DIFFICULTY TO ESTABLISH VEGETATIVE COMPATIBILITY OF JAPANESE ISOLATES OF *VERTICILLIUM DAHLIAE* KLEB. USING MELANIN-SYNTHESIS DEFICIENT MUTANTS

HIDEYUKI NAGAO,* DAI WAKATABE, AND TSUTOMU IIJIMA¹

Faculty of Horticulture, Chiba University, Matsudo 271, Japan

¹*Tokyo Metropolitan Agricultural Experiment Station, Tachikawa 180, Japan*

(Received February 1, 1994; Accepted July 14, 1994)

Japanese isolates of *Verticillium dahliae* were formerly designated as tomato pathotype and non-tomato pathotype by pathogenicity tests. We attempted to classify 18 Japanese isolates based on vegetative compatibility. Only three isolates of the tomato pathotype induced compatible *alm-brm* mutants. A non-secreting *brm* mutant was not induced from isolate of the non-tomato pathotype. Complementation was not achieved by a pair of mutants from the tomato pathotype and those from the non-tomato pathotype. From these results, Japanese isolates of *V. dahliae* could not be separated into any vegetative compatibility groups using melanin synthesis-deficient mutants.

Verticillium dahliae Kleb. is an important vascular wilt pathogen of various plant species. The fungus has been characterized by the pathogenicity in some plants (7,22,24). In Japan, *Verticillium* wilt has been recognized from the early 1970s (15). Iijima categorized Japanese isolates of *V. dahliae* as “tomato pathotype” and “non-tomato pathotype” according to the pathogenicity on tomato (susceptible cultivar Beiju) (14). Hagiwara further proposed six groups for Japanese isolates based on pathogenicity for eggplant, tomato, pepper, Chinese cabbage, and soybean (9). Horiuchi et al. (13) have described a system for classifying Japanese *V. dahliae* isolates using a differential set of host plants. However, this differential host method is not conclusive. Employing a new differential host for the pathogenicity test with the formerly determined isolates, a new categorization would be possible. For example, parsley (33) and Chinese lantern plant (*Physalis alkekengi* var. *franchetii*) (14) showed more different susceptibilities than the host plants in proposed groups did. Increasing the kinds of

* Address reprint requests to: Dr. Hideyuki Nagao, Faculty of Horticulture, Chiba University, 648 Matsudo, Matsudo 271, Japan.

differential host plants may make the recognition of pathogenic groups more complicated. In addition, *V. dahliae* and *V. albo-atrum* do not clearly show host specificity as known in *Fusarium* with some exceptions (12, 17, 22).

Heterokaryon formation is used as the criterion for compatibility of many imperfect fungi (4, 5, 8, 35). Heterokaryon compatibility groups suggested the existence of diverse genetic populations in imperfect fungi (6). It was examined by using complementary auxotrophic mutants (23, 31) or color mutants (28). *V. dahliae* formed black microsclerotia where melanin granules concentrated in cell wall layers (36). Three albino mutants (*alm*) and four brown mutants (*brm*) were determined in their different mutants. Complementary relationships among these mutants were reported (2). No microsclerotia mutant (*nms*) does not form microsclerotia on the hypha and showed a white colony (2). Although *nms* also was one of the microsclerotia mutants, it is not complementary with either *alm* or *brm*. Heterokaryon compatibility groups were rephrased vegetative compatibility groups (VCGs) by Puhalla (29) and VCGs have been demonstrated in *V. dahliae* through the use of these microsclerotia color mutants induced with ultraviolet light. The defoliating and non-defoliating isolates from cotton were clearly distinguished into different VCGs (27). However many pathotypes of *V. dahliae* were considered to be vegetatively incompatible based on these kinds of mutants (29). Recently, pathotypes of *V. dahliae* previously designated as VCGs using microsclerotia color mutants were reassessed using nitrate non-utilizing (*nit*) mutants. It was reported that many pathotypes were found to be compatible using *nit* mutants (19, 20, 32).

In this paper, we examined vegetative compatibility groups for Japanese isolates of *V. dahliae* using melanin-synthesis-deficient mutants and their relationships to the pathotypes that had been categorized by pathogenicity for the differential host plants.

MATERIALS AND METHODS

Cultural conditions. Isolates of *Verticillium dahliae* Kleb. and their sources are shown in Table 1. An isolate which was virulent on *Crucifera* (Chinese cabbage, radish, rape and so on) has been identified as *V. dahliae* var. *longisporum* (16) and as diploid (13, 18, 21). Home-made potato-sucrose agar (PSA) was used for maintenance of isolates and determination of colony diameter. Potato-carrot-dextrose agar (PCDA) and minimal medium (MM) were prepared according to Puhalla's method (26, 30). Instead of sanguinarine nitrate (29), 200 ppm of sanguinarine sulfate was added to potato-dextrose-agar (PDA) (Difco Laboratories, Detroit, MI, U.S.A.) as the sanguinarine medium (25). To determine the loci of *brm* mutants, the tester mutants were grown on sucrose-nitrate agar and in 50 ml of liquid sucrose-nitrate medium in 250-ml Erlenmeyer flasks (2, 3). All cultures were incubated at 25°C in darkness.

Induction of mutants. Conidia of each isolate were obtained from 14-day-old colonies. Scraped mycelia were suspended in 10 ml of sterilized distilled water and

Table 1. Characteristics of isolates of *Verticillium dahliae*.

Isolate ^a	Source of isolation	Growth rate on PSA ^b	Tolerance to sanguinarine ^c
LE103 (T) ^d	Tomato	4.55	0.81
ST1 (T)	Potato	4.40	0.25
HE6 (T)	Okra	4.25	0.24
LE8602 (T)	Tomato	3.65	0.77
*84011 (T) (B) ^e	Chinese cabbage	2.98	0.67
CV1 (NT)	Watermelon	5.15	NT
SM821 (NT)	Eggplant	4.75	0.50
22720 (NT)	Chrysanthemum	4.23	1.06
SM931 (NT)	Eggplant	4.01	NT
22710 (NT)	Chrysanthemum	3.87	ND
SM321 (NT)	Eggplant	2.81	NT
AC406 (NT)	Udo	2.34	0.92
*84034 (NT) (A)	Eggplant	1.92	0.99
CM208 (NT)	Chrysanthemum	1.53	0.96
*84023 (NT) (C)	Eggplant	1.48	0.71
*84013 (NT) (D)	Chinese cabbage	2.96	NT
*84010 (NT) (D)	Chinese cabbage	1.86	0.41
*V316 (NT) (D)	Chinese cabbage	3.02	NT

^a All isolates were identified and previously designated by Iijima (14). Isolates with an asterisk were kindly supplied by Hagiwara (9).

^b All cultures were incubated at 25°C in darkness.

^c The value was calculated by the formula; (colony diameter on PDA + sanguinarine)/(colony diameter on PDA). ND, isolate did not grow on PDA; NT, not tested.

^d Tomato (*Lycopersicon esculentum*; cv. Beiju) was used as a differential host by Iijima (14). T, tomato pathotype; NT, non-tomato pathotype.

^e A, B, C, and D represent the host specificity grouping according to Hagiwara (9).

filtered through a sterilized glass filter (20–30 µm pore size). The concentration of conidia was determined with a Thoma's hemacytometer and adjusted depending on the intensity of irradiation to obtain a survival curve. Conidia were spread on plates containing MM or PSA with a sterile glass rod. A sterilizing lamp (Toshiba GL15) was used as the source of ultraviolet (UV) light at 254 nm. Dosage was measured with a Black-Ray ultraviolet intensity meter (UVP, Inc., U.S.A.). The average intensity of the UV light was ca. 20 ergs/mm². After 10 days, the numbers of surviving colonies were counted and colonies were selected for their abnormalities in color and diffusion of pigments into the medium. Mutants were transferred on PSA two or three times because some of them slowly reverted to producing black microsclerotia. Mutants were verified by a single spore isolation and the color of the microsclerotia on PCDA was confirmed by visual and microscopic observations. Mutant phenotypes were designated with the genetic symbols albino microsclerotia mutant (*alm*), brown microsclerotia mutant (*brm*), and no microsclerotia mutant (*nms*) according to Bell et al. (2).

Pairing of color mutants. Pairings were made either between *alm* mutants or between an *alm* and a *brm* mutant. Colonies were grown for 14 days on PCDA.

Mycelial blocks were cut with a 5-mm-diameter cork borer and paired 10 mm apart from each other on PCDA. Formation of a zone of black microsclerotia on the contact line was recognized as complementation. Each pairing was conducted at least twice. In some pairings, the cellophane method was used to examine exuding intermediates in the melanin biosynthetic pathway (27).

RESULTS

Comparison of growth characteristics

Growth rates among the isolates were almost similar on PSA at 25°C. Tolerance to sanguinarine of some isolates of the tomato pathotype were very sensitive. On the other hand, most of the isolates among the non-tomato pathotype were resistant (Table 1).

*Survival of *V. dahliae* with UV irradiation and its frequency of melanin-deficient mutations*

Eighteen isolates of *V. dahliae* were irradiated on MM with several different UV doses; 0.001% of colony survived at 5,000 ergs/mm². The frequency of *alm* mutants of the tomato pathotype represented ca. 0.6% of the surviving colonies. However *alm* mutants from the non-tomato pathotype occurred less frequently (<0.25%). All *brm* mutants of the non-tomato pathotype were the melanin intermediate secreting type. *Brm* mutants cultured on sucrose-nitrate medium did not markedly change the medium color that should reflect difference of loci (2). No stable mutant was obtained from diploid isolates (84010, 84013 and V316).

*Pairings of color mutants of tomato pathotype of *V. dahliae**

Twenty-nine color mutants were induced by UV irradiation from five tomato pathotype (Table 2). For each isolate, complementations of *alm* and *brm* mutants were tried to be determined. Complementary *alm-brm* mutants were induced from LE103 (221, 222, 3103, 3104, 3123, and 3253), LE8602 (3503, 3506, and 3508), and ST1 (8105, 8109, and 8112). For isolate HE6, all mutants obtained were *nms*. Complementary mutants (tester) were selected in each isolate. Then we examined complementation between these mutants to determine the relationships among isolates of the tomato pathotype (Table 3). LE103 and LE8602 were complemented in all *alm* and *brm* combinations. LE103 and ST1 were complemented in certain *alm* and *brm* combinations. LE8602 and ST1 were complemented with both *brm* mutants. None of the mutants used secreted any melanin precursors. For isolate 84011, *alm* mutants obtained did not show any complementation toward these testers. Isolates LE103, LE8602, and ST1 of the tomato pathotype showed compatibility with each other. However, results of complemental reaction depended upon the mutants and isolates used. These results did not clearly show "tomato pathotype" as one of the diverse populations in *V. dahlinae*.

Table 2. Nature of mutants of *Verticillium dahliae* by UV irradiation.

Isolate	Mutant induced	Dose of UV irradiation (erg/mm ²)	Designation of phenotype
LE103	221	14,400	<i>alm</i>
	222	14,400	<i>alm</i>
	3103	3,000	<i>brm</i>
	3104	3,000	<i>alm</i>
	3123	2,400	<i>alm</i>
	3125	2,400	<i>nms</i>
	3237	3,000	<i>nms</i>
	3237S	3,000	<i>nms</i>
	3239	3,000	<i>nms</i>
	3253	3,000	<i>alm</i>
	3287	3,000	<i>nms</i>
ST1	8105	4,600	<i>brm</i>
	8109	4,600	<i>brm</i>
	8112	4,600	<i>alm</i>
	8132	4,600	<i>alm</i>
HE6	7101	3,800	<i>nms</i>
	7102	3,800	<i>nms</i>
	7105	3,800	<i>nms</i>
	7108	3,800	<i>nms</i>
	7110	3,800	<i>nms</i>
	7110B	3,800	<i>nms</i>
	7115	3,800	<i>nms</i>
LE8602	3503	3,040	<i>brm</i>
	3506	3,040	<i>alm</i>
	3508	3,040	<i>alm</i>
84011	H4109	4,600	<i>alm</i>
	H4201	4,800	<i>alm</i>
	H4202	4,800	<i>alm</i>
	H4203	4,800	<i>alm</i>
CV1	J201	4,800	<i>brm</i>
SM821	6201	3,008	<i>alm</i>
	6208	3,008	<i>alm</i>
	6217	3,008	<i>alm</i>
	6230	3,008	<i>alm</i>
	6240	2,700	<i>alm</i>
	6244	2,700	<i>brm</i>
SM931	6301	3,000	<i>alm</i>
	6302	3,760	<i>brm</i>
AC406	5201	2,400	<i>brm</i>
	5203	7,008	<i>brm</i>
	5205	4,512	<i>brm</i>
	5209	4,640	<i>nms</i>
	5210	4,640	<i>alm</i>
CM208	4211	4,560	<i>brm</i>
	4305	4,640	<i>brm</i>
	4401	4,800	<i>alm</i>
	4402	4,800	<i>alm</i>

Table 3. Complementation test among mutants of the tomato pathotype of *Verticillium dahliae*.

		LE103			LE8602			ST1			
		3123	3253	3103*	3506	3508	3503*	8112	8132	8105*	8109*
LE103	222	—	—	+	—	—	+	—	—	—	NT ^b
	3123	**	—	+	—	—	+	—	—	NT	NT
	3253		**	+	—	—	+	+	+	—	+
	3103* ^a			**	—	—	—	+	+	+	—
LE8602	3506				**	—	+	—	—	—	NT
	3508					**	+	—	—	—	NT
	3503*						**	—	—	+	NT
ST1	8112							**	—	+	+
	8132								**	—	—
	8105*									**	+

^a Mutants asterisked were *brm* mutants.

^b +, formation of a zone of black microsclerotia on the contact line; —, no formation of a zone of black microsclerotia on the contact line; NT, not tested. Pairing was conducted twice.

Pairings of color mutants of non-tomato pathotype of *V. dahliae*

Five of the 10 isolates of non-tomato pathotype were induced color mutants. Nine *alm* mutants and eight secreting *brm* mutants were obtained in these isolates. Non-secreting *brm* mutant could not be induced in our experiments. Therefore we could not determine the compatibility and the tester in non-tomato pathotype. Complementation between isolates of the non-tomato pathotype could not be achieved. Although the complementation test among mutants of the non-tomato pathotype could not be achieved, we tried to test complementation between the tomato pathotype and the non-tomato pathotype. Complementary *alm-brm* pairings did not succeed in any combination between the tomato pathotype and non-tomato pathotype of *V. dahliae*.

DISCUSSION

We compared the cultural characteristics of *V. dahliae* between the tomato pathotype and the non-tomato pathotype described in Japan by Iijima (14). There were no significant differences in colony growth rate among the isolates. In *V. albo-atrum*, colony growth rates reflected the difference of alfalfa pathotype and potato pathotype (1). However, it was not the same case in Japanese isolates of *V. dahliae*. Puhalla and Hummel (29) reported the relationships of the VCGs to antibiotic resistance. In their experiment, employing sanguinarine nitrate, v-c P2 including seven tomato isolates was sensitive and v-c P5 including four pepper isolates was very resistant. Our isolates also showed a range of sensitivities but the results were not sufficiently definitive to use sensitivity to sanguinarine compounds as a criterion for distinguishing the pathotypes.

We examined three steps to verify the vegetative compatibility: 1) to obtain both *alm* and *brm* compatible mutants from each isolate that showed obvious

complementary reactions of black microsclerotia, 2) to check the selected mutants that must be non-secreting, 3) to pair an *alm* mutant and a *brm* mutant (tester) from different isolates.

The results of our experiments suggested the difficulty of using the melanin-synthesis-deficient mutants. For the first step experiment, we obtained the *alm-brm* complementary mutants from solely three isolates. In the non-tomato pathotype, both complementary *alm-brm* mutants could not be induced. Thus, the non-tomato pathotype could not confirm the ability of self-compatibility. Although we verified the ability of complementation for these three isolates, the nature of the *alm* and *brm* mutants were different. Each *alm* and *brm* mutants reacted differently depending on the particular pairing (Table 3). In our experiments, it was impossible to determine the genetical differences from the complementation and culture characteristics. *Alm* mutants could not be distinguished as *alm* 1, 2 or 3 based on the complementation tests. For *brm* mutants, Bell et al. (2) distinguished them with the results of complementation tests and culture characteristics on the sucrose-nitrate medium. In *V. dahliae*, the number of loci which control melanin synthesis has not clearly been agreed upon. Bell et al. (2) reported three *alm* and four *brm* loci. On the contrary, Kasyanenko and Portenko in 1978 reported that 22 *alm*, 6 *brm* and 5 *chm* loci had been found in *V. dahliae* (10).

At the second step, *brm* mutants of three self-compatible isolates were non-secreting. Secreting *brm* mutants were obtained in some isolates of the non-tomato pathotype (Table 2).

At the final step, three self-compatible isolates of tomato pathotype showed complementation among the isolates. However, other isolates that could uniquely induce *alm* mutants did not show complementary reaction with these three isolates. At least these three isolates of the tomato pathotype are regarded as the same vegetative compatibility group.

Formations of heterokaryosis was governed by different factors. Heale (11) suggested that many hyphal fusions were required to produce a visible heterokaryotic reaction in combinations with albino-brown microsclerotial color mutants. Our isolates of both the tomato and non-tomato pathotypes showed hyphal anastomoses when each isolate was incubated on water agar. Therefore, isolates with the ability to produce hyphal fusion may fail to yield a sufficient hyphal fusion, as Heale suggested. We wondered if additional mutation occurred in the pathway of melanin synthesis for unsuccessful *alm* mutants. Pairings between the secreting *brm* and these *alm* mutants of the non-tomato pathotype produced black microsclerotia. Thus, the melanin mutants of these isolates were supposed to retain the normal pathway of melanin synthesis. Another impeding factor was incompatibility genes which could exist in either the nucleus or as extrachromosomal factors (34). However it was not the aim of this study to demonstrate the contribution of incompatibility genes.

Mutant induction by a mutagen has the disadvantage of the occurrence of unexpected changes. We are concerned about artificial effects on these complicated

results. Recently, VCGs of *V. dahliae* using microsclerotial color mutants were reassessed using nitrate non-utilizing (*nit*) mutants. Many pathotypes were supposed to be compatible by *nit* mutants (19,20,32). We continue to study the vegetative compatibility relationships among Japanese isolates of *V. dahliae* using *nit* mutants.

We gratefully thank Dr. A. P. Keinath, Clemson University, U.S.A., for his kind reading of our manuscript and helpful advice. This research was supported in part by a Grant-in-Aid for Encouragement of Young Scientists No. 1760042 (1989) from the Ministry of Education, Science and Culture of Japan.

REFERENCES

- 1) Basu, P. K. and Bulter, G., Colony growth response to temperature and pathogenicity of isolates of *Verticillium albo-atrum* from alfalfa in Canada. *Can. J. Plant Pathol.*, **13**, 112–115 (1991).
- 2) Bell, A. A., Puhalla, J. E., Tolmosoff, W. J., and Stipanovic, R. D., Use of mutants to establish (+)-scytalone as an intermediate in melanin biosynthesis by *Verticillium dahliae*. *Can. J. Microbiol.*, **22**, 787–799 (1976).
- 3) Brandt, W. H., Morphogenesis in *Verticillium*: Effects of light and ultraviolet radiation on microsclerotia and melanin. *Can. J. Bot.*, **42**, 1017–1023 (1964).
- 4) Brooker, N. L., Leslie, J. F., and Dickman, M. B., Nitrate non-utilizing mutants of *Colletorichum* and their use in studies of vegetative compatibility and genetic relatedness. *Phytopathology*, **81**, 672–677 (1991).
- 5) Correll, J. C., Gordon, T. R., and McCain, A. H., Genetic diversity in California and Florida populations of the pitch canker fungus *Fusarium subglutinans* f. sp. *pini*. *Phytopathology*, **82**, 415–420 (1992).
- 6) Croft, J. H. and Jinks, J. L., Aspects of the population genetics of *Aspergillus nidulans*. In *Genetics and Physiology of Aspergillus*, ed. by Smith, J. E. and Pateman, J. A., Academic Press, New York (1977), p. 339–360.
- 7) De Vay, J. and Pullman, G. S., Epidemiology and ecology of diseases caused by *Verticillium* species, with emphasis on *Verticillium* wilt of cotton. *Phytopathol. Mediterr.*, **23**, 95–108 (1984).
- 8) Elmer, W. H., Vegetative compatibility groups of *Fusarium proliferatum* from asparagus and comparisons of virulence, growth rates, and colonization of asparagus residues among groups. *Phytopathology*, **81**, 852–857 (1991).
- 9) Hagiwara, H., Differentiation of the pathogenicity of *Verticillium dahliae* in Japan. *Plant Prot.*, **44**, 299–303 (1990).
- 10) Hastie, A. C. and Heale, J. B., Genetics of *Verticillium*. *Phytopathol. Mediterr.*, **23**, 130–162 (1984).
- 11) Heale, J. B., *Verticillium* spp., the cause of vascular wilts in many species. In *Advances in Plant Pathology*, Vol. 6, *Genetics of Plant Pathogenic Fungi*, ed. by Sidhu, G. S., Academic Press, San Diego (1988), p. 291–312.
- 12) Heale, J. B. and Isaac, I., Wilt of lucerne caused by species of *Verticillium*. IV. Pathogenicity of *V. albo-atrum* and *V. dahliae* to lucerne and other crops; Spread and survival of *V. albo-atrum* in soil and in weeds; Effect upon lucerne production. *Ann. Appl. Biol.*, **52**, 439–451 (1963).
- 13) Horiuchi, S., Hagiwara, H., and Takeuchi, S., Host specificity of isolates of *Verticillium dahliae* towards Cruciferous and Solanaceous plants. In *Biological Control of Soil-Borne Plant Pathogens*, ed. by Hornby, D., C. A. B International, London (1990), p. 285–298.
- 14) Iijima, T., Studies on *Verticillium* wilt of tomato. *Bull. Tokyo Metrop. Agric. Exp. Stn.*, **16**, 63–128 (1983).

- 15) Iijima, T. and Abe, Z., Occurrence of *Verticillium* wilt of tomato. *Ann. Phytopathol. Soc. Jpn.*, **39**, 131 (1973).
- 16) Ingram, R., *Verticillium dahliae* var. *longisporum*, a stable diploid. *Trans. Br. Mycol. Soc.*, **51**, 339–341 (1968).
- 17) Isaac, I., *Verticillium* wilt of Brussels spouts. *Ann. Appl. Biol.*, **45**, 276–283 (1957).
- 18) Jackson, C. W. and Heale, J. B., Relationship between DNA content and spore volume in sixteen isolates of *Verticillium lecanii* and two new diploids of *V. dahliae* (= *V. dahliae* var. *longisporum* Stark). *J. Gen. Microbiol.*, **131**, 3229–3236 (1985).
- 19) Joaquim, T. R. and Rowe, R. C., Reassessment of vegetative compatibility relationships among pathotypes of *Verticillium dahliae* using nitrate-non utilizing mutants. *Phytopathology*, **80**, 1160–1166 (1990).
- 20) Joaquim, T. R. and Rowe, R. C., Vegetative compatibility and virulence of pathotypes of *Verticillium dahliae* from soil and potato plants. *Phytopathology*, **81**, 552–558 (1991).
- 21) Nagao, H., Taguchi, T., Nishimura, K., Tanaka, R., and Horiuchi, S., Ploidy analysis for *Verticillium dahliae* by flow cytometry. Proc. 35th Anniversary Meet. Mycol. Soc. Jpn, p. 47 (1991).
- 22) Nelson, R., The specific pathogenesis of the *Verticillium* that causes wilt of peppermint. *Phytopathology*, **37**, 17 (1947).
- 23) O'Garro, L. W. and Clarkson, J. M., Heterokaryon incompatibility and heterozygous diploid production in tomato wilt isolates of *Verticillium dahliae*. *J. Gen. Microbiol.*, **134**, 2977–2984 (1988).
- 24) Pegg, G. F., *Verticillium* diseases. *Rev. Plant Pathol.*, **53**, 157–182 (1974).
- 25) Presley, J. T., Growth response of *Verticillium albo-atrum* to sanguinarine in nutrient agar. *Phytopathology*, **59**, 1968–1969 (1969).
- 26) Puhalla, J. E., Differences in sensitivity of *Verticillium* species to ultraviolet irradiation. *Phytopathology*, **63**, 1488–1492 (1973).
- 27) Puhalla, J. E., Classification of isolates of *Verticillium dahliae* based on heterokaryon incompatibility. *Phytopathology*, **69**, 1186–1189 (1979).
- 28) Puhalla, J. E., A visual indicator of heterokaryosis in *Fusarium oxysporum* from celery. *Can. J. Bot.*, **62**, 540–545 (1984).
- 29) Puhalla, J. E. and Hummel, M., Vegetative compatibility groups within *Verticillium dahliae*. *Phytopathology*, **73**, 1305–1308 (1983).
- 30) Puhalla, J. E. and Mayfield, J. E., The mechanism of heterokaryotic growth in *Verticillium dahliae*. *Genetics*, **76**, 411–422 (1974).
- 31) Puhalla, J. E. and Spieth, P. T., A comparison of heterokaryosis and vegetative incompatibility among varieties of *Gibberella fujikuroi* (*Fusarium moniliforme*). *Exp. Mycol.*, **9**, 39–47 (1985).
- 32) Strausbaugh, C. A., Schroth, M. N., Weinhold, A. R., and Hancock, J. G., Assessment of vegetative compatibility of *Verticillium dahliae* tester pathotypes and isolates from California potatoes. *Phytopathology*, **82**, 61–68 (1992).
- 33) Takeda, K., Ishizuka, T., and Akanuma, R., Classification of the pathogen of *Verticillium* yellows of celery by pathogenicity. *Proc. Kanto-Tosan Plant Prot. Soc.*, **35**, 71–72 (1988).
- 34) Typas, M. A., Heterokaryon incompatibility and interspecific hybridization between *Verticillium albo-atrum* and *Verticillium dahliae* following protoplast fusion and microinjection. *J. Gen. Microbiol.*, **129**, 3043–3056 (1983).
- 35) Venter, S. L., Theron, D. J., Steyn, P. J., Ferreira, D. I., and Eicker, A., Relationship between vegetative compatibility and pathogenicity of isolates of *Fusarium oxysporum* f. sp. *tuberosi* from potato. *Phytopathology*, **82**, 858–862 (1992).
- 36) Wheeler, M. H., Tolmsoff, W. J., and Meola, S., Ultrastructure of melanin formation in *Verticillium dahliae* with (+) -scytalone as a biosynthetic intermediate. *Can. J. Microbiol.*, **22**, 702–711 (1976).