Inheritance of Compatibility and Sex in *Gibberella baccata*

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ABSTRACT

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Isolates of *Gibberella baccata* (=*Fusarium lateritium*) were mated and produced perithecia on carrot agar at 22 C under mixed cool-white and black fluorescent lights on a 12-hr alternating light/dark schedule. Of the cross-fertile isolates, most were hermaphrodites, although three were males and one was tentatively identified as a female. Data from test crosses indicated that the two compatibility groups were allelomorphs not linked to the gene(s) controlling sexual expression. Isolates proved interfertile irrespective of origin and host.

Gibberella baccata (Wallr.) Sacc. (=*Fusarium lateritium* Nees) has a broad host range and is distributed worldwide. It causes a canker and dieback on a wide range of woody plants (3). In Africa, notably Nigeria and Benin, where *Celosia argentea* L. is grown as a popular staple leaf vegetable, *F. lateritium* is the causal agent of a serious leaf and stem spot (2).

G. baccata has been reported to be heterothallic (7,8) or heterothallic and homothallic (3) and forms perithecia in culture (1–3) and in nature (14). Homothallic strains rarely produce perithecia on agar but will produce them on materials such as sterile wheat straw (3). In studies of *F. lateritium* from Nigeria, Afanide et al (1) found all isolates that were tested to be self-sterile; perithecia were produced only from matings between stromatic and nonstromatic isolates. From these results, they suspected that a compatibility factor was present.

To date, only random matings of this fungus have been performed, and the nature of the compatibility and sexual systems remain unclear. The purpose of this work was to elucidate the nature of the compatibility and sexual system in *G. baccata* by means of a series of crosses and testcrosses.

MATERIALS AND METHODS

Isolates of *G. baccata* from the collection of the Fusarium Research Center were utilized in a series of eight crosses and two testcrosses. Media and cultural conditions were varied in an attempt to identify conditions that would yield optimum results even for minimally fertile isolates.

Media used were potato-dextrose agar (PDA) (17), γ-irradiated carnation leaf agar (CLA) (5), V-8 juice agar (V8A), wheat straw agar (WSA), and wheat stem piece agar (WSPA) prepared as described by Fisher et al (5). Stem pieces of *Triticum aestivum* cultivar Michigan Amber/#8 Chancellor, CI 15888, 5 cm long, were dried in an oven at 45–55 C and sterilized with 2.7 megarads of gamma irradiation from a cobalt 60 source or kept in the refrigerator until they were sterilized for 1 hr with ethanol (WSP-EtOH), a treatment that stimulated production of perithecia of *Gibberella zeae* (Schw.) Petch. (18).

Media containing carrot included carrot extract agar (CEA) (6) prepared with double-distilled water instead of tap water and carrot potato-dextrose agar (CPDA). The latter was prepared in the same manner as the CEA with the addition of 250 g of scrubbed potato chunks to the carrots before steaming and the addition of 20 g of dextrose to the neutralized potato-carrot extract at pH 7.0–7.2. Carrot puree agar (CA) was prepared by steaming 200 g of scrubbed carrot pieces and 5 g of agar separately in 150 ml of double-distilled water for 20 min. The steamed carrots were then blended into a fine suspension in a Waring blender, added to the water agar, and enough double-distilled water was added to bring the volume to 500 ml.

All cultures were grown on 10-ml agar slants in test tubes at a constant temperature of 23 C with a 12-hr photoperiod. Cool-white fluorescent (Echelon Corp. F40+12/28/RFL) or black fluorescent (Sylvania F40-BLB) lamps were used in various combinations at a height of 42 cm above the cultures. The cool-white fluorescent lamps emitted a spectrum from 310 to 700 nm and the black fluorescent lamps emitted a spectrum from 300 to 500 nm (11). For all matings, each isolate was started from a single conidium (17) from lyophilized material and the tubes were sealed with cigarette paper (16) to exclude culture mites while allowing for adequate air exchange (13).

Isolates acting as males (the spermatizing agent) in matings were started from single conidia and placed on CLA and grown under cool-white fluorescent lamps. Isolates acting as females in matings were placed on any of several media described above, and grown under any of the different light conditions previously noted.

Spermatization was carried out 21–30 days after the cultures were initiated. A heavy conidial suspension of the male parent, free of mycelial debris, was prepared in sterile double-distilled water; 0.33 ml of this suspension was spread evenly over the surface of the female culture. The tubes were returned to the light banks and resealed after about 2 days when excess water had evaporated. Each of the eight matings, except number four, was set up as a complete diallel.

Random F_1 progeny were recovered from L88 × L90 from cross number five and L90 × L86 from cross number seven for testcross analysis. Mature ascospores were present 23 days after spermatization. Random ascospores were obtained from perithecia that had been cleaned in sterile water by removing any debris, conidia, and loose mycelium. The cleaned perithecia were transferred to a sterile glass slide, crushed, and checked for the presence of mature ascospores. If mature ascospores were present,

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the crushed perithecia were washed onto a large (8.5-cm diameter) petri dish of 2% water agar. The following day, germinated ascospores were identified under a microscope, marked, and transferred to PDA slants. All progeny from a given cross were placed on PDA that had been prepared at the same time.

These tubes were placed under cool-white fluorescent lamps for 2 wk then were evaluated for colony morphology. At this time each progeny isolate was assigned a Lateritium Filial (LF) number.

Single spores of all F_1s to be used in the testcross were placed on CA slants. The testcrosses were set up so that each F_1 isolate acted as a male for the female parent in the original cross, and as a female for the male parent in the original cross. The original male parent was grown on CLA under fluorescent lamps, while the rest of the isolates were grown under mixed light sources. Each tube in the cross was checked for mature perithecia 3 wk after spermatization and periodically for an additional 2 mo.

RESULTS

Mating type and sex. Of the 29 isolates tested, 16 crossed successfully with one or more of the other isolates. As indicated in Fig. 1, none of the isolates produced the perfect state when selfed (indicated by the dashed diagonal line). Instead, the isolates segregated into two groups that were intrasterile and interfertile. The majority of the isolates tested proved to be hermaphroditic, while four were unisexual. Isolates L83, L86, and L95 only acted as males in crosses, while L55 appeared to be a female, although it was so in only one cross and that designation is tentative. Accordingly, fertile isolates were separated into two incompatibility groups, mating type + (mt+) and mating type − (mt−) as shown in Table 1.

The number of loci involved in coding for mating type (mt) was determined by testcross analysis. Of 77 random F_1s from the cross L88 (mt+ hermaphrodite) × L90 (mt− hermaphrodite), 64 proved to be fertile. Thirty-two of the fertile F_1s crossed successfully with L88 indicating the F_1s were mt− and 32 crossed successfully with L90 indicating the F_1s were mt+. This 1:1 ratio of mt+:mt− would be expected if mating type is controlled by one gene with two alleles ($\chi^2 = 0.008$, d.f. = 1, and $P = 9$ [Yates' correction factor]) (15).

The second testcross involved 169 randomly selected progeny from the cross L90 (mt− hermaphrodite) × L86 (mt+ male). The results of this testcross are shown in Table 2 and support the hypothesis that mating type is controlled by one gene with two alleles which is not linked to the gene or genes controlling expression of sexual type.

Cultural requirements for production of perithecia. Perithecia were produced most abundantly when the maternal isolate was grown under a mixture of cool-white and black fluorescent light. In general, CA was the most suitable medium for production of perithecia although isolates L70, L73, L75, and L76 fruited more vigorously on γ-irradiated WSPA. Mature perithecia appeared 24–55 days after spermatization depending on the isolates involved. Perithecia developed in the same length of time in reciprocal crosses.

TABLE 1. Mating type designations and sources of isolates of *Gibberella baccata* used in this study

Isolate	Origin	Host	Sex[a]	Mating type
L55	Louisiana	Elm canker	F	+
L70	Zimbabwe	*Coffea arabica* berries	FM	−
L73	Zimbabwe	*Coffea arabica* berries	FM	+
L75	Zimbabwe	*Coffea arabica* berries	FM	−
L76	Zimbabwe	*Coffea arabica* berries	FM	−
L83	Papua New Guinea	Coffee berries[b]	M	−
L85	Papua New Guinea	Coffee berries	FM	−
L86	Papua New Guinea	Coffee berries	M	+
L87	Papua New Guinea	Coffee berries	FM	+
L88	Papua New Guinea	Coffee berries	FM	+
L89	Papua New Guinea	Coffee berries or twigs	FM	−
L90	Papua New Guinea	Coffee berries or twigs	FM	−
L91	Papua New Guinea	Coffee berries or twigs	FM	−
L92	Papua New Guinea	Coffee berries or twigs	FM	+
L95	BBA Berlin 62458 (Dr. Gerlach)	*Coffea arabica*	M	−
L107	Zimbabwe	Coffee[b]	FM	+

[a] F = female, M = male, and FM = hermaphrodite.
[b] It was not possible to determine the species of *Coffea* from New Guinea.

DISCUSSION

The sexual system of *G. baccata* appears to parallel that of *F. solani* f. sp. *cucurbitae* (4,10) and *F. moniliforme* (12) in that it exhibits compatibility heterothallism. Whether it will also display sexual heterothallism remains uncertain. As with the above two species, different isolates do vary in sexual expression. While most fertile isolates were hermaphrodites, three male isolates (L83, L86, and L95) were found and L55 acted only as a female in the cross in which it was used. However, since not all isolates were equally fertile, the results of one cross are not sufficient to designate an isolate as male or female.

Initial crosses of hermaphrodite and male isolates of *F. solani* f. sp. *cucurbitae* resulted in a one-to-one ratio of both sexual types in the progeny (9). This implied that one gene with two alleles controlled sexual expression. In such a situation, identical to the L90 × L86 cross presented here, recombinant sexual types would not be detectable. As with *F. solani* f. sp. *cucurbitae*, which possesses separate genes for maleness and femaleness (4,10), determination of sexual heterothallism for *G. baccata* will rely on the recovery of hermaphrodite and neuter recombinant progeny from crosses of male and female isolates. When a consistently fertile female isolate of *G. baccata* is identified, this experiment will need to be performed.

Compatibility or mating type in *G. baccata* is controlled by one gene with two alleles as indicated by the testcross results. As shown in Fig. 1, isolates segregated into two groups that were interfertile and intrasterile. Although Booth (3) stated that homothallic strains exist, our results agree with those of Afanide et al (1), and no self-fertile isolates were observed.

The relationship between the compatibility locus and the gene(s) for sexual expression was explored in the testcross from the progeny of L90 × L86 (Table 2). If the gene or genes controlling sex and mating type are unlinked, equal numbers of mt− hermaphrodites, mt+ hermaphrodites, mt− males, and mt+ males would be expected. However, when using the F_1 progeny as the spermatizing agent in a testcross with isolate L90 as the female, one would be unable to differentiate mt+ hermaphrodites and males.

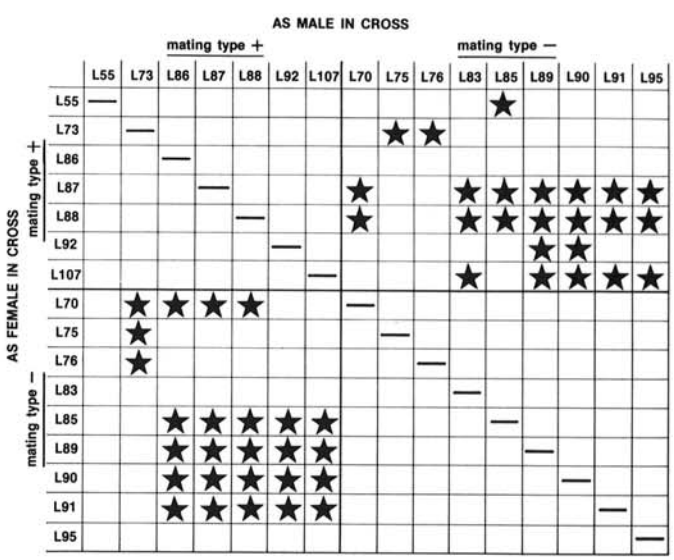

Fig. 1. Results of diallel crosses of isolates of *Gibberella baccata*. A successful cross resulting in the production of perithecia with mature ascospores is indicated by a star while isolates that did not produce perithecia when selfed are indicated by a dash.

TABLE 2. Results of testcross of 160 F_1 progeny from the cross of isolates L90 × L86 of *Gibberella baccata*

Parents		Expected progeny genotypes: Fertile cross possible with: FM[b] mt−	M mt−	FM mt+	M mt+	F_1s expected in a fertile cross (no.)	F_1s observed in a fertile cross (no.)	χ^2[a] df = 1
Female	Male							
L90 (mt− FM) ×	F_1s	no	no	yes	yes	85	89	0.14
F_1s	× L86 (mt+ M)	yes	no	no	no	42	39	0.15

[a]Chi square (χ^2) for a 1:1 ratio is 3.84 at $P = 0.05$ according to Yates' correction factor (15).
[b]FM = hermaphrodite and M = male.

These two progeny genotypes would be rated together, resulting in a 1:1 ratio of successful to unsuccessful matings in the first testcross. Only one-fourth of the 169 progeny, the mt− hermaphrodites, would be expected to act successfully in a testcross when used as the female with isolate L86 (mt+) as the male. The half of the progeny that was mt+ could, of course, not cross with another mt+ isolate. The remaining quarter of the progeny, the mt− males, would not have acted successfully in the testcrosses as they were performed. These data illustrate that the genes for mating type and sexual expression are inherited independently.

Perithecia of *G. baccata* have been reported to occur in nature (14), and indeed isolates L70 and L73 from the same location (Zimbabwe) and host (berries of *Coffea arabica*) were able to cross. Isolates from different locations and hosts also proved fertile. Isolate L55, recovered from an elm with branch dieback in Louisiana successfully crossed with L85, an isolate from coffee berries from New Guinea. This situation resembles that reported for *F. moniliforme* (12).

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