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Vegetative compatibility groupings of *Verticillium dahliae* from cotton in mainland China

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Abstract

One hundred and fourteen isolates of *Verticillium dahliae* obtained from cotton and eggplant in mainland China were successfully assigned to two vegetative compatibility groups (VCGs) except for one self-incompatible isolate. Eleven isolates were strongly compatible with T9, the tester strain of the cotton defoliating pathotype, forming a linear growth of wild type with abundant microsclerotia and dense mycelia between compatible nitrate-nonutilizing mutants. The remaining 102 isolates were grouped into the non-defoliating VCG2, although the strength of the reaction varied; some isolates were strongly compatible with the tester strain while others were only slightly compatible. All VCG1 isolates including T9 showed the same defoliating symptom in greenhouse inoculation tests. This study confirmed the presence of the defoliating pathotype (VCG1) of *V. dahliae* in mainland China.

Introduction

Since *Fusarium* wilt in cotton has been successfully controlled by means of resistant cultivars, *Verticillium* wilt caused by *Verticillium dahliae* Kleb has become a major constraint in cotton production in mainland China (Shen, 1992). Several nation-wide outbreaks of *Verticillium* wilt occurred in China in 1993, 1995, and 1996. The disease occurred in limited areas in the 1980s (Gu et al., 1988). Currently, the disease is present in all major cotton cultivated areas in mainland China including Xingjiang which is regarded as the most important cotton growing area in the country.

Vegetative compatibility or anastomosis has been widely documented among several fungi and has proved to be a powerful tool in determining fungal genetic diversity (Puhalla, 1979, 1985; Anagnostakis et al., 1986; Correl et al., 1987; Joaquim and Rowe, 1990, 1991; Kistler, 1997; Leslie et al., 1996). Certain isolates that can anastomose and form heterokaryons with one another are assigned to a vegetative compatibility group (VCG). Isolates that are incapable of anastomosing with one another are considered to be vegetatively incompatible. Puhalla (1979) identified 16 VCGs in *V. dahliae* among a worldwide collection from several hosts using microsclerotial color mutants. Later, several researchers reassessed VCGs and confirmed the existence of only four VCGs using the nitrate-nonutilizing (*nit*) mutants method (Joaquim and Rowe, 1990; Strausbaugh et al., 1992). The vegetative compatibility of *V. dahliae* isolates from the United States and other western countries has been extensively studied (Chen, 1994; Corsini et al., 1985; Puhalla and Hummel, 1983; Katan and Katan, 1988; Strausbaugh, 1993; Daayf et al., 1995).

Although the pathogen causing *Verticillium* wilt in mainland China had been identified to be *V. dahliae* and not *V. albo-atrum* (Shen, 1992), no further information has been reported on the phenotypes of this fungus other than pathogenicity tests and colony characteristics (Gu et al., 1988). This study reports the vegetative compatibility groups of *V. dahliae* isolated from China, mainly from Jiangsu province since the 1980s.

Materials and methods

Maintenance of Verticillium *isolates.* One hundred and fifty isolates of *V. dahliae*, mainly from Jiangsu province, China were obtained and identified at the

Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, China. Isolates were maintained at 4 °C on potato dextrose agar (PDA) slants and subcultured every 6 months. The tester strains of the defoliating pathotype, T9, and the non-defoliating pathotype, P207, were supplied by Beijing Agricultural University.

Generation of nit *mutants.* The influence of different concentrations of potassium chlorate on induction of *nit* mutants was determined. Nitrate nonutilizing (*nit*) mutants were induced by culturing wild-type isolates on minimal medium (MM) (Puhalla, 1985) amended with 0.7 g of asparagine and different concentrations of potassium chlorate, viz. 15, 17.5, 20, 22.5, 25, 27.5 and 30 g l^{-1} in petri dishes at 25 °C. The potassium chlorate concentration of 22.5 g l^{-1} was chosen for further induction of *nit* mutants. After an incubation period of 1–2 weeks, chlorate-resistant sectors were subcultured on MM. On this medium, all colonies characterized by an expansive but thin mycelial growth were considered to be *nit* mutants.

Characterization of nit *phenotypes.* The phenotypes of all *nit* mutants were further characterized by growing them on MM and MM amended with sodium nitrite (0.4 g l^{-1}) or hypoxanthine (0.5 g l^{-1}). *Nit* mutants which were unable to utilize nitrate but able to use nitrite and hypoxanthine were designed as *nit* 1. *Nit* mutants incapable of using nitrate and hypoxanthine but capable of using nitrite were referred to as *nit* M (Correll et al., 1987; Joaquim and Rowe, 1990, 1991). *Nit* O was used to represent all other phenotypes.

Assignment of isolates to VCGs. All *nit* 1 mutants were paired with *nit* M mutants of the tester strains, and *nit* M mutants were paired with the *Nit* 1 mutants of the tester strains. The tester strains were T9 and P207, representatives of the defoliating and non-defoliating types, respectively.

Two *nit* mutants were placed 1.0–1.5 cm apart on MM in 9.0-cm-diameter petri plates. Heterokaryon formation between complementary mutants was detected when prototrophic growth developed at the mycelial interface between the *nit* mutants positioned at the center of the plate. Vegetative compatibility between isolates was determined by the formation of heterokaryons. Each pairing was repeated at least three times. Furthermore, the mutants derived from JC1 and SY12 from China were paired with all other *nit* mutants.

Pathogenicity tests. Pathogenicity studies were conducted to determine whether isolates identified as VCG1 (defoliating groups) would cause defoliation of inoculated cotton plants in the greenhouse. Seven isolates identified as VCG1, viz. VD8, JC1, JC4, JC5, SY11, SY12, and T9, and two non-defoliating isolates, BP2 and DF14, were selected. Inocula of all the above isolates were obtained by incubating cultures in wheat-seed media. Wheat seed was soaked in water for 48 h at room temperature. Drained wheat-seed was packed into flasks (400 g drained wheat-seed per flask) and then autoclaved at 121 °C for 1 h. A flask was inoculated with 10 ml of conidial suspension (10^8 spores ml^{-1}) of the test isolates and then incubated at 25 °C for 2 weeks. These wheat-seed media were air-dried and used as inocula. The *Verticillium*-susceptible cotton cultivar, Shi-2 was used as a check. Aluminum boxes ($45 \times 33 \times 20$ cm) containing an equal proportion of clay soil and sand were prepared. Dried wheat-seed inocula (100 g box^{-1}) were thoroughly mixed with the soil before sowing. Cotton plants were grown at a $25 \pm 2/18 \pm 2$ °C (day/night) regime in the greenhouse. The disease symptoms were observed daily. Each treatment contained 30 plants and the experiment was repeated twice.

Results

Development of nit *mutants.* The concentrations of potassium chlorate in minimal medium influenced the development of *nit* mutants for most isolates. Among the potassium chlorate concentrations tested, 22.5 g l^{-1} yielded the most *nit* mutants and was chosen for inducing *nit* mutants in further studies. On MM, all *nit* mutants were characterized by an expansive but thin mycelial growth.

Recovery of nit *phenotypes.* The *nit* mutants were divided into three phenotypes based on their growth on MM supplemented with various nitrogen sources. Among all mutants tested, 51.3%, 32.7% and 16.0% were characterized as *nit* 1, *nit* M and *nit* O, respectively. Both *nit* 1 and *nit* M mutants required for VCG tests were recovered from 58 isolates from the 1980s, 36 from 1991 and 20 from 1994 and 1995 (Table 1).

Table 1. Isolates of *Verticillium dahliae* obtained from cotton and eggplant in mainland China*

Number	Isolate	Year	Crop	Geographic origin	VCG
1–4	JV1001-4[1]	1980s[2]	Cotton	Dafeng county, Jiangsu	VCG2
5–10	JV1006-11	1980s	Cotton	Chiyang county, Jiangsu	VCG2
11	VD8	1980s	Cotton	Nantong county, Jiangsu	VCG1
12–21	JV2001-10	1980s	Cotton	Nantong county, Jiangsu	VCG2
22–24	JV2012-14	1980s	Cotton	Qidong county, Jiangsu	VCG2
25	JV2024	1980s	Cotton	Ludong county, Jiangsu	VCG2
26	JV2025	1980s	Cotton	Haimen county, Jiangsu	VCG2
27	JV2048	1980s	Cotton	Haimen county, Jiangsu	VCG2
28–29	JV3001-2	1980s	Cotton	Tongshan county, Jiangsu	VCG2
30	JV3003	1980s	Cotton	Nanjing city, Jiangsu	VCG2
31	JV3004	1980s	Cotton	Fengxian county, Jiangsu	VCG2
32	JV3005	1980s	Cotton	Pixian county, Jiangsu	VCG2
33	JV3006	1980s	Cotton	Sheyang county, Jiangsu	VCG2
34–38	JV4001-5	1980s	Cotton	Changsu county, Jiangsu	VCG2
39–42	JV4007-10	1980s	Cotton	Changsu county, Jiangsu	VCG2
43	JV4012	1980s	Cotton	Changsu county, Jiangsu	VCG2
44	JV4014	1980s	Cotton	Changsu county, Jiangsu	VCG2
45	JV4016	1980s	Cotton	Zhangjiagang county, Jiangsu	VCG2
46	JV4017	1980s	Cotton	Kunshan county, Jiangsu	VCG2
47	JV4018	1980s	Cotton	Nantong county, Jiangsu	Self-IC[4]
48	JV5001	1980s	Cotton	Tianmen county, Hubei	VCG2
49	JV5002	1980s	Cotton	Jingzhou county, Hubei	VCG2
50	JV5003	1980s	Cotton	Liaoling	VCG2
51	JV5004	1980s	Cotton	Shaanxi	VCG2
52	JV5005	1980s	Cotton	Hebei	VCG2
53	JV5006	1980s	Cotton	Xiangjiang	VCG2
54	JV5008	1998s	Cotton	Shandong	VCG2
55	JV6001	1980s	Eggplant[3]	Dafeng county, Jiangsu	VCG2
56	JV6002	1980s	Eggplant	Nantong county, Jiangsu	VCG2
57	JV6004	1980s	Eggplant	Nantong county, Jiangsu	VCG2
58	JV6006	1980s	Eggplant	Nantong county, Jiangsu	VCG2
59	JC1	1991	Cotton	Changsu county, Jiangsu	VCG1
60–61	JC2-3	1991	Cotton	Changsu county, Jiangsu	VCG2
62	JC4	1991	Cotton	Changsu county, Jiangsu	VCG1
63	JC5	1991	Cotton	Changsu county, Jiangsu	VCG1
64	JC6	1991	Cotton	Changsu county, Jiangsu	VCG2
65–67	JC7-9	1991	Cotton	Changsu county, Jiangsu	VCG2
68–75	SY1-8	1991	Cotton	Nantong county, Jiangsu	VCG2
76	SY10	1991	Cotton	Nantong county, Jiangsu	VCG2
77	SY11	1991	Cotton	Nantong county, Jiangsu	VCG1
78	SY12	1991	Cotton	Nantong county, Jiangsu	VCG1
79–81	SY13-15	1991	Cotton	Nantong county, Jiangsu	VCG2
82–87	DF1-6	1991	Cotton	Dafeng county, Jiangsu	VCG2
88–93	DF9-14	1991	Cotton	Dafeng county, Jiangsu	VCG2
94	BP2	1991	Cotton	Nanjing city, Jiangsu	VCG2
95	TS1	1994	Cotton	Tongshan county, Jiangsu	VCG1
96	TS2	1994	Cotton	Tongshan county, Jiangsu	VCG1
97–100	TS3-6	1994	Cotton	Tongshan county, Jiangsu	VCG2
101–102	HB1-2	1994	Cotton	Hebei	VCG2

Table 1. Continued

Number	Isolate	Year	Crop	Geographic origin	VCG
103	HB3	1994	Cotton	Hebei	VCG1
104	HB4	1994	Cotton	Hebei	VCG1
105–106	HB5-6	1994	Cotton	Hebei	VCG2
107–109	CY1-3	1995	Cotton	Sheyang county, Jiangsu	VCG2
110–113	SX1-4	1995	Cotton	Shaanxi	VCG2
114	HuB1	1995	Cotton	Tianmen county, HuBei	VCG1

* Isolates isolated and stored at the Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, China.
[1] JV1001-4 represents JV1001, JV1002, JV1003 and JV1004 respectively.
[2] Isolates from the 1980s were collected from 1980 to 1985.
[3] Eggplants located near heavily *Verticillium*-wilted cotton fields.
[4] Self-IC: self-incompatible.

VCGs of V. dahliae. Vegetative compatibility between unknown isolates and known VCG tester strains was indicated by the development of linear wild-type growth with abundant microsclerotia and/or dense mycelia at the interface between the mutants. Of all 114 isolates from which complementary *nit* 1 and/or *nit* M mutants were found, eleven isolates were assigned to VCG1. All of these were strongly compatible with the tester strain, T9, forming linear wild-type growth with abundant microsclerotia and dense mycelia. One hundred and two isolates were assigned to VCG2. These were compatible with P207, although some formed weak linear wild-type growth. One isolate, JV 4018, was self-incompatible (Table 1) (Jacobson et al., 1988).

JC1 and SY12 yielded both *nit* 1 and *nit* M mutants and were further tested for compatibility with all other *nit* mutants. These two isolates demonstrated the same compatibility with other VCG1 isolates as did the tester strain T9.

Among the 58 isolates obtained from cotton and eggplant in the 1980s, only one isolate, VD8 from Nantong county, Jiangsu province (Figure 1) was of the defoliating type corresponding to Puhalla's VCG 1. The other 56 isolates were of the non-defoliating type except for JV4018, which was found to be self-incompatible and incompatible with both tester strains. Five isolates obtained from two counties (four sites) in Jiangsu (Figure 1) in 1991 were classified as the defoliating type, and the other 31 isolates were of the non-defoliating type. The percentage of defoliating isolates increased from 1.7% in the 1980s (1980 to 1985) to 13.8% in 1991. Among the 20 isolates obtained during 1994 and 1995, five isolates from Jiangsu, Hebei and Hubei provinces (Figure 1) were of the defoliating type, VCG1. The remaining isolates were of the non-defoliating type. The percentage of defoliating isolates further increased to 16.7% in 1994 and 1995.

Pathogenicity tests. Cotton plants inoculated with VCG1 isolates (VD8, JC1, JC4, JC5, SY11, SY12 and T9) in the soil showed defoliating symptoms at the squaring stage. Some plants defoliated at early seedling stages, leaving bare stems. At maturity, these plants were totally defoliated. In contrast, inoculation with non-defoliating type isolates, DF4 and BP2, led to typical symptoms of leaf chlorosis without defoliation.

Discussion

Defoliating symptoms in cotton were reported in Nantong and Changsu county, Jiangsu province in China in 1983 (Gu et al., 1988; Shen, 1992). Phenotypes of *V. dahliae* isolates were described based on the pathogenicity to different plant species or cultivars and/or colony growth characteristics (Gu et al., 1988; Shen, 1992). The present study confirms the existence of *V. dahliae* isolates of the defoliating type (VCG1) in mainland China using vegetative compatibility analysis. Recovery of defoliating isolates increased from one county (one site) in the 1980s to two counties (four sites) in 1991. In 1995, two additional provinces, Hubei and Hebei were found to have defoliating isolates. The recent nation-wide distribution of huge quantities of cotton seed without implementation of

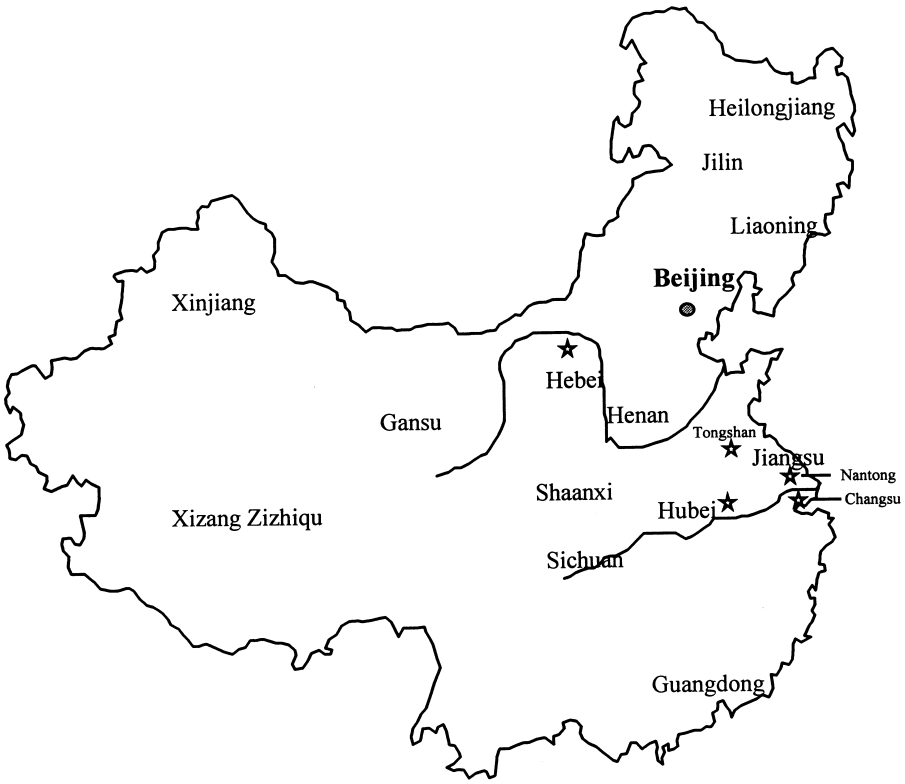

Figure 1. Locations in mainland China (⋆) from which isolates of *Verticillium dahliae* belonging to the defoliating pathotype were found.

proper quarantine procedures before distribution may have contributed to the widespread dissemination of the defoliating type.

The present study confirmed that all isolates identified as belonging to VCG1 were strongly virulent to cotton, and caused defoliating symptoms. Defoliating symptoms in cotton due to *Verticillium* wilt have become increasingly widespread since the 1980s in mainland China and are currently found in major cotton cultivated areas. This phenomenon is likely due to the widespread distribution of the defoliating pathotype of *V. dahliae.*

Four isolates obtained from eggplant near highly infected cotton fields were identified as VCG2 and the symptoms in eggplant were similar to those in cotton. The host range of *V. dahliae* is very broad (Horner, 1954; Krikun and Bernier, 1987; Dobinson et al., 1994; Subbarao et al., 1995) and it is suggested that *V. dahliae* isolates from eggplant and cotton may be very similar in genetics, and pathogenicity (Johnson et al., 1980). However, it appears that a greater genetic variation may exist in VCG2 as compared with VCG1, since some isolates were strongly compatible with the tester strains while others were not.

Isolates from more diverse geographic areas in China need to be studied further and the nature of vegetative compatibility among isolates from mainland China determined at a molecular level.

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