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The Perfect State of *Pyricularia oryzae* Cav. in Culture

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加藤 肇*・山口富夫*・西原夏樹**：イネいもち病菌の培地上における完全世代の形成

Webster documented the perfect state of *Pyricularia aquatica* Ingold as *Massarina aquatica* in 1965[1]. Hebert, in 1971, succeeded in inducing perithecium formation in *P. grisea* (Cke.) Sacc. occurring on crabgrass in matings of monoconidial isolates. Despite numerous trials, the cultures of *P. oryzae* Cav. originating from rice failed to produce perithecia[2].

The present paper gives an account of studies on the production of mature perithecia in *P. oryzae* mated with *Pyricularia* sp. from ragi or finger millet, *Eleusine coracana* Gaertn.

Twenty-three monoconidial isolates of *P. oryzae* from the collection of the National Institute of Agricultural Sciences in Japan were used. These isolates had been obtained from naturally infected rice leaves and panicles from various localities in Japan. Fourteen monoconidial isolates from infected leaves and seeds of ragi and three isolates each from *Eleusine indica* (L.) Gaertn., *Setaria viridis* (L.) P. Beauv. var. *minor* (Thunb.) Ohwi, and *Eragrostis curvula* (Schrad.) Nees from Japan were also used. The isolates were cultured on potato sucrose agar in test tubes.

To supply mycelial fragments for mating experiments, each isolate was grown in a nutrient solution consisting of 5g Difco yeast extract, 0.5g K_2HPO_4, 0.5g KH_2PO_4, 0.5g $MgSO_4 \cdot 7H_2O$, 0.05g $CaCl_2$, 10g glucose, and 1000ml deionized water. A 100ml Erlenmeyer flask containing a tiny block of mycelium, 45ml of medium, and thirty glass beads 7mm in diameter was incubated at 28C in darkness for 10 days. During the period of incubation, the flask was manually shaken once a day to fragment the growing mycelium. The mating techniques used were similar to those of Hebert[2]. Approximately 2ml of each suspension of mycelial fragments was poured onto small pieces of rice straw previously disinfected with propylene oxide and embedded in modified Sachs' nutrient agar[2] in a 9cm petri dish. All of the possible pairings were made. The mycelial fragments of paired isolates and single isolate were incubated for 20 to 30 days at 20C with illumination ranging from 330 to 680nm in wavelength and with an intensity of 5500erg/cm^2·sec. in a 12hr photoperiod.

Thirteen of fourteen isolates from ragi belonged to two compatibility groups, designated as *A* and *a*. All isolates except isolate FI 1-1 exhibited intergroup fertility (Table 1). Of the total, four isolates belonged to group *A* and nine to group *a*. Up to now, nine isolates from rice mated with the isolates from ragi belonging to group *a* have yielded mature perithecia (Table 2).

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1) Webster, J. (1965), Trans. Brit. Mycol. Soc. 48：449-452.
2) Hebert, T. T. (1971), Phytopathology 61：83-87.

Table 1. Production of perithecia of *Pyricularia* sp. in matings of monoconidial isolates from ragi

Locality obtained	Isolate	Production of perithecium														Group of compatibility
		KUR 1-2	KUR 2-5	KEN 1-5	KEN 2-5	CHI 1-5	CHI 2-1	FI 1-1	FI 1-3	KAG 3-1	KAG 4-1	TOK 1-1	TOK 2-1	MZ 1-1	MZ 1-2	
Tochigi	KUR 1-2	N	PS	N	N	N	P	N	PS	PS	PS	P	P	N	N	A
	KUR 2-5		N	N	N	N	N	N	N	N	N	N	N	PS	PS	a
Tokyo	KEN 1-5			N	N	P	N	N	N	N	N	N	N	PS	PS	a
	KEN 2-5				N	P	N	N	N	N	N	N	N	P	PS	a
Chiba	CHI 1-5					N	P	N	P	PS	PS	PS	P	N	N	A
	CHI 2-1						N	N	N	N	N	N	N	P	PS	a
Fukui	FI 1-1							N	N	N	N	N	N	N	N	?
	FI 1-3								N	N	N	N	N	PS	PS	a
Kagawa	KAG 3-1									N	N	N	N	PS	PS	a
	KAG 4-1										N	N	N	PS	PS	a
Tokushima	TOK 1-1											N	N	PS	PS	a
	TOK 2-1												N	P	N	a
Miyazaki	MZ 1-1													N	N	A
	MZ 1-2														N	A

The symbol N indicates the mating to be sterile, P to have produced immature perithecia, and PS to have produced mature perithecia with ascospores.

Table 2. Frequency of perithecium formation in matings of monoconidial isolates of *Pyricularia oryzae* from rice and *Pyricularia* sp. from ragi

Isolates from rice*			Frequency			
			Perithecium formation with ragi isolates** of compatibility group			Ascospore formation with ragi isolates of compatibility group *a*
Locality	Isolate	Race	*A*	*a*	*?*	
Kumamoto	Ken73-01	JT-2	0/4	9/9	0/1	4/9
Akita	TH63-454	JC-1	0/4	9/9	0/1	2/9
Nagano	Naga87	JC-3	0/4	9/9	0/1	4/9
Niigata	Ken66-18	JN-1	0/4	4/9	0/1	1/9
Tokyo	Ken62-03	JN-1	0/4	6/9	0/1	1/9
Fukushima	Ken59-49	JN-2	0/4	2/9	0/1	1/9
Hiroshima	Chu66-45	JN-2	0/4	4/9	0/1	1/9
Aichi	Ina168	JN-4	0/4	9/9	0/1	5/9
Ishikawa	Naga61-14	JN-5	0/4	1/9	0/1	1/9

* Twenty-three monoconidial isolates from rice were used.
** Fourteen monoconidial isolates from ragi indicated in Table 1 were used.

Perithecia, singly or in groups, were partially embedded or formed on segments of rice straw or sometimes in Sachs' agar (Fig. 1). The mature perithecium was brown to dark brown and globose, measuring 57-150μm (mean 110μm) in diameter. The outer layer consisted of almost angular cells measuring 9×16μm. A well-developed ostiolate beak, measuring 50-1000μm (mean 160μm) in length and 33-95μm (mean 50μm) in diameter, was produced. The centrum was occupied by a mass of hyaline paraphyses which disappeared at maturity. Asci in a fascicle arose from

the base of the central cavity. The asci were cylindrical to subclavate, unitunicate shortstipitate, and evanescent at maturity; they measured 8—12×53—70μm(mean 9×60μm) and contained from one to eight ascospores. The apex of the ascus was not stained by Melzer's iodine reagent and constituted a light-refractive ring. Ascospores were hyaline, fusiform, straight to slightly curved, and without sheaths. Mature ascospores measured 4—6×14—24μm (mean 5×20μm), and typically had three septa. Germ tubes produced appressorium-like round organs, measuring 6μm in diameter.

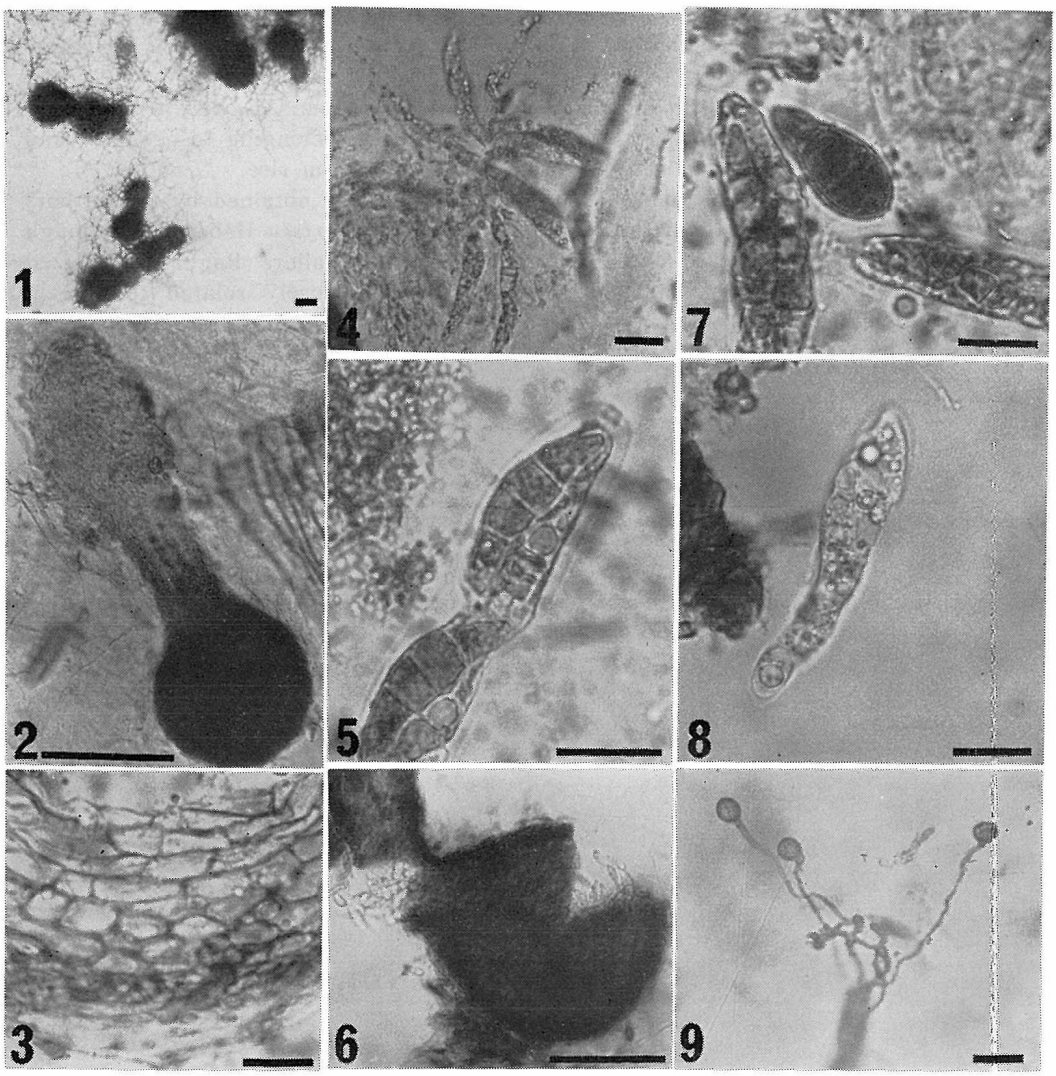

Fig. 1. Perithecium production in *Pyricularia oryzae* from rice mated with *Pyricularia* sp from ragi. 1) Juvenile perithecia cultured for 7 days in modified Sachs' nutrient agar with fragments of rice straw. 2) Mature perithecium with ostiolate beak. 3) Wall structure of peridium. 4) Asci in a fascicle cultured for 18 days. 5) Ascus and ascospores stained with iodine reagent. 6) Forcible discharge of ascospores from perithecium cultured for 30 days. 7) A comparison between conidium and asci stained with iodine reagent. 8) Juvenile ascus. 9) Appressorium-like organs developed at the tip of germ tubes from ascospores. The scale bars represent 10μm for photographs of 5), 7), and 8); 20μm for those of 3), 4), and 9); and 100μm for those of 1), 2), and 6).

Under the conditions mentioned above, immature perithecia developed in 6–7 days, immature asci developed in 10 days, and viable ascospores were formed in 20 days. Isolates derived from single ascospores were demonstrated to be physiologically heterothallic. Cultures arising from these ascospores produced typical conidia. To examine the pathogenicity of each of parental and monoascosporic isolates, seedlings of rice, 'Norin 8' and 'Aichi-asahi', and of ragi, 'Iiya-zairai' and 'Yukijirushi-kei', were used. Cross-inoculation tests of isolates indicated that each isolate infected its own host. By using conidia derived from monoascosporic cultures, nine cultures were classified into three groups: pathogenic to rice, pathogenic to ragi, and pathogenic to neither plant, respectively. Further studies on the genetic basis of pathogenicity are in progress.

Three isolates each from *E. indica*, *S. viridis* var. *minor* and *E. curvula* developed mature perithecia in matings with the isolates from ragi belonging to group *a*, and failed to develop perithecia in matings with the isolates from rice.

The morphology and the measurements of each organ obtained by the authors were in general agreement with those of *Ceratosphaeria grisea* Hebert[2], although the size of the perithecium produced in our crosses was smaller. Ragi is recognized as an indicator plant in a savannah area and as a plant closely related to rice in its history of cultivation. The results obtained may warrant further research on the establishment of in a single population of genotypes having a certain host-specificity in pathogenesis.

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