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FULL PAPER

Yoshiyuki Ebihara · Hideyuki Nagao · Seiji Uematsu
Jyoji Moriwaki · Etsuo Kimishima

First report of *Verticillium tricorpus* isolated from potato tubers in Japan

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Abstract In 1998, *Verticillium* sp. (CE98Vt1 and CE98Vt2) were isolated from discolored vascular structures of potato tubers sold at a market in Chiba Prefecture. These isolates were identified as *Verticillium tricorpus* on the basis of cultural and morphological characteristics and PCR diagnosis. This observed vascular discoloration of the potato tuber was demonstrated in three cultivars (Touya, Toyoshiro, and Waseshiro) among eight cultivars by inoculation to seedlings. External and internal symptoms of these isolates were not distinct in potato plants. The virulence of these isolates to potato was very low as compared with *Verticillium dahliae*. These two isolates were not pathogenic to Chinese cabbage, eggplant, green pepper, larkspur, parsley, snapdragon, soybean, tobacco, and tomato. This is the first report of *V. tricorpus* from potato in Japan.

Key words Potato tubers · Vascular discoloration · *Verticillium tricorpus*

Introduction

In 1998, vascular discolorations were found in potato (*Solanum tuberosum* L.) tubers (cultivar and place of production were unknown) at a market in Chiba Prefecture, Japan. Although these tubers did not exhibit any external symptoms, their vascular bundle showed discoloration and a brown ring just below the epidermis when cut in cross section.

Genus *Verticillium* is frequently isolated from discolored tissues of tubers. In Japan, *V. albo-atrum* Reinke & Berthold, *V. dahliae* Klebahn, and *V. nigrescens* Pethybridge were reported as pathogens of potato and induced similar symptoms (Saito et al. 1981; Iijima 1981; Kitazawa and Sato 1984). However, the potato isolates were different from *V. albo-atrum*, *V. dahliae*, and *V. nigrescens* in colony pigmentation and the types of resting structure. These new isolates showed yellow pigmentation on potato dextrose agar (PDA) at the beginning of incubation and produced three types of resting structures: chlamydospore, dark resting mycelia, and microsclerotia.

In this study, the morphology, cultural characteristics, pathogenicity, and polymerase chain reaction (PCR) diagnosis were investigated to identify these new isolates. Preliminary results were reported elsewhere (Ebihara et al. 1999).

Materials and methods

Isolates

The two potato isolates and *Verticillium* spp. examined are listed in Table 1.

Morphology

The morphology of phialides, conidia, and resting structures of CE98Vt1 and CE98Vt2 on PDA were compared with a reference isolate of *V. tricorpus* (IMI71799) and published descriptions of *Verticillium* spp.

Lengths of 50 randomly observed phialides were measured after 10–14 days incubation. Lengths and widths of 100 conidia were measured after 10–14 days incubation. Widths of 50 resting mycelia were measured after 21–28 days incubation. Lengths and widths of 50 microsclerotia and chlamydospores were measured after 21–28 days incubation.

Y. Ebihara (✉) · S. Uematsu
Chiba Prefectural Agriculture Research Center, Southern Horticulture Institute, 1762 Yamamoto, Tateyama, Chiba 294-0014, Japan
Tel. +81-470-22-2603, Fax +81-470-22-2604
e-mail: y.ebhr3@ma.pref.chiba.jp

H. Nagao
Agro-biological Science, Ibaraki, Japan

J. Moriwaki
National Agricultural Reserch Center, Niigata, Japan

E. Kimishima
Ministry of Agriculture, Forestry and Fisheries Japan, Yokohama Plant Protection Station, Kanagawa, Japan

Table 1. Origins of *Verticillium* spp.

Isolates	Species	Source	Locality	Provider[a]
Vaa	*V. albo-atrum*	Unknown	Japan, Tokyo	1
SVPI 94221	*V. albo-atrum*	Potato	Japan, Hokkaido	2
84034	*V. dahliae*	Eggplant	Japan, Mie	3
84011	*V. dahliae*	Chinese cabbage	Japan, Aichi	3
84023	*V. dahliae*	Eggplant	Japan, Nagano	3
86101	*V. dahliae*	*Chenopodium album*	Japan, Gunma	3
SVPI 94146	*V. dahliae*	Potato	Japan, Hokkaido	2
84010	*V. longisporum*	Chinese cabbage	Japan, Nagano	3
Vnc 2	*V. nigrescens*	Cosmos	Japan, Tokyo	1
SVPI 94078	*V. nigrescens*	Potato	Japan, Hokkaido	2
IMI 71799	*V. tricorpus*	Tomato	England, unknown	4
CE98Vt1	*V. tricorpus*	Potato tuber	Japan, unknown	–
CE98Vt2	*V. tricorpus*	Potato tuber	Japan, unknown	–

[a] 1, T. Iijima; 2, T. Komatsu; 3, H. Hagiwara; 4, Yokohama Plant Protection Station

CE98Vt1 and *V. albo-atrum* (Vaa) were inoculated to autoclaved tomato stem and incubated on water agar (WA) for 2 months; the bases of conidiophores were then observed.

Growth test

Mycelial growth of the three isolates (CE98Vt1, CE98Vt2, and IMI71799) was evaluated at 5.0° to 35.0°C at intervals of 2.5°C. A 4-mm plug was taken from the edge of an actively growing colony, transferred to a PDA dish, and incubated at each temperature. Cultures were incubated in the dark for 14 days, and the diameters of all colonies were then measured. There were two replicate plates per treatment, and each experiment was repeated twice.

Pathogenicity test to potato tuber

CE98Vt1 and CE98Vt2 were inoculated on wounded potato tubers (cv. Waseshiro). Potato tubers were hollowed out to the center with 4-mm cork borer, or the surface was scratched with a wire. Both isolates were incubated for 3–4 weeks; 1 cm^2 of PDA culture disk was then plugged into the center of the potato, in the case of hollowed tubers, or put on the scratched sites. In addition, moistened hydrophilous cotton was affixed over each inoculated site of the scratched tubers for 3–4 days. Inoculated tubers were kept in a moistened metal basin and placed at room temperature (15°–25°C) for 30 days. Four tubers were used for each treatment.

Pathogenicity test to seedlings

CE98Vt1, CE98Vt2, and each pathotype of *V. dahliae* and *V. longisporum* (C. Stark) Karapapa, Bainbr. & Heal were used (see Table 1). Host specifity and five pathotypes were reported in *V. dahliae* (Horiuchi et al. 1990). Isolates pathogenic to each tested plant were used in this study. Inocula of these isolates were prepared with incubating cultures on PDA in 90-mm petri dishes for 21–28 days at 25°C. Conidial suspensions of each isolate were prepared by adding 10 ml distilled water to each of the plates and scraping the cultures with chopsticks. The conidial density of each isolate was adjusted to 10^7 conidia/ml.

Pathogenicity tests were conducted on potato, eggplant (*Solanum melongena* L.), tomato (*Lycopersicon esculentum* Mill.), green pepper (*Capsicum annuum* L.), tobacco (*Nicotiana glutinosa* and *N. tabacum* L.), Chinese cabbage (*Brassica campestris* L.), soybean (*Glycine max* (L.) Merr.), parsley (*Petroselinum crispum* Nym.), snapdragon (*Antirrhinum majus* L.), and larkspur (*Consolida ambigua*).

The roots of 3- to 4-week-old seedlings were dipped in each inoculum (100 ml) for 30 min. Three to 11 seedlings of each host were inoculated per isolate. These inoculated seedlings were replanted in plastic pots (10 cm diameter) containing sterilized soil. For potatoes, unglazed pots were used instead of plastic pots. Remaining inocula were poured on the soil surface at a rate of 10 ml per plant. For comparison, each pathotype of *V. dahliae* and *V. longisporum* was inoculated to each host seedling. 84034 was inoculated to potato, eggplant, tobacco, parsley, snapdragon, and larkspur; 84011, 84023, 86101, and 84010 were inoculated to tomato, green pepper, soybean, and Chinese cabbage, respectively. The roots of uninoculated control seedlings were dipped in water and maintained in the same way as the inoculated plants. Experiments were conducted in a greenhouse (20°C ± 5°C) during October 1998 to May 1999.

At 45 to 120 days after inoculation, all plants were gently uprooted, and disease severity was assessed from both external (foliar damage) and internal (degree of vascular discoloration) symptoms. Foliar damage was evaluated by the following scale: 0 = no symptom; 1 = yellowing of lower leaves; 2 = wilt or necrosis of leaves; 3 = wilt or necrosis of all leaves; 4 = dead. Vascular discoloration was evaluated by the following scale: 0 = no discoloration; 1 = browning localized below hypocotyls level; 2 = browning of vessels but not of the adjacent tissues; 3 = browning of both vessels and adjacent tissues; 4 = dead. Mean values of external and internal symptoms were calculated based on replicates. To reisolate inoculated pathogens from plants or tubers, 10-mm sections of tissues (hypocotyls or tuber vessels) were stripped off the epidermis and washed with running water

for 10 min. Washed tissues were placed on WA and cultured at 25°C for 7–14 days in the dark.

Tests 1 and 2 were conducted on potato (cv. Waseshiro). The method of test 1 is as just mentioned. In test 2, external symptom was assessed at 35–90 days with the lapse of time. Final reisolations were conducted after the death of plants. Tests 1 and 2 were conducted twice.

Polymerase chain reaction assays using *V. tricorpus*-specific primers

CE98Vt1 and CE98Vt2 were compared with *V. tricorpus* (IMI71799) and other plant pathogenic *Verticillium* species by PCR, using *V. tricorpus*-specific primers (Moukhamedov et al. 1994). Total DNA was extracted from mycelia by the modified methods of Adachi et al. (1993). The extracted DNA was confirmed by gel electrophoresis on 0.7% agarose in TAE buffer (Sambrook et al. 1989) at 100 V for 30 min. The gel was stained with ethidium bromide and the bands were visualized with a UV transilluminator. PCR reactions were undertaken with primers internal transcribed spacer (ITS)1 (5′-TCCGTAGGTGAACCTG CGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), reported by White et al. (1990), and *V. tricorpus*-specific primers from the ITS region (5′-CGCCGGTACATCAG TCTC-3′ and 5′-ACTCCGATGCGAGCGAA-3′), reported by Moukhamedov et al. (1994). These primers were supplied by Espec Origo Service Corp. (Ibaraki, Japan). The amplification was conducted in 20-μl reaction mixtures in 50 mM KCl, 20 mM Tris-Cl (pH 8.4), 2 mM MgCl, 200 μM each of the four deoxynucleotide triphosphates, 0.25 μM each primer, 20 ng templates, and 0.8 units Taq DNA polymerase (Takara, Tokyo, Japan). The PCR amplification reactions were performed with a thermal cycler MP (Takara). The cycle parameters were 30 cycles, consisting of denaturation at 95°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min. The PCR products were analyzed by gel electrophoresis on 2% agarose (NuSieve 3:1 Agarose; FMC Bioproducts, Rockland, ME, USA) in 0.5 × TBE buffer (Sambrook et al. 1989) at 50 V for 30 min. The gel was stained with ethidium bromide, and the bands were visualized with a UV transilluminator.

Results

Morphology

Morphological data are shown in Table 2. CE98Vt1 and CE98Vt2 formed hyaline and erect conidiophores, with one to six (mostly three to four) phialides arising verticillately at each node (Fig. 1). Conidia were hyaline, unicellular (occasionally one-septate), ellipsoidal to subcylindrical, produced monophialidically at the apices of phialides (conidiogenous cells), formed in a false head. The isolates produced three types of resting structures: chlamydospore (Fig. 2), dark resting mycelia (Fig. 3), and microsclerotia (Fig. 4). The bases of conidiophores of two potato isolates

Fig. 1. Conidiophores of *Verticillium tricorpus*. *Bar* 30 μm

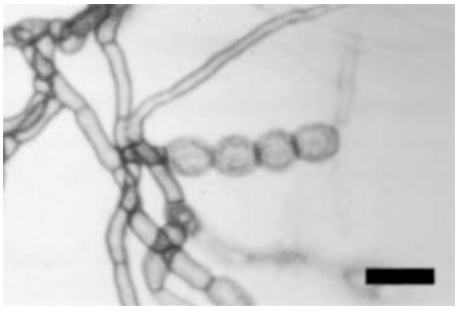

Fig. 2. Chlamydospore of *Verticillium tricorpus*. *Bar* 10 μm

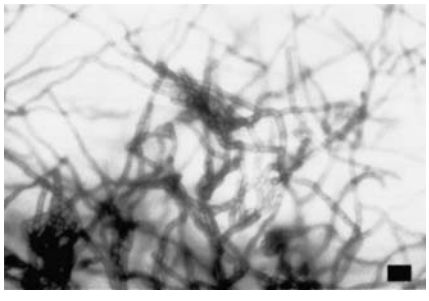

Fig. 3. Dark resting mycelia of *Verticillium tricorpus*. *Bar* 10 μm

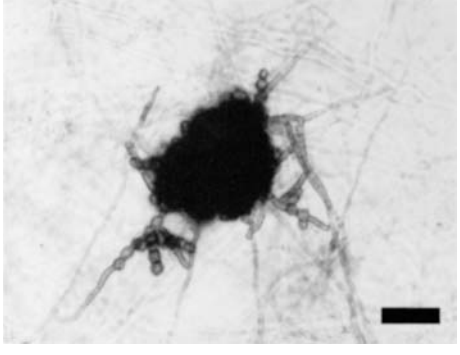

Fig. 4. Microsclerotia of *Verticillium tricorpus*. *Bar* 20 μm

Table 2. Comparison of morphological characters of the present fungus (CE98Vt1 and CE98Vt2) with *Verticillium* spp.

	Size of conidia (μm)	Length of phialide (μm)	Width of dark resting mycelia (μm)	Size of microsclerotia (μm)	Size of chlamydospore (μm)	Coloring of the base of conidiophore	Colony color
CE98Vt1	3.0–10.0 × 1.5–4.5 (5.4 × 2.5)[a]	16.3–38.3 (24.8)	2.0–7.5 (3.8)	55–160 × 40–110 (101.8 × 74.5)	4.0–10.0 × 3.0–8.0 (7.2 × 6.1)	Hyaline	Yellow to black
CE98Vt2	3.0–10.0 × 1.5–3.8 (5.5 × 2.6)	Nd[b]	2.0–6.0 (3.9)	40–140 × 35–90 (84.1 × 63.6)	4.5–10.5 × 3.5–7.5 (7.3 × 6.0)	Hyaline	Yellow to black
V. tricorpus IMI 71799	2.6–7.2 × 1.3–4.0 (4.7 × 2.4)	15.9–38.5 (25.4)	2.1–5.7 (4.2)	24–59 × 20–44 (44.5 × 31.7)	4.7–9.9 × 3.8–8.3 (7.2 × 6.0)	Hyaline	Yellow to black
V. tricorpus[c]	3.5–10.0 × 1.5–3.5	12.0–25.0	3.5–7.0	60–85	7.5–11.0	Hyaline	Yellow to black
V. albo-atrum[d]	3.5–10.5 × 2.0–4.0	20.0–30.0 (–50)	3.0–7.0	Absent	Absent	Darkened	White to black
V. dahliae[e]	2.5–8.0 × 1.4–3.2	16.0–35.0	Absent	15–50 (–100)	Absent	Hyaline	White to black
V. longisporum[f]	7.0–9.0 ± 0 .08	Nd[b]	Absent	Nd[b] (Irregular and elongate)	Absent	Hyaline	White to black
V. nigrescens[g]	4.0–8.0 × 1.5–2.5	20.0–35.0 (–50)	Absent	Absent	5.5–8.0 (–10.0)	Hyaline	White to brown

[a] Average size of each structure is placed in parentheses below the size range
[b] Nd, no description
[c] Hawksworth (1970b)
[d] Hawksworth and Talboys (1970a)
[e] Hawksworth and Talboys (1970b)
[f] Karapapa et al. (1997)
[g] Hawksworth (1970a)

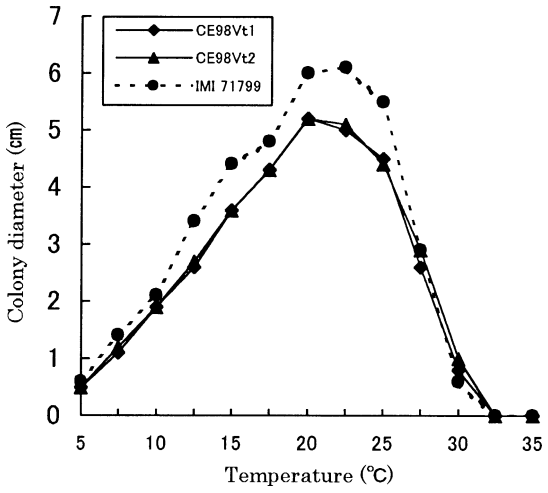

Fig. 5. Growth of mycelial colonies of two potato isolates and *Verticillium tricorpus* (IMI 71799) on PDA after 2 weeks incubation at different temperatures

were hyaline, but those of *V. albo-atrum* (Vaa) were obviously darkened after 2 months. Morphological characters of these two potato isolates were indistinguishable from reference isolates of *V. tricorpus* (IMI71799) and from the descriptions of *V. tricorpus* (Isaac 1953; Hawksworth 1970b).

Growth test

CE98Vt1, CE98Vt2, and the reference isolate of *V. tricorpus* (IMI71799) were able to grow between 5.0° and 30.0°C with an optimum at 20.0°–22.5°C (Fig. 5). Cultured colonies on PDA were yellow up to about 1–2 weeks. The older parts of colonies gradually became black after 2–3 weeks. These growth data were similar to one another and published descriptions of *V. tricorpus* (Isaac 1953).

Pathogenicity to potato tuber

Inoculations of stored potato tubers were all negative. Neither method of inoculation induced either vascular discoloration or rot of tubers.

Pathogenicity to potato plant

Results of inoculation tests to potato plants are shown in Table 3. CE98Vt1 and CE98Vt2 had no marked effect on foliar conditions among eight cultivars. Discoloration of the vascular bundles of stems was observed in some plants of all cultivars when inoculated with CE98Vt1 or CE98Vt2. Reisolation rates from stems were notably high. These two potato isolates were reisolated from 40%–80% of inoculated plants on each cultivar. Vascular discolorations in tuber vessels were also observed on newly formed tubers in three cultivars (cv. Toyoshiro, Touya, and Waseshiro) among eight cultivars. Inoculated potato isolates were reisolated from newly formed tubers of seven cultivars ex-

Table 3. Pathogenicity of Japanese isolates of *V. tricorpus* on eight cultivars of potatoes

Cultivar	Days after inoculation	CE98Vt1					CE98Vt2					*V. dahliae*					Uninoculated control				
		n[a]	Plant			Tuber	*n*[a]	Plant			Tuber	*n*[a]	Plant			Tuber	*n*[a]	Plant			Tuber
			External index	Internal index	Re. (%)[b]	Discolored plant (%)[c]	Re. (%)[d]		External index	Internal index	Re. (%)[b]	Discolored plant (%)[c]	Re. (%)[d]		External index	Internal index	Re. (%)[b]	Discolored plant (%)[c]	Re. (%)[d]		External index	Internal index	Re. (%)[b]	Discolored plant (%)[c]	Re. (%)[d]
Dansyaku	55	5	0.6	1.2	100	0	60	4	0.5	0.3	75	0	0	5	2.2	2.0	80	40	40	4	0.8	0.0	0	0	0
Kitaakari	55	5	0.2	0.0	60	0	0	5	0.0	0.4	80	0	20	5	1.4	2.0	100	40	0	5	0.2	0.0	0	0	0
May-queen	55	5	0.2	0.4	40	0	0	5	0.6	0.6	80	0	0	5	2.0	1.8	80	20	0	4	0.8	0.0	0	0	0
Nishiyutaka	55	5	0.2	0.2	100	0	40	6	0.7	0.7	50	0	33	5	2.4	2.0	100	20	20	5	0.4	0.0	0	0	0
Sayaka	55	5	0.2	0.0	80	0	20	5	0.2	0.2	80	0	80	5	3.0	2.0	80	60	20	5	0.4	0.0	0	0	0
Touya	55	5	0.8	1.0	60	20	0	5	0.4	0.4	80	20	80	5	3.0	2.4	60	20	0	5	0.4	0.0	0	0	0
Toyoshiro	55	5	0.6	0.2	40	20	20	5	0.6	0.8	100	0	20	5	1.4	2.0	100	0	20	5	0.0	0.0	0	0	0
Waseshiro																									
Test 1	45–55	10	0.0	0.1	100	0	0	9	0.1	0.0	78	0	22	12	3.1	2.8	100	42	25	10	0.2	0.0	0	0	0
Test 2[e]	70–90	13	Nt[f]	Nt[f]	69	23	46	13	Nt[f]	Nt[f]	69	15	46	11	Nt[f]	Nt[f]	100	55	82	13	Nt[f]	Nt[f]	0	0	0

[a] Number of tested plants
[b] % of plant that inoculated pathogen were reisolated (Re.) from stem
[c] % of plant of which tuber was discolored
[d] % of plant that inoculated pathogen were reisolated from tuber
[e] In Waseshiro test 2, research of tuber and reisolation from stem and tuber were conducted after death of plants
[f] Nt, not tested

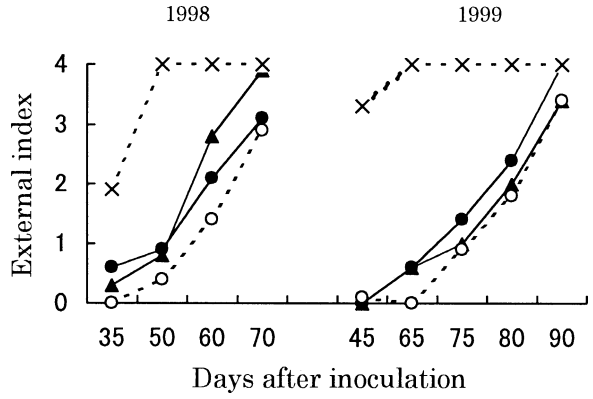

Fig. 6. Development of external index of potato (cv. Waseshiro) in test 2, conducted during October 1998 to January 1999 (*left*) and January to April 1999 (*right*): CE98Vt1 (▲), CE98Vt2 (●), 84034 (×), uninoculated control (○)

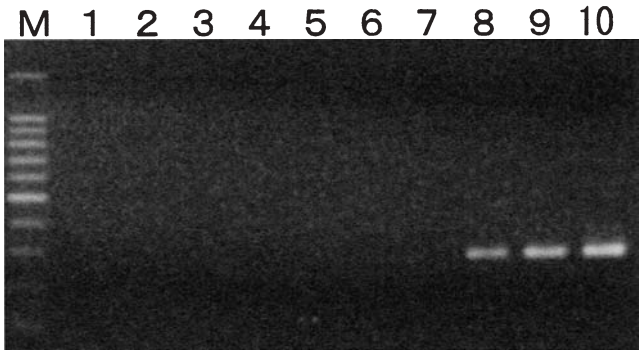

Fig. 7. Agarose gel electrophoresis of PCR products using *Verticillium tricorpus*-specific primers. *Lanes 1–10* are PCR products from isolates *V. albo-atrum* (Vaa and SVPI 94221), *V. dahliae* (84034 and SVPI 94146), *V. longisporum* (84010), *V. nigrescens* (Vnc2 and SVPI 94078), *V. tricorpus* (IMI 71799), CE98Vt1 (potato isolate), and CE98Vt2 (potato isolate), respectively; the molecular size marker (*M*) is 100-bp DNA Ladder (TaKaRa)

cept cv. May queen. *V. dahliae* produced distinct external and internal symptom of all tested cultivars and was reisolated from stems of all tested cultivars.

In cv. Waseshiro (test 2), developments of external symptoms were observed until the end of plant growth (Fig. 6). External symptoms, that is, yellowing and curling of lower leaves, were observed on some plants only after a protracted cultivation period. However, those symptoms were not always produced and were often unclear. Even if external symptoms were produced, the development of disease was very slow, such that yellowing of inoculated plants occurred only slightly more than with uninoculated controls. This observation is evidence that the virulence of these two potato isolates to potato was very low as compared with *V. dahliae*. All the potato plants inoculated with *V. dahliae* were dead after 50–60 days inoculation.

Pathogenicity to several crops

Neither CE98Vt1 nor CE98Vt2 produced any symptoms on tested crop plants (Table 4). However, both isolates were reisolated from these inoculated plants, and the percentage of reisolated plants in each host was quite high. Of the inoculated plants, 40%–100% were infected. In contrast, *V. dahliae* produced severe symptoms on inoculated hosts, except *N. tabacum* cv. Xanthi nc.

PCR amplification of *V. tricorpus*-specific region

Electrophoretic patterns of PCR-amplified fragments are shown in Fig. 7. *V. tricorpus*-specific primers amplified a 337-bp fragment of DNA extracted from CE98Vt1, CE98Vt2, and *V. tricorpus* (IMI71799). The primers failed to amplify the DNA extracted from *V. albo-atrum* (Vaa and SVPI 94221), *V. dahliae* (84034 and SVPI 94146), *V. longisporum* (84010), and *V. nigrescens* (Vnc2 and SVPI 94078). The genomic DNA of all tested isolates was successfully amplified with the universal primers ITS1/ITS4 (data not shown).

Discussion

CE98Vt1 and CE98Vt2 isolated from vascular discolored potato tubers were identified as *V. tricorpus* on the basis of cultural and morphological characteristics and PCR diagnosis. *V. tricorpus* formed young yellow colonies on PDA up to 1–2 weeks and produced three types of resting structures: chlamydospore, dark resting mycelia, and microsclerotia. *V. albo-atrum* produced only dark resting mycelia, *V. dahliae* and *V. longisporum* formed only microsclerotia, and *V. nigrescens* had only chlamydospores. The bases of conidiophores were hyaline, but those of *V. albo-atrum* were obviously darkened; they can grow at 30°C but *V. albo-atrum* cannot. These characteristics distinguish *V. tricorpus* from the other four closely related species. Furthermore, PCR diagnosis using *V. tricorpus*-specific primers is likely to be very effective to distinguish *V. tricorpus* from these species.

In Japan, *V. tricorpus* has not been detected. This fungus was one of the specially designated pests of quarantine significance and was being monitored for potential invasion into Japan by the Ministry of Agriculture, Forestry and Fisheries. Seed quarantine is routinely performed with imported tomato seeds. In this study, *V. tricorpus* was isolated from uncooked potato sold at a market in Japan. All uncooked potatoes sold in Japan are of domestic origin, so it seems likely that *V. tricorpus* inhabits somewhere in Japan.

V. tricorpus was isolated from tomato, potato, mint, cantaloupe, cotton, and weeds (Isaac 1953; Isaac and Harrison 1968; MacGarvie and Hide 1966; Skotland 1971; Huisman 1988; Korolev and Katan 1999). Also, this fungus has been reported as the wilt pathogen of tomato (Isaac 1953; Uys et al. 1997) and snapdragon (Isaac 1956), and as the causal agent of dry rot of stored potato (Thanassoulopoulos and Giapanoglou 1994). Japanese isolates of *V. tricorpus* CE98Vt1 and CE98Vt2 were mildly pathogenic to potato. Vascular discoloration of potato tuber was observed after inoculation of seedlings. These isolates did not induce tuber

Table 4. Pathogenicity of Japanese isolates of *V. tricorpus* on several crops

Crop	Days after inoculation	CE98Vt1				CE98Vt2				*V. dahliae*				Uninoculated control			
		n[a]	External index	Internal index	Re. (%)[b]	*n*[a]	External index	Internal index	Re. (%)[b]	*n*[a]	External index	Internal index	Re. (%)[b]	*n*[a]	External index	Internal index	Re. (%)[b]
Eggplant (cv. Senryo-2-go)	45	5	0.0	0.0	100	5	0.0	0.0	100	5	3.0	3.0	100	5	0.0	0.0	0
	85	5	0.2	0.0	40	5	0.4	0.0	40	5	3.2	2.6	100	5	0.2	0.0	0
Tobacco																	
(*N. glutinosa*)	70	10	0.0	0.1	100	11	0.0	0.0	100	10	3.8	3.8	90	11	0.0	0.0	0
(*N. tabacum*)	70	10	0.0	0.1	90	10	0.0	0.1	100	10	0.0	0.0	40	10	0.0	0.0	0
Parsley (cv. Grand)	80	10	0.7	0.0	50	10	0.4	0.0	50	6	2.7	2.7	100	10	0.7	0.0	0
Snapdragon																	
(cv. Tall giant mixed)	60	10	0.0	0.1	70	Nt[c]	Nt[c]	Nt[c]	Nt[c]	6	3.5	3.0	100	10	0.0	0.0	0
(cv. F1 light pink three)	80	10	0.0	0.0	80	10	0.0	0.0	70	10	1.6	1.5	90	10	0.0	0.0	0
(cv. Maryland bright yellow)	80	10	0.0	0.0	80	10	0.0	0.0	60	10	1.5	1.5	90	10	0.0	0.0	0
Larkspur (cv. mixed)	45	10	0.1	0.0	70	10	0.1	0.1	40	8	2.6	2.6	63	10	0.0	0.0	0
Tomato (cv. Ponderosa)	60	17	0.0	0.0	100	18	0.0	0.0	100	16	3.0	2.7	100	16	0.0	0.0	0
	120	5	0.6	0.0	100	5	0.2	0.2	100	5	3.0	3.0	100	5	0.4	0.0	0
Green pepper (cv. Ace)	45	5	0.0	0.0	100	5	0.0	0.0	100	5	4.0	3.0	100	5	0.0	0.0	0
	85	6	0.0	0.0	67	5	0.0	0.0	60	5	4.0	3.0	100	5	0.0	0.0	0
Soybean (cv. Tamahomare)	50	4	0.0	0.0	100	4	0.0	0.0	100	4	3.0	3.0	100	4	0.0	0.0	0
	80	5	0.0	0.2	80	5	0.0	0.6	40	3	3.0	3.0	100	5	0.0	0.0	0
Chinese cabbage	50	4	0.0	0.3	75	3	0.0	0.0	100	4	3.0	2.8	75	4	0.0	0.0	0
(cv. Taibyo-60-nichi)	80	4	0.0	0.3	75	4	0.0	0.5	50	4	3.0	2.5	75	4	0.0	0.0	0

[a] Number of tested plants
[b] % of plant that inoculated pathogen were reisolated from hypocotyl
[c] Not tested

rot by inoculation to stored tuber and were nonpathogenic to tomato, snapdragon, and other tested plants. Similarly, Smith (1965) and Skotland (1971) reported that their isolates were nonpathogenic to tomato. These data were inconsistent with previous reports (Isaac 1953, 1956; Uys et al. 1997; Thanassoulopoulos and Giapanoglou 1994). Each researcher reported the pathogenicity of *V. tricorpus* differently. Two reasons may account for these contradictions. First, inoculation and cultivation conditions would influence the pathogenicity of this fungus. Isaac (1956) reported the pathogenicity of *V. tricorpus* only showed under very wet conditions and when heavy applications of organic nitrogenous fertilizer had been used. Second, the pathogenicity of *V. tricorpus* would vary for each isolate. In the closely related species *V. albo-atrum* and *V. dahliae*, several pathotypes were classified (Correll et al. 1988; Horiuchi et al. 1990; Iijima 1983a,b). Therefore, several pathotypes may exist in *V. tricorpus*. Further sampling and inoculations are needed to clarify this question.

In this experiment, although both potato isolates showed no external and internal symptoms with inoculated plants, reisolation rates were quite high. Similarly, *V. tricorpus* was reisolated from eggplant, snapdragon, tobacco, lupin, and cotton without showing any symptoms (Isaac 1953; Skotland 1971; Huisman 1988; Korolev and Katan 1999). These data indicate that *V. tricorpus* can readily colonize several plants. Therefore, *V. tricorpus* may invade into Japan with latently infected plants.

In recent research, *V. tricorpus* was isolated from various plants in Japan: larkspur (Chikuo et al. 2001), delphinium (Chikuo et al. 2000; Ebihara et al. 2000), anemone, ranunculas, and tomato (Ebihara et al. 2000). This fungus may be extensively distributed in Japan. On the basis of these investigations, *V. tricorpus* can be eliminated from the manual for specially designated pests of quarantine significance in June 2001.

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