Vegetative Compatibility Groups in *Cercospora kikuchii*, the Causal Agent of Cercospora Leaf Blight and Purple Seed Stain in Soybean

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ABSTRACT

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Nitrogen nonutilizing (Nit) mutants were used to assess vegetative compatibility of 58 isolates of *Cercospora kikuchii*, 55 of which were isolated from soybean plants in Louisiana. Two isolates were vegetatively self-incompatible. Of 56 self-compatible isolates, 16 were assigned to six multimember vegetative compatibility groups (VCGs), 01 to 06, with 2 or 3 isolates in each VCG. The other 40 isolates each belonged to a distinct VCG. All six multimember VCGs contained isolates from different soy-

bean cultivars, and three included isolates from different locations. Only one of six multimember VCGs included isolates both from soybean leaves and seed, while the other five included isolates from only leaves or seed. The likelihood of tissue specificity or preference was discussed. All isolates and tested Nit mutants produced cercosporin on potato dextrose agar under light. Significantly different amounts of cercosporin were produced among wild-type isolates, and two Nit mutants produced significantly more cercosporin than their wild-type counterparts. All isolates produced typical *Cercospora* leaf blight symptoms on soybean plants in greenhouse pathogenicity tests.

Cercospora kikuchii (Matsumoto & Tomoyasu) M. W. Gardner infects leaves, stems, and pods of soybean (*Glycine max* (L.) Merr.), where it causes purple seed stain and Cercospora leaf blight (33,45,47). Purple seed stain on soybean was first reported in Korea in 1921 (45); whereas, in the United States, it was first reported in Indiana in 1924 (19). Purple seed stain is conspicuous and easily distinguished. It is characterized by irregular blotches that vary from light to dark purple and range from a tiny spot to covering the entire area of the seed coat. Purple seed stain does not reduce yields directly, but it may cause reduced stands (40). Under field conditions, symptoms of Cercospora leaf blight are observed at the beginning of and throughout seed set. Reddish purple, angular-to-irregular lesions occur on upper leaves exposed to sunlight. Numerous infections cause rapid chlorosis and necrosis of leaf tissue, resulting in defoliation, and can cause substantial yield loss (40).

More than 3,000 species have been listed in the genus *Cercospora*, of which some are synonyms (36). Some species previously placed in *Cercospora* have been segregated and reclassified into other genera (11–14,20,37,42). Most *Cercospora* spp., including *C. kikuchii*, have no known sexual stage, although a *Mycosphaerella* teleomorph has been identified for a few (5,6), such as the peanut pathogen, *Mycosphaerella arachidis* (*C. arachidicola*).

Little is known about the population structure of *C. kikuchii*. What are the roles of geographic separation and host genotype in maintaining diversity? Is there evidence for a sexual stage? Are there races in this fungus and, if so, how frequently do they arise? Vegetative compatibility has been useful in characterizing population structures of asexual fungi, including *Fusarium oxysporum* (1,3,16,17,26,27), *Colletotrichum* spp. (2,34), and *Verticillium*

spp. (7,24,25,28,39,43,44). Fungal strains able to undergo mutual hyphal anastomosis and form viable heterokaryons are assigned to the same vegetative compatibility group (VCG). Without sexual recombination, members of each VCG will form a genetically isolated subpopulation that will be subjected to standard population genetic forces such as selection, mutation, migration, and drift (21). On the other hand, members within a VCG have the potential to exchange genetic material through parasexual recombination.

The objective of this study was to examine population structure in *C. kikuchii* using vegetative compatibility grouping. A preliminary report was published (4).

MATERIALS AND METHODS

Isolation of *C. kikuchii* from soybean leaves and seed. In all, 28 soybean leaf samples showing typical Cercospora leaf blight symptoms and 27 seed samples showing purple stain symptoms were collected from three locations in Louisiana in 2000 and 2001. Of these, 28, 2, and 25 samples were collected from a single soybean field in Alexandria, Baton Rouge, and Winnsboro, LA, respectively. Each seed was separately soaked in sterile warm water (35°C) for 10 min, and the seed coat was peeled off with forceps. A single leaflet or seed coat from each sample was placed in a moist chamber at room temperature under diffuse daylight. A single conidium was picked off with a 0.20-mm-diameter pin under a dissecting microscope. The conidium was placed on sporulation medium, V8 agar (15) amended with 5 g of dried, senescent soybean leaf powder per liter (V8-SSLP), and incubated at 26°C under a 16-h photoperiod for 6 days. Morphological characters of conidia and conidiophores were observed with a compound microscope and used to confirm species identification.

Three isolates from other sources also were included in this study. Isolates NC and PR were provided by R. G. Upchurch (North Carolina State University, Raleigh). Isolate ATCC36864 was obtained from the American Type Culture Collection (Rock-

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TABLE 1. Isolates of *Cercospora kikuchii* used in this study listed according to vegetative compatibility groups (VCGs), host cultivar, tissue, and geographic origin

VCG, isolate ^a	Host cultivar	Tissue	Geographic origin ^b
01			
CKBR1	Hornbeck HBK R5588	Leaf	Baton Rouge, LA
DLL6013-1B	Asgrow AG6201	Leaf	Alexandria, LA
MRL6020-2B	SS RT 6299N	Leaf	Winnsboro, LA
02			
DLS5070-2B	Deltapine DP 5915 RR	Seed	Alexandria, LA
DLS5098-1B	Asgrow AG5902	Seed	Alexandria, LA
03			
DLS5070-3A	Deltapine DP 5915 RR	Seed	Alexandria, LA
DLS6020-4B	SS RT 6299N	Seed	Alexandria, LA
04			
DLS5012-1A	Asgrow AG5901	Seed	Alexandria, LA
DLS5070-2A	Deltapine DP 5915 RR	Seed	Alexandria, LA
MRS5012-1A	Asgrow AG5901	Seed	Winnsboro, LA
05			
DLS5012-4A	Asgrow AG5901	Seed	Alexandria, LA
MRL6020-4A	SS RT 6299N	Leaf	Winnsboro, LA
MRS5098-2B	Asgrow AG5902	Seed	Winnsboro, LA
06			
MRL5070-1B	Deltapine DP 5915 RR	Leaf	Winnsboro, LA
MRL5070-3A	Deltapine DP 5915 RR	Leaf	Winnsboro, LA
MRL6020-1A	SS RT 6299N	Leaf	Winnsboro, LA
SIVs ^c			
ATCC36864	Amsoy	Seed	Urbana, IL
CKBR2	SS RT 6299N	Leaf	Baton Rouge, LA
DLL5012-1A	Asgrow AG5901	Leaf	Alexandria, LA
DLL5012-3A	Asgrow AG5901	Leaf	Alexandria, LA
DLL5012-4B	Asgrow AG5901	Leaf	Alexandria, LA
DLL5070-2A	Deltapine DP 5915 RR	Leaf	Alexandria, LA
DLL5070-2B	Deltapine DP 5915 RR	Leaf	Alexandria, LA
DLL5070-4B	Deltapine DP 5915 RR	Leaf	Alexandria, LA
DLL5098-1A	Asgrow AG5902	Leaf	Alexandria, LA
DLL5098-1B	Asgrow AG5902	Leaf	Alexandria, LA
DLL5098-2B	Asgrow AG5902	Leaf	Alexandria, LA
DLL5098-3B	Asgrow AG5902	Leaf	Alexandria, LA
DLL6013-2B	Asgrow AG6201	Leaf	Alexandria, LA
DLL6020-1B	SS RT 6299N	Leaf	Alexandria, LA
DLL6020-3A	SS RT 6299N	Leaf	Alexandria, LA
DLL6020-4A	SS RT 6299N	Leaf	Alexandria, LA
DLS5070-1B	Deltapine DP 5915 RR	Seed	Alexandria, LA
DLS5098-3A	Asgrow AG5902	Seed	Alexandria, LA
DLS6013-1B	Asgrow AG6201	Seed	Alexandria, LA
DLS6013-3B	Asgrow AG6201	Seed	Alexandria, LA
DLS6013-4B	Asgrow AG6201	Seed	Alexandria, LA
DLS6020-4A	SS RT 6299N	Seed	Alexandria, LA
JL11	Eagle ES 41RR	Leaf	Winnsboro, LA
JL41	Eagle ES 41RR	Leaf	Winnsboro, LA
MRL5070-1A	Deltapine DP 5915 RR	Leaf	Winnsboro, LA
MRL6013-1A	Asgrow AG6201	Leaf	Winnsboro, LA
MRL6020-1B	SS RT 6299N	Leaf	Winnsboro, LA
MRL6020-3B	SS RT 6299N	Leaf	Winnsboro, LA
MRS5012-1B	Asgrow AG5901	Seed	Winnsboro, LA
MRS5012-3A	Asgrow AG5901	Seed	Winnsboro, LA
MRS5070-2A	Deltapine DP 5915 RR	Seed	Winnsboro, LA
MRS5070-3B	Deltapine DP 5915 RR	Seed	Winnsboro, LA
MRS5098-2A	Asgrow AG5902	Seed	Winnsboro, LA
MRS5098-3A	Asgrow AG5902	Seed	Winnsboro, LA
MRS6013-2A	Asgrow AG6201	Seed	Winnsboro, LA
MRS6013-3B	Asgrow AG6201	Seed	Winnsboro, LA
MRS6020-2B	SS RT 6299N	Seed	Winnsboro, LA
MRS6020-3B	SS RT 6299N	Seed	Winnsboro, LA
MRS6020-4A	SS RT 6299N	Seed	Winnsboro, LA
MRS6020-4B	SS RT 6299N	Seed	Winnsboro, LA
HSI ^d			
NC	...	...	NC
PR	...	Seed	PR

^a Of isolates from Louisiana, JL11 and JL41 were isolated from soybean leaf samples collected in 2000; other isolates were from leaf and seed samples collected in 2001.

^b LA = Louisiana, IL = Illinois, NC = North Carolina, and PR = Puerto Rico.

^c Single isolate vegetative compatibility groups.

^d Heterokaryon self-incompatible.

ville, MD). All 58 isolates (Table 1) were transferred to potato dextrose agar (PDA) and incubated at 26°C in darkness for 7 days, then stored at 4°C.

Pathogenicity tests. All isolates were tested as follows. Each isolate was transferred to a 10-cm-diameter V8-SSLP plate and incubated at 26°C under a 16-h photoperiod for 5 to 7 days. Colonies were flooded with approximately 10 ml of sterile distilled water and rubbed with a glass rod to dislodge conidia and mycelial fragments. Half a milliliter of this conidial and mycelial suspension was used to inoculate each V8-SSLP plate, with five plates per isolate. The plates were shaken gently to distribute the suspension across the agar surface. After incubation as described above for 6 days, conidia from each plate were harvested in 20 ml of sterile distilled water amended with 0.3% Tween 20. Colonies were rubbed gently to minimize mycelia in the suspension. Conidial concentrations for each isolate were determined with a hemacytometer. Except for isolates NC and PR, which produced very few conidia, the concentrations were 10⁴/ml or higher. Mycelia of these two isolates were harvested by scratching the agar surface, and the suspensions were triturated with a Waring blender. The mycelial suspensions for these two isolates were used as inoculum.

Three soybean seeds of cv. SS RT 6299N (Southern States Cooperative, Inc., Richmond, VA) were planted in each 1-liter plastic pot in a mixture of steam-disinfested soil:sand:Jiffy Mix (Jiffy Products of America, Inc., Batavia, IL), 1:1:2 (vol/vol/vol). Plants were fertilized with slow-release fertilizer (Osmocote, Marysville, OH). After 10 days, plants were thinned to one plant per pot. These plants were inoculated at the V3 growth stage (18). Conidia or mycelial suspensions from each isolate were applied using an atomizer onto soybean leaves from all directions until runoff. Three replicate plants were used for each isolate. After inoculation, plants were placed in a moist chamber built with transparent plastic sheeting on a greenhouse bench. The moist chamber was closed at 6:00 p.m. and opened at 10:00 a.m. every day. After the chamber was closed, plants were sprayed with a fine mist for 30 s. Temperature in the chamber ranged from 16 to 26°C at night and 22 to 31°C during the daytime, and relative humidity ranged from 90 to 100% when the chamber was closed and 40 to 90% when the chamber was open. After 7 days, the plastic coverings were removed, and plants were kept on the bench for another 14 days to allow for disease development. Leaves above the second trifoliate leaf were removed every 3 days so that the inoculated leaves received direct sunlight.

Generation and characterization of nitrate-nonutilizing mutants. Based on a preliminary study (4), PDA containing 3% KClO₃ was used to generate nitrate-nonutilizing (Nit) mutants from *C. kikuchii*. Chlorate-resistant sectors on this medium were transferred to minimal medium (MM) containing NaNO₃ at 2 g/liter (38). Nit mutants grew on MM with a thin, expansive mycelium. They were assigned to physiological phenotypes (*nit1*, *NitM*, and *nit3*) based on their growth on MM with NaNO₃, hypoxanthine, ammonium tartrate, or uric acid, which replaced NaNO₃ as the only nitrogen source (8). In initial tests, most Nit mutants did not grow on MM with NaNO₃ at 0.5 g/liter as the only nitrogen source. A subset of Nit mutants was tested on MM with different concentrations of NaNO₃ (0.05, 0.1, 0.25, and 0.5 g/liter), and NaNO₃ at 0.05 g/liter was found to support growth of these Nit mutants. This concentration of NaNO₃ was used to test all Nit mutants.

Vegetative compatibility grouping. A *nit1* mutant and a *NitM* mutant were chosen from each isolate. Complementation was assessed on MM. Nit mutants were paired on MM plates ≈2 cm apart and incubated at 26°C in darkness. For any two isolates, four pairings, between complementary Nit mutants and between Nit mutants of the same phenotype, were tested. Nit mutants from the same isolate were paired to test self-compatibility. Plates were scored for prototrophic growth 4 to 6 weeks after inoculation.

Complementation was evident by the formation of a wild-type growth zone formed where the two colonies met. Two isolates were assigned to the same VCG if heterokaryons formed in the two pairings between their complementary Nit mutants.

Selection of Nit testers. Within each multimember VCG, Nit mutants of compatible phenotypes were paired in all possible combinations with four replicates. The robustness of heterokaryon formation was estimated visually at 6 weeks. One *nit1* and one NitM that consistently formed strong heterokaryons were chosen as Nit testers for each VCG.

Cercosporin production. All 58 isolates and 140 Nit mutants, including the 116 Nit mutants used in complementation tests, were tested for cercosporin production. A 6-mm-diameter mycelial plug was transferred to the center of a 10-cm-diameter PDA plate and incubated at 25°C under a 16-h photoperiod (fluorescent light, 4,000 lux). Plates were observed for cercosporin production 7 days after inoculation by noting the production of a red or red-purple pigment in the agar. Cercosporin concentration was assessed for seven isolates and 16 Nit mutants using a method described by Jenns et al. (23). Three 6-mm-diameter mycelial plugs were cut from the colony margin 120° apart and soaked in 5 ml of 5 N KOH in darkness overnight. Absorbance of the soaking solutions was measured at 480 nm, which is the visible absorbance maximum of cercosporin in alkaline solution (48), using a spectrophotometer (Spectronic 601; Milton Roy, Rochester, NY). Three plates were used for each isolate and Nit mutant, and the experiment was conducted twice. Cercosporin production among isolates and Nit mutants was compared statistically using Tukey's pair-wise comparison test ($P = 0.05$) (Minitab release 13; Minitab Inc., State College, PA).

RESULTS

Isolation of *C. kikuchii* from soybean leaves and seed. Abundant conidia with minimal mycelia were produced on leaflets and seed coats in 48 to 72 h. In preliminary tests, we used whole soybean seed instead of seed coats for isolation. However, abundant mycelia grew on the seed, which made single-conidial isolation difficult. Three to five single-conidial isolations were made from each of the 55 samples, and one was randomly chosen to be included in this study. All isolates sporulated on V8-SSLP medium. Conidia and conidiophores typical of *C. kikuchii* (5) were readily observed with a compound microscope.

Pathogenicity tests. All 58 isolates caused leaf spot symptoms on soybean plants. Symptom expression was similar to that observed in the field. Pinpoint, reddish purple spots appeared on inoculated leaves 6 to 7 days after inoculation. The spots gradually enlarged to angular-to-irregular lesions. Symptoms such as chlorosis, necrosis, and a light purple, leathery appearance also were observed on inoculated leaves. Percentage of leaf surface showing symptoms was estimated visually 21 days after inoculation (data not shown).

Generation and characterization of Nit mutants. *C. kikuchii* colonies were olive green to light brown with concentric rings and sparse aerial mycelium on PDA containing 3% KClO₃. Radial growth was not greatly restricted. Chlorate-resistant sectors, which had dense aerial mycelium and occasionally produced reddish brown pigment, appeared in ≈3 weeks. These sectors were slightly faster growing, which was evident when they emerged from the colony perimeters. Sectors that grew on MM as expansive colonies with thin mycelium were presumed to be Nit mutants. Nit mutants were tested for phenotype until at least one *nit1* and one NitM were identified for each isolate. Five Nit mutants from the same isolate were transferred to an MM plate, and those that formed heterokaryons with neighboring Nit mutants were tested first. Of tested Nit mutants, 74% were *nit1* and 25% were NitM.

Assignment of *C. kikuchii* isolates to VCGs. Results of vegetative compatibility grouping are shown in Table 1. Isolates

NC and PR were self-incompatible. Of the 56 self-compatible isolates, 16 were grouped into six multimember VCGs, 01 to 06, whereas the other 40 isolates were not vegetatively compatible with any other isolates. There was no dominant VCG. Each multimember VCG included only two or three isolates. Among isolates in each VCG, pairings between Nit mutants of compatible phenotypes always formed prototrophic heterokaryons, whereas those between Nit mutants of the same phenotypes did or did not. Of the six multimember VCGs, three (VCGs 01, 04, and 05) included isolates from different locations, whereas the other three included isolates from single locations. Only VCG 05 included isolates from both soybean leaves and seed. The other five VCGs contained isolates from either soybean leaves or seed. All six VCGs included isolates from different soybean cultivars. In all, 28 and 25 isolates were recovered from a single soybean field in Alexandria and Winnsboro, LA, respectively; 25 and 22 VCGs were identified among isolates from Alexandria and Winnsboro, respectively. Three VCGs contained isolates from both fields.

Selection of Nit testers. Compared with other pairings in the same VCG, heterokaryon formation was significantly weaker (and occasionally discontinuous) in the following pairings: in VCG04, DLS5070-2A-1 (NitM)/MRS5012-1A-2 (*nit1*) and in VCG05, DLS5012-4A-1 (NitM)/MRS5098-2B-2 (*nit1*). For each VCG, one pair of Nit mutants, one *nit1* and one NitM, that consistently formed strong heterokaryons with Nit mutants of compatible phenotypes from isolates within the same VCG were chosen as Nit testers for future studies (Table 2).

Cercosporin production. All 58 isolates and 140 Nit mutants produced cercosporin. Significantly different amounts of cercosporin were produced among the seven wild-type isolates that were subjected to quantitative analysis, and two Nit mutants, one *nit1* and one NitM, produced significantly more cercosporin than their wild-type counterparts (Fig. 1).

DISCUSSION

This is the first study on vegetative compatibility of *C. kikuchii*. Clearly, this technique provides a very useful means to examine population structure of this fungus. *C. kikuchii* showed high VCG diversity, with 56 self-compatible isolates distributed among 46 VCGs with no dominant VCG. Previous studies generally revealed low VCG diversity in highly pathogenic groups in *F. oxysporum* and *Verticillium* spp., whereas nonpathogenic groups showed high VCG diversity. For example, in *F. oxysporum* f. sp. *lycopersici*, 106 of 107 isolates collected from Australia, Mexico, and several states in the United States were grouped into only four VCGs (32); in *F. oxysporum* f. sp. *radicis-lycopersici*, of a worldwide collection of 218 isolates, 215 were grouped into seven VCGs, some of which were further divided into subgroups (27); and in *F. oxysporum* f. sp. *melonis*, 107 of 119 isolates collected from four countries were included in four VCGs (22). On

TABLE 2. Nitrate nonutilizing (Nit) mutants of *Cercospora kikuchii* chosen as Nit testers for the six multimember vegetative compatibility groups (VCGs)

VCG	Nit	Phenotype
01	DLL6013-1B-9	NitM
	DLL6013-1B-8	<i>nit1</i>
02	DLS5070-2B-1	NitM
	DLS5070-2B-3	<i>nit1</i>
03	DLS5070-3A-1	NitM
	DLS6020-4B-2	<i>nit1</i>
04	DLS5012-1A-4	NitM
	DLS5012-1A-5	<i>nit1</i>
05	MRL6020-4A-1	NitM
	MRL6020-4A-5	<i>nit1</i>
06	MRL5070-3A-1	NitM
	MRL5070-3A-3	<i>nit1</i>

Fig. 1. Mean cercosporin concentrations produced by isolates of *Cercospora kikuchii* and nitrate nonutilizing (Nit) mutants on 6-mm plugs of potato dextrose agar. Cercosporin concentrations produced by wild-type isolates labeled with the same letter are not significantly different. Nit mutants that produced significantly different amounts of cercosporin compared with their wild-type counterparts are marked with “*”. All comparisons were based on Tukey’s pair-wise comparison tests ($P = 0.05$).

the other hand, 186 of 317 nonpathogenic *F. oxysporum* isolates from asymptomatic tomato roots collected in Louisiana were grouped into 48 multimember VCGs, and the other 131 isolates did not form heterokaryons with any isolates other than themselves (17). In *Verticillium* spp., low VCG diversity, similar to that observed in different formae speciales in *F. oxysporum*, was found in the highly pathogenic species *Verticillium albo-atrum* (7,25,39) and *V. dahliae* (24,28,43,44), whereas higher VCG diversity was observed in *V. nigrescens* and *V. tricorpus*, which are considered to be weak pathogens or saprotrophs (29). VCG diversity in *C. kikuchii* is similar to that observed in nonpathogenic *F. oxysporum* populations.

Sexually reproducing populations are expected to have high VCG diversity (31). In all, 7 to 15 heterokaryon incompatibility (*het* or *vic*) loci have been identified in fungi in which these loci have been most intensively studied (30). In a fungus with 10 *vic* loci and two alleles per locus, there will be 1,024 possible VCGs. If it reproduces sexually more often than selection or genetic drift can eliminate most VCGs, there always will be a large number of VCGs in its population. The large number of VCGs in *F. moniliforme*, which has a known sexual stage, was attributed to sexual reproduction (31). Leslie (31) suggested that high VCG diversity in nonpathogenic populations of *F. oxysporum* indicates that it may have a cryptic sexual stage.

When the sexual stage is lost, exchange of genetic materials is assumed to be limited to isolates in the same VCG, and VCGs become genetically isolated subpopulations. VCGs can be lost because of selection or genetic drift. If each VCG is equally fit, VCGs will be lost by chance to genetic drift, and the number of VCGs could be used as a measure of the age of the population with respect to the last sexual event (30). There is no information about the rate of extinction of VCGs. If the rate is not very high, and the sexual stage was lost recently, most VCGs will still exist in the population. High VCG diversity in *C. kikuchii* suggests that either this fungus has a covertly occurring sexual stage or it was lost relatively recently.

Of the 56 self-compatible *C. kikuchii* isolates, only one isolate from a soybean leaf was vegetatively compatible with two isolates

from soybean seed (VCG 05). One explanation for this scenario is that, because *C. kikuchii* has such a high VCG diversity, two isolates are more likely to be in different VCGs than in the same VCG. However, because five out of six multimember VCGs contained isolates from only leaves or seed, we hypothesize that tissue specificity or preference also played a role. Isolates in some VCGs may be more likely to infect soybean leaves while isolates in other VCGs are better adapted to seed infection, thus leading to the distinct VCG compositions between leaf and seed populations observed in this study. Previous studies support this hypothesis. When screening soybean cultivars for resistance, Walters (47) reported that some soybean cultivars were resistant to seed infection but susceptible to leaf infection. In other words, the isolates used in these inoculations were more likely to be found in leaves than in seed in these cultivars. Furthermore, Orth and Schuh (35) reported that the incidence of purple seed stain in 17 soybean cultivars was unrelated to disease severity on the foliage. Further studies are necessary to test this hypothesis.

Cercosporin plays a critical role in the ability of *C. kikuchii* and other cercosporin-producing *Cercospora* spp. to infect their hosts (9,10,46). Culture parameters, such as nitrogen sources and carbon:nitrogen ratio, have been shown to affect cercosporin production in several *Cercospora* spp., including *C. kikuchii* (10,41). However, the same conditions that enhance cercosporin production in one species or isolate may have negative or no effect on another (10). In this study, 2 of 16 Nit mutants, one *nit1* and one *NitM*, produced significantly more cercosporin than their wild-type counterparts. This suggests that certain mutations in the nitrate utilization pathway may enhance cercosporin production in some isolates of *C. kikuchii*, but mutations in the same locus or loci may not significantly affect cercosporin production in other isolates of the same species.

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LITERATURE CITED

- Appel, D. J., and Gordon, T. R. 1994. Local and regional variation in populations of *Fusarium oxysporum* from agricultural field soils. *Phytopathology* 84:786-791.
- Brooker, N. L., Leslie, J. F., and Dickman, M. B. 1991. Nitrate nonutilizing mutants of *Colletotrichum* and their use in studies of vegetative compatibility and genetic relatedness. *Phytopathology* 81:672-677.
- Cai, G., Gale, L. R., Schneider, R. W., Kistler, H. C., Davis, R. M., Elias, K. S., and Miyao, E. M. 2003. Origin of race 3 of *Fusarium oxysporum* f. sp. *lycopersici* at a single site in California. *Phytopathology* 93:1014-1022.
- Cai, G., and Schneider, R. W. 2002. Use of nitrate nonutilizing mutants to examine vegetative compatibility groups in *Cercospora kikuchii*. (Abstr.) *Phytopathology* 92(suppl.):S11.
- Chupp, C. 1954. A Monograph of the Fungus Genus *Cercospora*. Published by the author, Ithaca, NY.
- Corlett, M. 1991. An annotated list of the published names in *Mycosphaerella* and *Sphaerella*. *Mycol. Mem.* 18:1-328.
- Correll, J. C., Gordon, T. R., and McCain, A. H. 1988. Vegetative compatibility and pathogenicity of *Verticillium albo-atrum*. *Phytopathology* 78:1017-1021.
- Correll, J. C., Klittich, C. J. R., and Leslie, J. F. 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology* 77:1640-1646.
- Daub, M. E., and Ehrenshaft, M. 1997. The photoactivated toxin cercosporin: Toxicity, resistance, regulation and role in disease. Pages 37-66 in: *Toxins in Plant Disease Development and Evolving Biotechnology*. R. K. Upadhyay and K. G. Mukerji, eds. Science Publishers, Enfield, NH.
- Daub, M. E., and Ehrenshaft, M. 2000. The photoactivated *Cercospora* toxin cercosporin: Contributions to plant disease and fundamental biology. *Annu. Rev. Phytopathol.* 38:461-490.
- Deighton, F. C. 1967. Studies on *Cercospora* and allied genera. II. *Passalora*, *Cercosporidium*, and some species of *Fusicladium* on *Euphorbia*. *Mycol. Pap.* 112:1-80.
- Deighton, F. C. 1973. Studies on *Cercospora* and allied genera. IV. *Cercosporiella* Sacc., *Pseudocercosporiella* gen. nov. and *Pseudocercosporidium* gen. nov. *Mycol. Pap.* 133:1-62.
- Deighton, F. C. 1976. Studies on *Cercospora* and allied genera. VI. *Pseudocercospora* Speg., *Pantospora* Cif. and *Cercoseptoria*. *Mycol. Pap.* 140:1-168.
- Deighton, F. C. 1979. Studies on *Cercospora* and allied genera. VII. New species and redispositions. *Mycol. Pap.* 144:1-56.
- El-Gholl, N. E., Alfieri, S. A., Jr., Riding, W. H., and Schoulties, C. L. 1982. Growth and sporulation *in vitro* of *Cercospora apii*, *Cercospora arachidicola*, *Cercospora kikuchii*, and other species of *Cercospora*. *Can. J. Bot.* 60:862-868.
- Elias, K. S., and Schneider, R. W. 1991. Vegetative compatibility groups in *Fusarium oxysporum* f. sp. *lycopersici*. *Phytopathology* 81:159-162.
- Elias, K. S., Schneider, R. W., and Lear, M. M. 1991. Analysis of vegetative compatibility groups in nonpathogenic populations of *Fusarium oxysporum* isolated from symptomless tomato roots. *Can. J. Bot.* 69:2089-2094.
- Fehr, W. R., Caviness, C. E., Burmood, D. T., and Pennington, J. S. 1971. Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. *Crop Sci.* 11:929-931.
- Gardner, M. W. 1926. Indiana plant diseases, 1924. *Proc. Ind. Acad. Sci.* 35:237-257.
- Goodwin, S. B., Dunkle, L. D., and Zismann, V. L. 2001. Phylogenetic analysis of *Cercospora* and *Mycosphaerella* based on the internal transcribed spacer region of ribosomal DNA. *Phytopathology* 91:648-658.
- Hartl, D. L., and Clark, A. G. 1989. *Principles of Population Genetics*, 2nd ed. Sinauer Associates, Sunderland, MA.
- Jacobson, D. J., and Gordon, T. R. 1989. Further investigation of vegetative compatibility within *Fusarium oxysporum* f. sp. *melonis*. *Can. J. Bot.* 68:1245-1248.
- Jenns, A. E., Daub, M. E., and Upchurch, R. G. 1989. Regulation of cercosporin accumulation in culture by medium and temperature manipulation. *Phytopathology* 79:213-219.
- Joaquim, T. R., and Rowe, R. C. 1991. Vegetative compatibility and virulence of strains of *Verticillium dahliae* from soil and potato plants. *Phytopathology* 81:552-558.
- Katan, T. 2000. Vegetative compatibility in populations of *Verticillium*—An overview. Pages 69-86 in: *Advances in Verticillium, Research and Disease Management*. E. C. Tjamos, R. C. Rowe, J. B. Heal, and D. R. Fravel, eds. The American Phytopathological Society, St. Paul, MN.
- Katan, T., and Katan, J. 1988. Vegetative compatibility grouping of *Fusarium oxysporum* f. sp. *vasinfectum* from tissue and the rhizosphere of cotton plants. *Phytopathology* 78:852-855.
- Katan, T., Zamir, D., Sarfatti, M., and Katan, J. 1991. Vegetative compatibility groups and subgroups in *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Phytopathology* 81:255-262.
- Korolev, N., Katan, J., and Katan, T. 2000. Vegetative compatibility groups of *Verticillium dahliae* in Israel: Their distribution and association with pathogenicity. *Phytopathology* 90:529-536.
- Korolev, N., and Katan, T. 1999. Vegetative compatibility grouping in *Verticillium nigrescens* and *V. tricornutum*. *Mycol. Res.* 103:65-76.
- Leslie, J. F. 1990. Genetic exchange within sexual and asexual populations of the genus *Fusarium*. Pages 37-48 in: *Fusarium Wilt of Banana*. R. C. Ploetz, ed. The American Phytopathological Society, St. Paul, MN.
- Leslie, J. F. 1993. Fungal vegetative compatibility. *Annu. Rev. Phytopathol.* 31:127-150.
- Marlatt, M. L., Correll, J. C., and Kaufmann, P. 1996. Two genetically distinct populations of *Fusarium oxysporum* f. sp. *lycopersici* race 3 in the United States. *Plant Dis.* 80:1336-1342.
- Matsumoto, T., and Tomoyasu, R. 1925. Studies on the purple speck of soybean seed. *Ann. Phytopathol. Soc. Jpn.* 1:1-14.
- Nitzan, N., Hazanovsky, M., Tal, M., and Tsror (Lahkim), L. 2002. Vegetative compatibility groups in *Colletotrichum coccodes*, the causal agent of black dot on potato. *Phytopathology* 92:827-832.
- Orth, C. E., and Schuh, W. 1994. Resistance of 17 soybean cultivars to foliar, latent, and seed infection by *Cercospora kikuchii*. *Plant Dis.* 78:661-664.
- Pollack, F. G. 1987. An annotated compilation of *Cercospora* names. *Mycol. Mem.* 12:1-212.
- Pons, N., and Sutton, B. C. 1988. *Cercospora* and similar fungi on yams (*Dioscorea* species). *Mycol. Pap.* 160:1-78.
- Puhalla, J. E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. *Can. J. Bot.* 63:179-183.
- Rowe, R. C. 1995. Recent progress in understanding relationships between *Verticillium* species and subspecific groups. *Phytoparasitica* 23:31-38.
- Schuh, W. 1999. *Cercospora* blight, leaf spot, and purple seed stain. Pages 17-18 in: *Compendium of Soybean Diseases*. 4th ed. G. L. Hartman, J. B. Sinclair, and J. C. Rupe, eds. The American Phytopathological Society, St. Paul, MN.
- Shim, W.-B., and Dunkle, L. D. 2002. Identification of genes expressed during cercosporin biosynthesis in *Cercospora zeae-maydis*. *Physiol. Mol. Plant Pathol.* 61:237-248.
- Stewart, E. L., Liu, Z., Crous, P. W., and Szabo, L. J. 1999. Phylogenetic relationships among some cercosporoid anamorphs of *Mycosphaerella* based on rDNA sequence analysis. *Mycol. Res.* 103:1491-1499.
- Strausbaugh, C. A. 1993. Assessment of vegetative compatibility and virulence of *Verticillium dahliae* isolates from Idaho potatoes and tester strains. *Phytopathology* 83:1253-1258.
- Strausbaugh, C. A., Schroth, M. N., Weinhold, A. R., and Hancock, J. G. 1992. Assessment of vegetative compatibility of *Verticillium dahliae* tester strains and isolates from California potatoes. *Phytopathology* 82:61-68.
- Suzuki, K. 1921. Studies on the cause of "Shihan" of soybean. *Chosen Nakaiho* 16:24-28.
- Upchurch, R. G., Walker, D. C., Rollins, J. A., Ehrenshaft, M., and Daub, M. E. 1991. Mutants of *Cercospora kikuchii* altered in cercosporin synthesis and pathogenicity. *Appl. Environ. Microbiol.* 57:2940-2945.
- Walters, H. J. 1980. Soybean leaf blight caused by *Cercospora kikuchii*. *Plant Dis.* 64:961-962.
- Yamazaki, S., and Ogawa, T. 1972. The chemistry and stereochemistry of cercosporin. *Agric. Biol. Chem.* 36:1707-1718.