Determination of the Taxonomic Status of Tomato Crown and Root Rot Isolates in *Fusarium oxysporum* by DNA Homology*

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Abstract

The taxonomic status of Japanese tomato crown and root rot isolates ascribed to *Fusarium oxysporum* f. sp. *lycopersici* race J3 was determined on the basis of DNA-DNA reassociation kinetics. As compared with DNA homology among races of forma specialis *lycopersici*, isolates of race J3 showed relatively low homology (74-88%) with isolates of other races which caused typical vascular wilts of tomato, and shared high DNA homology (96-99%) within the race. By contrast, isolates of race J3 showed high DNA homology (95-100%) with an isolate of *F. oxysporum* f. sp. *radicis-lycopersici*, but exhibited low homology (69-87%) with isolates of other formae speciales of *F. oxysporum*. These results suggest that the causal fungi of tomato crown and root rot in Japan, previously proposed as race J3 of forma specialis *lycopersici*, should be designated as a different forma specialis: *F. oxysporum* f. sp. *radicis-lycopersici* (Jarvis and Shoemaker, 1978).

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Key words: tomato crown and root rot disease, *Fusarium oxysporum*, *Fusarium oxysporum* f. sp. *radicis-lycopersici*, molecular taxonomy, DNA relatedness.

INTRODUCTION

A crown and root rot of tomatoes caused by *Fusarium oxysporum* Schlecht. was first discovered in southern Japan in 1967³⁸⁾. Since then the disease has become a serious problem in the greenhouse tomato production, having been reported from numerous tomato-growing areas in Japan^{16,17,32)}. An identical disease also has been reported from U.S.A.^{22,27,30,31,35)}, Canada¹³⁾, Greece²⁴⁾, Israel¹⁸⁾ and Europe⁶⁾.

The designation for subspecific taxon of tomato crown and root rot isolates has not been used uniformly throughout the literature. Japanese researchers^{32,38)} designated a new race, J3, of *F. oxysporum* f. sp. *lycopersici*, based on its pathogenicity to the tomato differential variety Walter which is resistant to races J1 and J2 so far known in Japan. Races J1 and J2 are equivalent to races 1 and 2, respectively of U.S. and European workers, or to races 0 and 1 in the nomenclature proposed by Gabe¹⁰⁾. On the other hand, Jarvis and Shoemaker¹⁴⁾ have proposed a new forma specialis *radicis-lycopersici* for the fungus, because the disease symptoms it causes are clearly distinct from those caused by *F. oxysporum* f. sp. *lycopersici*.

Recently, a new *Fusarium* disease with typical vascular wilt symptoms on tomato cultivars resistant to race 2 has been reported from Australia and U.S.A., and a third race of *F. oxysporum* f. sp. *lycopersici* was assigned to the causal agent^{11,37)}. As a result, confusion has arisen in the

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subspecific classification of tomato crown and root rot isolates as race series of forma specialis *lycopersici*.

New methods in molecular biology now make possible the assessment of the subspecific classification in *F. oxysporum* at the molecular level^{8,15,25}. In a previous study²⁰, we suggested that the reassociation analysis of DNA may be useful in studies involving classification of formae speciales in *F. oxysporum*.

The purpose of this study was to determine genetical relatedness among races of forma specialis *lycopersici* and forma specialis *radicis-lycopersici* of *F. oxysporum* on the basis of DNA-DNA reassociation kinetics, as well as to elucidate the taxonomic status for the causal organism of tomato crown and root rot disease in Japan. A partial account of this work has previously been reported²¹.

MATERIALS AND METHODS

Fungal strain. Twenty-two isolates of *F. oxysporum* f. sp. *lycopersici* and nine isolates from other formae speciales of this species used in this study are listed in Table 1. The assignment of Japanese races (race J1, J2 and J3) of forma specialis *lycopersici* was made on the basis of Japanese differential system by the researchers who supplied the isolates. All isolates of race J3 used were collected from crown and root rot-affected tomato plants. The isolates of U.S.A. races (race 1, 2 and 3) of forma specialis *lycopersici* were provided from Dr. Jones, University of Florida. The isolate (CC#85) of forma specialis *radicis-lycopersici* was supplied by Dr. Jarvis, Agriculture Canada Research Station, Harrow. All isolates were grown in potato dextrose broth for 7 or 8 days at 28°C. The mycelia of each isolate were washed with distilled water, then filtered through a filter paper, frozen, and lyophilized.

Isolation of DNA. Extraction of fungal DNA was carried out according to a modified method¹⁹ of Britten *et al.*⁷ DNA purity was tested on the basis of absorbance ratio and DNA hyperchromicity. Analysis in CsCl/bisbenzimidazole density gradient centrifugation showed each DNA to contain less than 3.4% mitochondrial DNA.

DNA-DNA reassociation kinetics. The extent of DNA reassociation was determined spectrophotometrically, essentially based on the method reported by Seidler and Mandel³³ and Seidler *et al.*³⁴, as described previously¹⁹. DNA samples were sheared on ice by sonic treatment (Bronson Sonifier, Model B-12). Sheared DNA fragments had a molecular weight of approximately 5.5×10^5 daltons. Sheared DNA samples (75–85 µg/ml) were dissolved in $5 \times \text{SSC}$ (SSC: 150 mM NaCl, 15 mM sodium citrate, pH 7.0) containing 20% dimethylsulfoxide to promote reassociation. To determine relative homology of DNA unique sequences, a major portion of repetitive sequences was removed from the total DNA sample according to the technique described previously²⁰. DNA homology values were calculated using equation proposed by Seidler and Mandel³³.

RESULTS

Genetic relatedness between races of F. oxysporum f. sp. lycopersici

Percent homology values for hybridization reactions among races of forma specialis *lycopersici* are summarized in Table 2. All the isolates of race J3 showed relatively low homology values (74.4% to 88.9%) with isolates of other races (J1, J2, 1, 2 and 3). Isolates of Japanese races, J1 and J2, hybridized with isolates of American races, 1, 2 and 3 at consistently high rates (above 96.1%).

Genetic relatedness between races of F. oxysporum f. sp. lycopersici and other formae speciales of F. oxysporum

DNA relatedness between races of forma specialis *lycopersici* and nine other formae speciales

Table 1. List of isolates of *Fusarium oxysporum* used in this study

Isolate		Source ^{a)}
<i>f. sp. lycopersici</i>		
race J1	SUF 119	Shinshu Univ. (T. Matuo)
	Tomato V	Vegetable and Ornamental Crops Res. Sta.
	Tomato H	Higashi Nippon Gakuen Univ. (S. Kuninaga)
race J2	SUF 803	Shinshu Univ. (T. Matuo)
	IFO 31213	IFO, Shizuoka Agr. Expt. Sta. (T. Suzui; F3-1)
	F-1	Kochi Pref. Inst. Agr. Fores. Sci. (T. Kobayashi)
	F-8	"
race J3	FOly	Vegetable and Ornamental Crops Res. Sta.
	Mie	National Agr. Res. Ctr. (H. Hagiwara)
	KEF-2R1	Kanagawa Horticultural Expt. Sta. (N. Kita)
	KEF-2R4	"
	Y31	"
	Y11a	"
	h2	"
	F212	Kochi Pref. Inst. Agr. Fores. Sci. (T. Kobayashi)
	F213	"
	AFL 626	Univ. of Florida (J.P. Jones)
race 1	AFL 7388	"
race 2	AFL 548	"
	AFL 7399	"
race 3	AFL 5397	"
	AFL 8174	"
<i>f. sp. radialis-lycopersici</i>		
	CC#85	Agr. Canada Res. Sta. (W.R. Jarvis)
<i>f. sp. melongenae</i>		
	IFO 9968	IFO, Shinshu Univ. (T. Matuo)
<i>f. sp. nicotianae</i>		
	IFO 6386	IFO, Tottori Univ. (M. Nishimura)
<i>f. sp. melonis</i>		
	F.O.-1	Shizuoka Agr. Exp. St.
<i>f. sp. raphani</i>		
	R 772121	National Agr. Res. Ctr. (H. Hagiwara)
<i>f. sp. phaseoli</i>		
	IFO 9970	IFO, Shinshu Univ. (T. Matuo)
<i>f. sp. gladioli</i>		
	IFO 7166	IFO, Kobe Univ. (W. Yamamoto)
<i>f. sp. asparagi</i>		
	IFO 31382	IFO, NIAES (T. Suzui)
<i>f. sp. spinaciae</i>		
	F 58	Gifu Univ. (T. Naiki)

a) IFO : Institute for Fermentation, Osaka.

NIAES: National Institute of Agro-Environmental Sciences.

of this species was compared. As shown in Table 3, race J3 isolate (FOly) hybridized at perfect rates (97.7–99.8%) with isolate (CC#85) of forma specialis *radialis-lycopersici*, but at low rates (69.2–87.4%) with isolates of other formae speciales. Two isolates (SUF 119 and SUF 803) from race J1 and J2 showed a low relatedness (68.1–88.2%) with isolates from forma specialis *radialis-lycopersici* and other formae speciales.

Genetic relatedness between race J3 of *F. oxysporum f. sp. lycopersici* and *F. oxysporum f. sp. radialis-lycopersici*

DNA relatedness between race J3 of forma specialis *lycopersici* and forma specialis *radialis-*

Table 2. Genetic relatedness between races of *Fusarium oxysporum* f. sp. *lycopersici*

Race of f. sp. <i>lycopersici</i>	race J1 (SUF 119)	race 1 (AFL 626)	race J2 (SUF 803)	race 2 (AFL 548)	race J3 (FOly)	race 3 (AFL 5397)
race J1						
Tomato V	98.3 ^{a)}	96.7	97.9	98.2	84.2	97.9
Tomato H	96.1	N.D. ^{b)}	98.3	N.D.	79.3	98.9
race 1						
AFL 7388	97.6	98.2	98.7	98.9	86.8	99.1
race J2						
IFO 31213	99.1	96.8	98.2	97.3	88.2	97.8
F-1	97.9	98.2	98.9	99.1	82.9	98.6
F-8	N.D.	98.7	N.D.	N.D.	79.4	N.D.
race 2						
AFL 7399	98.2	N.D.	99.3	N.D.	74.9	99.0
race J3						
Mie	86.3	81.9	78.9	76.2	98.7	76.9
KEF-2R1	84.7	82.6	79.5	80.1	99.2	78.3
F212	86.3	88.9	74.4	81.6	99.3	79.9
race 3						
AFL 8174	99.3	N.D.	98.4	N.D.	79.3	98.8

a) Homology values (%) are average of two determinations, and were calculated from the equation described by Seidler and Mandel³³⁾.

b) Not determined.

Table 3. Genetic relatedness between race of *Fusarium oxysporum* f. sp. *lycopersici* and other formae speciales of *Fusarium oxysporum*

Forma specialis	race J1 (SUF 119)	<i>lycopersici</i> race J2 (SUF 803)	race J3 (FOly)
<i>radicis-lycopersici</i> (CC#85)	86.3–88.2 ^{a)}	78.2–85.3	97.7–99.8
<i>melongenae</i> (IFO 9963)	80.3–82.8	79.2–84.1	81.8–87.4
<i>nicotianae</i> (IFO 6386)	84.7–86.0	80.4–83.6	82.8–86.2
<i>melonis</i> (F.O.-1)	68.4–70.4	73.8–76.2	71.8–73.1
<i>raphani</i> (R772121)	73.5–76.9	68.1–70.0	71.9–75.2
<i>phaseoli</i> (IFO 9970)	76.6–80.1	72.8–75.6	75.7–80.3
<i>gladioli</i> (IFO 7166)	75.6–77.6	71.8–73.8	69.2–72.2
<i>asparagi</i> (IFO 31382)	73.1–75.5	78.5–80.4	72.2–76.9
<i>spinaciae</i> (F 58)	68.4–70.8	70.3–74.0	74.1–76.3

a) The range of homology values (%) is the results of two determinations and was calculated from the equation described by Seidler and Mandel³³⁾.

lycopersici was further determined. All the isolates of race J3 examined hybridized at consistently high rates (95.3–100%) with isolate CC#85 of forma specialis *radicis-lycopersici*, as shown in Table 4.

Table 4. Genetic relatedness between race J3 of *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium oxysporum* f. sp. *radicis-lycopersici*

Race J3 of f. sp. <i>lycopersici</i>	f. sp. <i>radicis-lycopersici</i> (CC#85)
Mie	96.8–99.6 ^{a)}
KEF-2R1	98.9–100
KEF-2R4	97.3–98.1
F212	98.3–99.7
F213	96.1–98.7
Y31	96.3–97.8
Y11a	98.3–99.0
h2	95.3–96.8

a) See footnote in Table 3.

Table 5. Relationships between DNA homology and subspecific taxa of *Fusarium oxysporum*

Spectroscopic method of Kuninaga <i>et al.</i> (1989) ²⁰⁾ (% homology)	Taxonomic statement
95–100	The same forma specialis or a race within the same forma specialis
85–94	Closely related but different forma specialis
60–84	Different forma specialis
0–59	Different species

DISCUSSION

Although most pathogenic forms of *Fusarium oxysporum* show a high degree of host specificity, some forms are pathogenic to a wide range of host plants either of the same family^{1,3)} or across many families^{2,4)}. Identifying formae speciales and physiological races of this species solely from pathogenicity tests, therefore, is difficult and often inconclusive. Disagreement on classification exists in the literature as to whether strains of *F. oxysporum* causing tomato crown and root rot disease be categorized as a new forma specialis^{14,29)} or as a physiological race within forma specialis *lycopersici*^{22,31,32,38)}.

We showed in a previous paper²⁰⁾ that the DNA reassociation technique used in this study provided a reliable criterion to delimit subspecific taxa in *F. oxysporum*. In Table 5, we suggest how DNA homology values obtained with the method should be interpreted.

The present investigations revealed that the Japanese race J3 of *F. oxysporum* f. sp. *lycopersici* share only 74–88% DNA homology with other races, including American races, of this forma specialis. Judging from the criteria shown in Table 5, it is suggested that the causal fungus of tomato crown and root rot should be classified into a single forma specialis distinct from forma specialis *lycopersici*.

The data presented here demonstrated further that all the tomato crown and root rot pathogens examined are genetically identical with strain of *F. oxysporum* f. sp. *radicis-lycopersici* and are distinct from other formae speciales in this species. These results coincide with current views^{9,28)} that tomato crown and root rot isolates has been assigned to its own single-member vegetative compatibility group in Puhalla's²⁸⁾ classification of strains of *F. oxysporum*.

Symptoms of *Fusarium* crown and root rot disease are quite unlike those caused by races of *F. oxysporum* f. sp. *lycopersici*^{13,27,29,32,35,38)}. The disease is also favored by cool soil temperature, in contrast to races of forma specialis *lycopersici*^{13,32,38)}. In view of these differences, Jarvis and Shoemaker^{12,14)} have proposed that *Fusarium* crown and root rot pathogen be designated new forma specialis *radicis-lycopersici*, based on its mode of pathogenesis, its tempera-

ture relations, and its close association with *Lycopersicon* spp. Rowe²⁹⁾ has supported Jarvis and Shoemaker's designation despite his observations that tomato crown and root rot isolates are pathogenic, on inoculation, to host plants outside the Solanaceae. Our results presented here certainly demonstrate the validity of subspecific classification of this pathogen as a new single forma specialis to distinguish it from the organisms causing typical *Fusarium* wilt. Accordingly, we propose that tomato crown and root rot isolates found in Japan should be referred to as *F. oxysporum* f. sp. *radicis-lycopersici*.

It is recently reported that two formae speciales, *radicis-lycopersici* and *lycopersici* are quite different with respect to protein composition⁵⁾, antibiotic tolerance²³⁾ and complement of fatty acids²³⁾.

The present study indicated that tomato crown and root rot isolate (FOly) showed comparatively high homology in DNA sequence with strains of formae speciales *lycopersici*, *melongenae* and *nicotianae* pathogenic to host plants from the Solanaceae. In a previous paper²⁰⁾, similar results were also observed between *Fusarium* strains from the Cruciferae or the Cucurbitaceae. These results suggest that formae speciales in *F. oxysporum* parasitizing host plants within the same botanical family may show phylogenetically close relationships with each other.

Japanese races in *F. oxysporum* f. sp. *lycopersici* are described by the designation of J1 or J2, distinct from that in U.S.A., because a set of tomato differential cultivars or lines used for identification of races is different in both countries. Since race J2 was found to be pathogenic to tomato cultivars having the *Lycopersicon pimpinellifolium* Accession 160 factor (I-factor) for resistance to race J1³⁶⁾, it seems that races J1 and J2 correspond to races 1 and 2, respectively. A correlation between these races from both countries is not, however, examined closely. This study showed these races, including new race 3 recently reported, to be closely homogeneous. It is reported that each of races (1 and 2) can be distinguished serologically²⁶⁾. Further investigations must be undertaken to explore alternative or complementary methods to expedite race classification of this forma specialis.

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和 文 摘 要

国永史朗・横沢菱三：DNA-DNA 再会合反応速度解析によるトマト根腐れ萎ちょう病菌の分類的位置の検討

トマト根腐れ萎ちょう病の病原菌は、わが国では *Fusarium oxysporum* f. sp. *lycopersici* の race J3 とされてきたが、諸外国では、別の分化型である *radicis-lycopersici* に分類されている。この分類学的な不一致を解決する目的で、DNA-DNA 再会合反応速度解析法を用いて、f. sp. *lycopersici* と f. sp. *radicis-lycopersici* 間の遺伝的関係を検討した。その結果、f. sp. *lycopersici* race J3 の菌株は本分化型の他のレース (J1, J2, 1, 2, 3) の菌株とは明らかに遺伝的に異なり (74~88% の DNA 相同性)、f. sp. *radicis-lycopersici* の菌株と遺伝的に一致する (96~99% の DNA 相同性) ことが確認された。以上のことから、わが国においてもトマト根腐れ萎ちょう病の病原菌は *Fusarium oxysporum* f. sp. *radicis-lycopersici* とすべきであると考えられた。