected by the fungus (Kim, 2000). Thus, appearance of *F. oxysporum* f. sp. *radicis-lycopersici*, which had new pathogenicity at specific area could not ignored.

Rowe (1980) and Menzies et al. (1990) reported that *F. oxysporum* f. sp. *radicis-lycopersici* had pathogenicity on Solanaceae, Leguminosae, Cucurbitaceae, and Chenopodiaceae in addition to *Lycopersicon* spp. The analysis of physiological and pathological characteristics including pathogenicity of isolate of *F. oxysporum* f. sp. *radicis-lycopersici* in Florida had high possibility to apply on domestic isolates since the isolates belong to the same VCG group have the same pathogenicity.

From these reasons, continuous investigations of pathogenicity variation containing VCG of Korean and foreign isolates and analysis of physiological, ecological, and genetic characteristic, considered to be used as useful data for prediction of damage and establishment of proper control plans.

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