Inter-Isolate Heterokaryosis in Pyricularia oryzae

Javad Fatemi and R. R. Nelson

Associate Professor, Department of Plant Protection, College of Agriculture, Pahlavi University, Shiraz, Iran, and Evan Pugh Professor, Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

Contribution 1011, Department of Plant Pathology, The Pennsylvania Agricultural Experiment Station.
The authors wish to thank T. T. Hebert, F. M. Latterell, and M. A. Marchetti for supplying the cultures.
Accepted for publication 5 June 1978.

ABSTRACT

FATEMI, J., and R. R. NELSON. 1978. Inter-isolate heterokaryosis in Pyricularia oryzae. Phytopathology 68: 1791-1794.

Heterokaryons were synthesized between: (i) monoconidial cultures, (ii) monoconidial cultures and field isolates, and (iii) field isolates of *Pyricularia oryzae*. Heterokaryotic hyphae appeared as a solid line of white mycelial tufts where contact occurred between colonies of compatible isolates when they were paired on an agar medium containing polished rice plus rose bengal. A mycelial fragment was taken from each of many tufts and allowed to grow. Hyphal tips from the resulting colonies provided cultures that were allowed to sporulate. Fifty or more monoconidial isolates were obtained from each hyphal tip isolate. Both parental types and some nonparental types were recovered in most instances. Thirteen of 20 different combinations involving 12 different field isolates produced heterokaryons. The percentage of heterokaryon formation varied from 1.5 to 17.75. Heterokaryons also were formed between monoconidial progeny of one isolate (race IB - 1, Texas) and field isolates. In addition, 7.6% of the crosses among 21 different field isolates produced tufts presumed to be heterokaryons.

Attempts to produce heterokaryons between different strains of fungal species have not been consistently successful. Grindle (5, 6) demonstrated that wild isolates in the *Aspergillus nidulans* group formed heterokaryons with spore color mutants derived from themselves. He observed few examples of inter-strain and none of inter-species compatibility. Furthermore, inter-strain compatibility was confined to strains from the same locality and even they formed heterokaryons more frequently with their own spore color mutants. Compatible isolates usually were identical or very similar in colony morphology regardless of the locality from which they were obtained.

Heale (9) suggested that heterokaryon incompatibility may exist between particular host strains of *Verticillium albo-atrum*.

Parmeter et al (11) recognized four anastomosis groups in *Thanatephorus cucumeris*. Anastomosis occurred between isolates from the same group, but not between isolates from different groups. Thus, the group appeared to be genetically isolated and incapable of nuclear exchange. Stretton and Flentje (12, 13) synthesized heterokaryons between isolates of *T. cucumeris* with similar and different pathogenic capacities. However, they found that inter-isolate synthesis of heterokaryons was increasingly difficult as genotypes became more diverse. Later Bolkan and Butler (2) demonstrated that heterokaryotic field isolates of this fungus interacted with each other and produced new heterokaryons. Anderson et al (1) found that heterokaryon formation in *T. cucumeris* is controlled by two closely linked genes which they called H factor. Homokaryons carrying H factor different at either or both genes, when paired produced tufts of heterokaryotic hyphae.

There is little published information concerning synthesis of heterokaryons between different strains of *Pyricularia oryzae* Cav. Genovesi (4) was not able to synthesize heterokaryons between two mutants derived from two different strains. He concluded that the wide geographic separation of the isolates had resulted in the evolution of genetic differences which prevented hyphal fusion and heterokaryons.

This paper reports the potential and frequency of inter-isolate heterokaryosis in *P. oryzae*.

MATERIALS AND METHODS

Twenty-three field isolates, and monoconidial progeny of 12 of them, were used in this investigation, including: 300, 301, 302 (race IG-1, Texas), 303 (race IB-1, Texas), 304 (race IG-1, Louisiana), 305 (race IB-4, Louisiana), 306 (race IB-6, Louisiana), 307, 308 (race unknown, Peru), 309 (race IB-54, Louisiana), 310 (race IC-17, Pakistan), 311 (race IB-49, Panama), 312 (race ID-13 Italy), 313 (race IA-65, greenhouse mutant of race IA-109, from The Philippines), 314 (race IG-1, Taiwan), 315 (race IG-1, Nicaragua), 316 (race 0, nonpathogenic, buff mutant of race IA-111 from Cambodia), 317 (race IB-33, mutant of race ID-14 from The Philippines), 318 (race IB-45, Guyana), 319 (race IB-49, Louisiana), 320 (race IG-1, Dominican Republic), 351 (race ID-13, Arkansas), and 355 (race IB-49, Louisiana).

Cultures used throughout these studies were maintained on potato-dextrose agar (PDA) in test tubes and petri dishes. Crosses were made on an agar medium

00032-949X/78/000 321$3.00/00

containing polished rice plus rose bengal (polished rice 20.0 g; agar 15.0 g; rose bengal 33 mg; distilled water 1 liter). Isolates were paired by placing the inoculum on opposite sides of a petri dish containing the medium. Inoculated plates were placed in brown paper bags and incubated at 21 ± 1 C for 2 wk. Some pairings of cultures produced a solid line of hyphae in the form of mycelial tufts (Fig. 1). Anastomosis was observed frequently between hyphae taken from the zone of contact between isolates that produced tufts but not between cultures without such zones of tufts. A small mycelial fragment from the tufted zone was transferred to petri dishes containing PDA and hyphal tip isolates were obtained from the resulting colonies. At least 50 single conidial cultures were isolated and plated on PDA in petri dishes. The resulting cultures were compared with the two parental isolates that produced the tufted reaction. We believe heterokaryosis was demonstrated if different monoconidial isolates from hyphal tip cultures were representative of both "parental" types and/or if new colony morphologies were recovered.

RESULTS

Synthesis of heterokaryons between monoconidial progeny of different field isolates.—Twenty monoconidial cultures from each of isolates 301, 303, 304, 305, 306, 307, 309, 310, 311, 312, 351, and 355 were paired with each other in 20 different combinations totaling 8,000 crosses (Table 1). Thirteen of the combinations produced white mycelial tufts at the line of contact between the paired isolates (Fig. 1). Colonies originating from the tufts frequently were different from either parental type. Single conidia isolated from these colonies usually produced cultures similar to parental types but occasionally, a colony different from either parental type was observed. The percentage of heterokaryon formation varied from 1.5 between the progeny of isolates 307 from Peru and 312 from Italy to 17.75 between progeny of isolates 307 and 309 from Louisiana. Progeny of isolate 307 did not produce heterokaryons when paired with isolate 310 from Pakistan. Progeny of 301 from Texas produced heterokaryons with most isolates with which it was paired, whereas progeny of isolate 304 from Louisiana (the same race as 301), did not do so with most isolates.

The success of heterokaryons formation between various single-spore progenies (Table 1 and 2) reveals that at least two groups of isolates exist in nature. Monoconidial progeny in one group produced heterokaryons among themselves and with progeny of other isolates (isolates 301, 310). Progeny of another group, produced heterokaryons with the progeny of another isolate, but not among themselves (304, 307, and 351).

Synthesis of heterokaryons between monoconidial progeny of isolates 303 (IB-1, Texas) and field isolates.—The previous experiment showed that heterokaryons were formed between homokaryons derived from different field isolates. The reactions

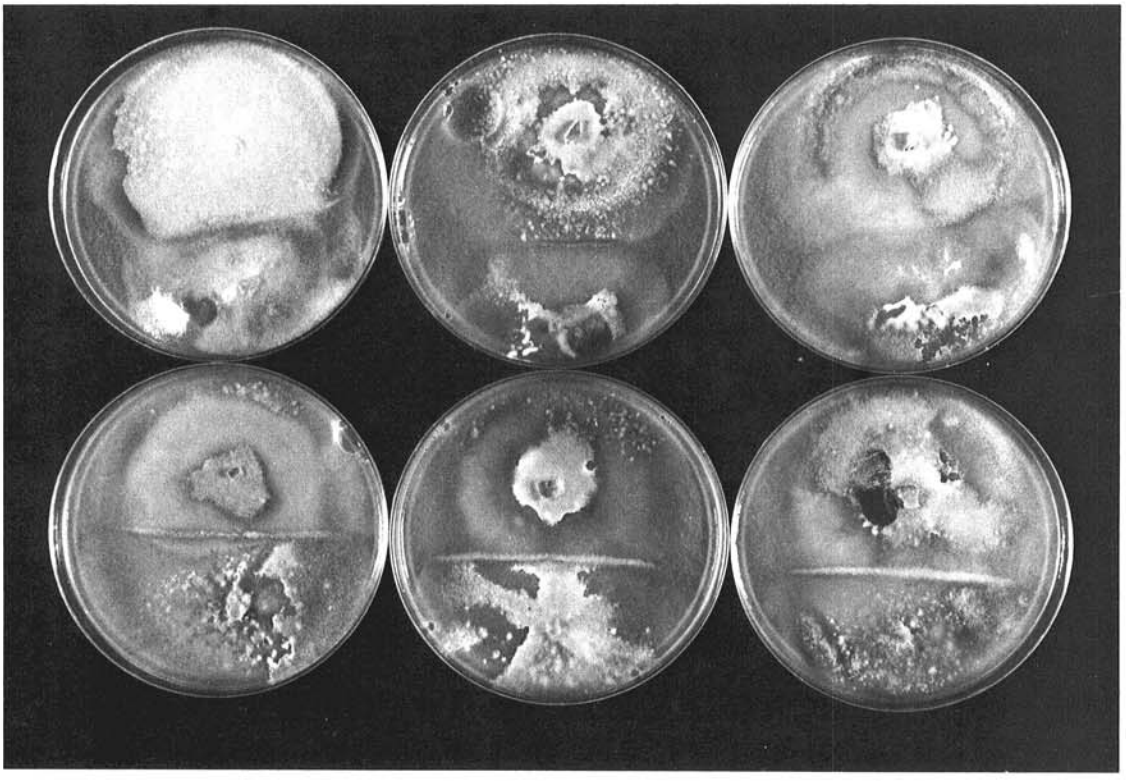

Fig. 1. Reaction between compatible isolates, tuft formation (bottom row) and noncompatible isolates, no tuft formation (top row) of *Pyricularia oryzae*.

between homokaryotic monoconidial cultures and heterokaryotic field isolates also were investigated. Twenty monoconidial cultures from isolate 303 were paired in all possible combinations with 21 field isolates. The field isolates consisted of different races. Of 420 crosses, 47 (11.1%) produced mycelial tufts indicating the formation of new heterokaryons. Fourteen of the 21 field isolates produced tufts with one or more of 14 of 20 homokaryons. Field isolates 303, 305, 307, 310, 314, 315, and 318 did not form heterokaryons with any of the homokaryons, although progeny of isolates 305, 307, 310, and 314 formed heterokaryons with each other or with the progeny of another isolate (Table 2). No tufts were formed between isolate 303 and its own monoconidial progeny, although all but four of the progeny 303 produced tufts with one or more other field isolates tested.

Synthesis of heterokaryons between field isolates.—The potential for exchange of nuclei between two field isolates was investigated by pairing 21 field isolates in all possible combinations. Sixteen of 210 combinations involving 14 different field isolates produced tufts indicating formation of new heterokaryons. Positive reactions were obtained in pairings of the following isolates: 303 × 318; 305 × 310, 311, and 316; 306 × 108 and 320; 307 × 311 and 320; 308 × 310 and 311; 309 × 311; 310 × 312; 311 × 321 and 313; and 312 × 317 and 320.

Analysis of heterokaryons and recovery of parental and new types.—Cultures obtained by isolating hyphal tips from mycelial tufts from 33 crosses were compared with parental isolates for colony types. Cultures from 25 of the crosses produced colony types morphologically different from either of the homokaryons used to synthesize the heterokaryons, indicating that association of the two nuclear types made the heterokaryons. At least 50 single spores were isolated from sixteen of these heterokaryons and colonies originating from these were compared with the parental isolates. The two parental types only were recovered from five of the heterokaryons, one parental type only, from five other heterokaryons, both parental types and one nonparental type from four heterokaryons, and one parental and one nonparental type from each of two heterokaryons. One parental type predominated the cultures obtained from heterokaryons yielding only the two parental types, with the frequency ranging from 74–97.4%.

Interaction between isolates that did not produce tufts.—Two types of behavior were observed between paired isolates that did not produce mycelial tufts at point of contact. A zone of inhibition, occurred in some pairings in which no hyphae were observed. In other pairings the hyphae of both isolates intermingled, but no noticeable reaction was observed. When hyphal fragments were taken from the zone of contact and processed as previously described, all monoconidial cultures were of one parental type.

DISCUSSION

This investigation revealed that monoconidial cultures derived from different field isolates of *P. oryzae* produce heterokaryons, sometimes with a frequency greater than that observed in pairings between progeny of the same isolate. Neither the geographic source nor the colony type of the isolates appear to affect the frequency of heterokaryon formation. Further, there appears to be no geographic separation of races as suggested by Genovesi (4). Frequency of heterokaryon formation between progeny of two races varies among races and may be influenced by the genetic make up and complexicity of the races. These results differ from those of Stretton and Flentje (12, 13) in their research with *T. cucumeris*, in which unrelated, diverse isolates did not form

TABLE 1. Frequency of heterokaryon formation between single-spore progeny of different races of *Pyricularia oryzae*

Isolates paired	Race and origin	Heterokaryon formation (%)
301 × 303	(IG-1, Texas) × (IB-1, Texas)	6.0
301 × 310	(IG-1, Texas) × (IC-17, Pakistan)	6.5
301 × 311	(IG-1, Texas) × (IB-49, Panama)	10.75
301 × 312	(IG-1, Texas) × (ID-13, Italy)	1.75
301 × 304	(IG-1, Texas) × (IG-1, Louisiana)	0
301 × 351	(IG-1, Texas) × (ID-13, Arkansas)	0
304 × 303	(IG-1, Louisiana) × (IB-1, Texas)	2.75
304 × 307	(IG-1, Louisiana) × (Unknown, Peru)	0
304 × 310	(IG-1, Louisiana) × (IC-17, Pakistan)	0
304 × 311	(IG-1, Louisiana) × (IB-49, Panama)	2.5
304 × 312	(IG-1, Louisiana) × (ID-13, Italy)	0
304 × 351	(IG-1, Louisiana) × (ID-13, Arkansas)	0
305 × 306	(IB-4, Louisiana) × (IB-6, Louisiana)	14.0
307 × 309	(Unknown, Peru) × (IB-54, Louisiana)	17.75
307 × 310	(Unknown, Peru) × (IC-17, Pakistan)	0
307 × 311	(Unknown, Peru) × (IB-49, Panama)	3.5
307 × 312	(Unknown, Peru) × (ID-13, Italy)	1.5
307 × 351	(Unknown, Peru) × (ID-13, Arkansas)	3.0
311 × 312	(IB-49, Panama) × (ID-13, Italy)	14.5
351 × 355	(ID-13, Arkansas) × (IB-45, Louisiana)	15.2

TABLE 2. Percentage of heterokaryon formation between single-spore progeny of the same and different races of *Pyricularia oryzae*

Pairings	Heterokaryon formation (%)
301 × 301	0.68
304 × 304	0
307 × 307	0
310 × 310	3.20
351 × 351	0
301 × 304	0
301 × 307	0
301 × 310	6.50
391 × 351	0
304 × 307	0
304 × 310	0
304 × 351	0
307 × 310	0
307 × 351	3.0
310 × 351	1.0

heterokaryons or only did so with difficulty. The frequency with which isolates form heterokaryons with other isolates would be an important factor in determining the extent and kind of variation that results. Isolates whose progeny are compatible with the progeny of many other isolates should have a greater chance for survival and adaptability.

The successful formation of new heterokaryons between certain homokaryons and field isolates and the resulting exchange of nuclei is an additional means for the species to increase its pathogenic potential and survival in nature.

Although no correlation exists between pathogenicity and colony types (10), several colony types can be recognized by isolating conidia from a field isolate. These results and observations suggest that heterokaryons may carry several genetically different nuclear types.

The ability of homokaryotic and heterokaryotic isolates to exchange nuclei with other isolates, regardless of the geographic origin, may account for occurrence of many races and the continual production of new ones.

The recovery of a single morphological type from the heterokaryon might be due to uneven distribution of the nuclei as reported for *Verticillium* species (7, 9) or the instability of the heterokaryon as reported by Garnjobst (3) and Hastie (7, 8).

LITERATURE CITED

1. ANDERSON, N. A., H. M. STRETTON, J. V. GROTH, and N. T. FLENTJE. 1972. Genetics of heterokaryosis in Thanatephorus cucumeris. Phytopathology 62:1057-1065.
2. BOLKAN, A. H., and E. E. BUTLER. 1974. Studies on heterokaryosis and virulence of Rhizoctonia solani. Phytopathology 64:513-522.
3. GARNJOBST, L. 1953. Genetic control of heterokaryosis in Neurospora crassa. Am. J. Bot. 40:607-614.
4. GENOVESI, A. D. 1975. Heterokaryosis and the parasexual cycle in Pyricularia oryzae Cav. M.S. Thesis, Texas A&M University, College Station, Texas. 48 p.
5. GRINDLE, M. 1963. Heterokaryon compatibility of unrelated strains in the Aspergillus nidulans group. Heredity 18:191-204.
6. GRINDLE, M. 1963. Heterokaryon compatibility of closely related wild isolates of Aspergillus nidulans. Heredity 18:397-405.
7. HASTIE, A. C. 1962. Genetic recombination in the Hop-Wilt fungus Verticillium albo-atrum. J. Gen. Microbiol. 27:327-382.
8. HASTIE, A. C. 1964. The parasexual cycle in Verticillium albo-atrum. Genet. Res. 5:305-315.
9. HEALE, J. B. 1966. Heterokaryon synthesis and morphogenesis in Verticillium. J. Gen. Microbiol. 54:419-427.
10. LATTRELL, F. M. 1975. Phenotypic stability of pathogenic races of Pyricularia oryzae and its implications for breeding of blast resistant varieties. Pages 199-234 *in* Horizontal resistance to blast disease of rice. CIAT publication, Series CE-9, Cali, Colombia, S.A. 246 p.
11. PARMETER, J. R., JR., R. T. SHERWOOD, and W. D. PLAT. 1969. Anastomosis grouping among isolates of Thanatephorus cucumeris. Phytopathology 59:1270-1278.
12. STRETTON, H. M., and N. T. FLENTJE. 1972. Inter-isolate heterokaryosis in Thanatephorus cucumeris. I. Between isolates of similar pathogenicity. Austr. J. Biol. Sci. 25:293-303.
13. STRETTON, H. M., and N. T. FLENTJE. 1972. Inter-isolate heterokaryosis in Thanatephorus cucumeris. II. Between isolates of different pathogenicity. Austr. J. Biol. Sci. 25:305-308.