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Mating type of isolates derived from the spermogonial state of *Puccinia coronata* var. *coronata*

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Hyphal confrontation between two haploid cultures originating from single basidiospores was used to determine the mating type of *Puccinia coronata* var. *coronata*. Pairs of 15 single-basidiospore cultures were placed approximately 1 mm apart on the medium in all possible combinations. Hyphae of the pairs of colonies came into contact with each other in all combinations approximately two weeks after confrontations. When the nuclear number of hyphal cells in a contact zone was investigated one month after confrontation, monokaryotic hyphae were observed in selfing combination. On the other hand, dikaryotic hyphae were observed in 90.5% of crossing combinations between different cultures. Two isolates are considered compatible if dikaryotic hyphae are present in the contact zone but incompatible if they are absent. The mating type of the fungus was found to be characterized by multiple-allelomorphic tetrapolar incompatibility controlled by the "A" and "B" incompatible factors.

Key Words——axenic culture; mating type; *Puccinia coronata*: rust fungus.

Introduction

Craigie (1927) reported for the first time in rust fungi that *Puccinia helianthi* Schw. is heterothallic. Since then, heterothallism has been found in 23 species of rust fungi (Buller, 1950; Hunt, 1985; Yamazaki and Katsuya, 1988a). Craigie (1927) also stated that if basidiospores had the opposite sex, "+" and "−", and are sown close together on a leaf, the pustules arising from the two infections soon coalesce, and binucleate aeciospores are produced through the mating process. This observation is also reviewed by Buller (1950). From this observation, the mating type of the rust fungi is generally accepted to be a two allelomorphic bipolar mating system. However, Yamazaki and Katsuya (1988b) reported that the mating type of *Cronartium quercuum* (Berk.) Miyabe ex Shirai is characterized by multiple-allelomorphic tetrapolar incompatibility. Therefore, we need to investigate the mating type of other rust fungi.

Axenic cultures derived from single basidiospores are suitable for making many clones to investigate the mating type of rust fungi. Most mating studies of rust fungi have been performed by using host plants, because axenic cultures derived from single basidiospores are difficult to establish. Mating experiments on rust fungi using single-basidiospore cultures have been reported only for *Gymnosporangium asiaticum* Miyabe ex G. Yamada (Tetsuka and Katsuya, 1984). Tetsuka and Katsuya (1984) concluded that the mating type of *G. asiaticum* was a two allelomorphic bipolar mating system.

The purpose of this study is to determine the mating type of *P. coronata* Corda var. *coronata* using axenic cultures.

Materials and Methods

Fifteen axenic cultures were used: isolate numbers Pccs-4, Pccs-6, Pccs-8, Pccs-15, Pccs-23, Pccs-24, Pccs-27, Pccs-32, Pccs-36, Pccs-45, Pccs-46, Pccs-47, Pccs-50, Pccs-59 and Pccs-60. These axenic cultures were isolated from leaf segments of the host plants, *Berchemia racemosa* Sieb. et Zucc. or *B. berchemiaefolia* Koidz., with a single lesion which seemed to originate from a single basidiospore. They were maintained by transferring small pieces of colonies to fresh medium at approximately 2.5 month intervals and were incubated at 20 C in the dark (Narisawa et al., 1992). For the first 3 months of incubation, axenic colonies were white and felty; however, soon after, approximately half the number of colonies changed from white and felty to orange and glossy (Narisawa et al., 1992). At the time of the experiment, all colonies of 15 cultures had a rough, glossy surface and consisted of long, narrow hyphal cells. The nuclear condition in the hyphae of these cultures was haploid and monokaryotic (Narisawa et al., 1992) over 3 years.

The medium used for confrontation contained a 25% concentration of Murashige and Skoog's minerals, vitamins, and glycine (Murashige and Skoog, 1962); lab-

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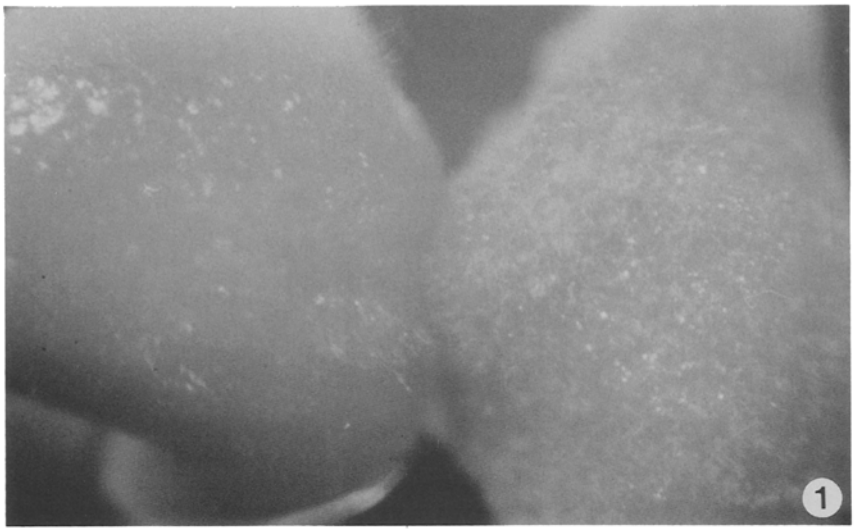
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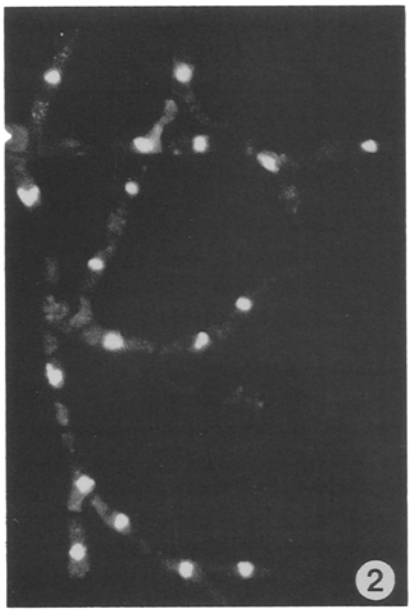
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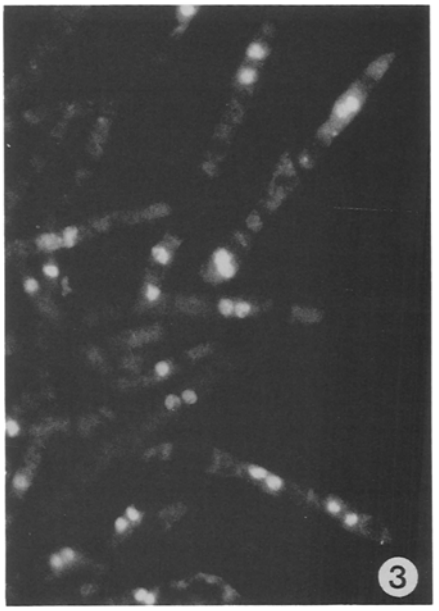
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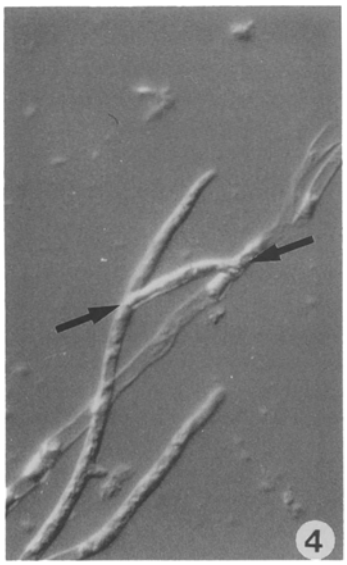
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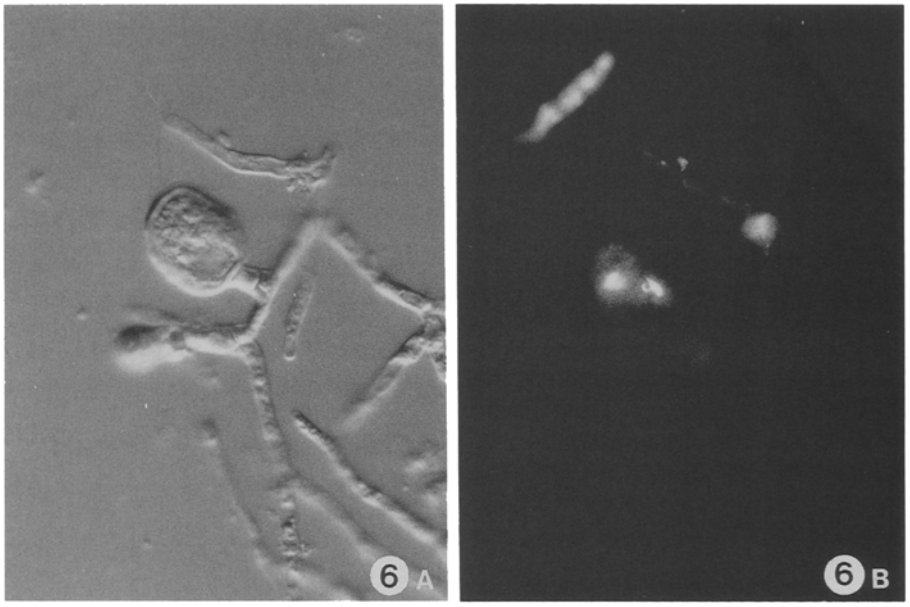

Fig. 6. A: Smooth-surfaced spore-like body formed in contact zone.
B: Dikaryotic spore-like body formed in contact zone.

lemco broth (Oxioid), 2.0 g; peptic peptone (U.S. Biochemical Co.), 2.0 g; sucrose, 40.0 g; and bacto agar (Difco), 8.0 g per liter. The pH of the medium was adjusted to 5.8–5.9 with 1 N HCl before autoclaving at 121°C for 10 min.

Pairs of single-basidiospore cultures were placed approximately 1 mm apart on the medium in petri plates (55 mm in dium.) and incubated at 20°C in the dark. After 1 month, small pieces of mycelia approx. 2 × 2 mm were cut and removed from different sites in the hyphal colonies in and out of the contact zone. These were stained with 4,6-diamidino-2-phenylindole (DAPI) at a concentration of 100 ng/ml to investigate the nuclear numbers of hyphal cells, as reported by Narisawa et al. (1992). Two cultures are considered compatible if dikaryotic hyphae are present in the contact zone but incompatible if they are absent. Mating experiments on fifteen cultures were performed in all possible combinations and replicated 3 times. When the dikaryotic hyphae were observed in the contact zone, pieces of mycelia approx. 10 × 10 mm were cut from the contact zone, transferred to fresh medium and incubated at 20°C in the dark. The cultures were maintained for more than 1 year by this method. At about 2.5 month intervals, when the colonies were transferred to fresh medium, small pieces of mycelia were removed to observe the nuclear numbers in the hyphae.

Results and Discussion

Hyphae of the two colonies (cultures) came into contact with each other in all combinations approximately two weeks after confrontation. The two colonies were recognized macroscopically to be fused one month after confrontation (Fig. 1). The nuclear numbers of the hyphal cells in and out of the contact zone were investigated one month after confrontation. Monokaryotic hyphae were observed in all selfing combinations (Fig. 2). On the other hand, dikaryotic hyphae were observed in 90.5% of crossing combinations between different cultures (Fig. 3). Dikaryotic hyphae were observed only in the contact zone. Hyphae of the subcultures derived from the mass of dikaryotic hyphae in the contact zone were dikaryotic for more than 1 year.

When the small pieces of colonies in the contact zone were observed by light microscope, anastomosis was observed in almost all mating combinations (Fig. 4). The cells of dikaryotic hyphae were broader and shorter (4.1 μm and 17.4 μm on average) (Fig. 5) than the cells of monokaryotic hyphae (2.6 μm and 49.0 μm on average). dikaryotic spore-like bodies which were subglobose, with a smooth surface, were sometimes observed on the apex of the dikaryotic hyphae (Fig. 6). Dikaryotic cultures of *P. coronata* var. *coronata* were established from leaves of *Calamagrostis arundinacea* Roth var. *brachyrica* infected with urediniospores (unpublished data). The colonies of the cultures also consisted

Fig. 1. The two fused colonies of *Puccinia coronata* var. *coronata* one month after confrontation (Pccs-23 × Pccs-60).
Fig. 2. Monokaryotic hyphae observed in selfing combinations (Pccs-47 × Pccs-47).
Fig. 3. Dikaryotic hyphae observed in crossing combinations (Pccs-15 × Pccs-60).
Fig. 4. Anastomosis between monokaryotic hyphae observed in contact zone (arrows).
Fig. 5. Broad, short dikaryotic cells of hyphae formed in contact zones.

Table 1. Results of matings between 15 cultures originating from a single basidiospore of *Puccinia coronata* var. *coronata*.

Isolate number (Pccs)	8	32	45	59	23	47	24	46	60	27	4	6	15	36	50	Mating type estimated
8	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	A_1B_1
32		−	−	+	+	+	+	+	+	−	+	+	+	+	+	A_1B_2
45			−	+	+	+	+	+	+	+	+	+	+	+	+	A_1B_3
59				−	+	+	+	+	−	+	+	+	+	+	+	A_2B_1
23					−	−	+	+	+	+	+	+	+	+	+	A_3B_4
47						−	−	+	+	+	+	+	+	+	+	A_3B_5
24							−	−	+	+	+	+	+	+	+	A_4B_5
46								−	−	+	+	+	+	+	+	A_4B_6
60									−	+	+	+	+	+	+	A_2B_6
27										−	+	+	+	+	+	A_5B_2
4											−	+	+	+	+	Each of the isolates has a different incompatibility factor from the other 14 isolates
6												−	+	+	+	
15													−	+	+	
36														−	+	
50															−	

"+" and "−" indicate the presence and the absense of dikariotic hyphae, respectively.

of broad, short dikaryotic hyphal cells (5.2 μm and 15.6 μm on average) (unpublished data). Tetsuka and katsuya (1984) reported that the cells of dikaryotic hyphae produced by confrontation of monokaryotic colonies of *Gymnosporangium asiaticum* had almost the same morphology as the monokaryotic hyphae of the fungus. In *P. coronata* var. *coronata*, however, the hyphae on the medium in the haploid and diploid stages were morphologically distinguishable.

Allen (1930) reported that *Puccinia coronata* Corda var. *avenae* (spermogonial and aecial state on *Rhamnus catharitica* L., uredinial and telial state on *Avena sativa* L.) is heterothallic. She used eight *Rhamnus* plants bearing one infection each originating from a single basidiospore. They were carefully isolated. Seven of the 8 remained sterile, that is, produced no aeciospores. On another plant bearing 6 infections, the basidiospores were well mixed. Five of the 6 produced open aecia. In the present study, the nuclear condition of 15 cultures originating from single basidiospores was haploid and monokaryotic for over 3 years after isolation. Monokaryotic hyphae were observed in all selfing combinations, whereas dikaryotic hyphae were observed in crossing between different cultures as a result of hyphal confrontation. We demonstrated that *P. coronata* var. *coronata* is heterotallic from the results of the nuclear condition of the cultures and the confrontation on the medium.

The mating pattern for 15 cultures originating from single basidiospores of *P. coronata* var. *coronata* are shown in Table 1. Matings of 3 combinations, i.e., Pccs-8 × Pccs-32, Pccs-8 × Pccs-45 and Pccs-8 × Pccs-59, showed an incompatible reaction. If the mating system of the fungus is characterized by a bipolar incompatibility, Pccs-8, Pccs-32, Pccs-45 and Pccs-59 should have the same mating type as "+" or "−". Mating of 2 combinations, i.e., Pccs-32 × Pccs-59 and Pccs-45 × Pccs-59, showed a compatible reaction, however. It is suggested that the fungus does not have bipolar incompatibility mating systems. Yamazaki and Katsuya (1988b) reported that the mating system of *Cronartium quercuum* is characterized by multiple-allelomorphic tetrapolar incompatibility controlled by the identical "A" and "B" incompatible factors. This was the first report of the multiple-allelomorphic tetrapolar incompatibility mating system in rust fungi. The $A \neq B \neq$ interaction was considered to be compatible, whereas the $A \neq B$, $A = B \neq$ and $A = B =$ interactions were considered to be incompatible. In line with this compatible or incompatible reaction, the mating type of all 15 cultures of *P. coronata* var. *coronata* was estimated by multiple-allelomorphic tetrapolar incompatibility (Table 1). Consequently, the mating system of *P. coronata* var. *coronata* is considered to be characterized by multiple-allelomorphic tetrapolar incompatibility controlled by the "A" and "B" incompatible factors.

Yamazaki and Katsuya (1988b) determined the mating type of *C. quercuum*, which causes galls on red pine (*Pinus densiflora* Sieb. et Zucc.) stems and branches derived from basidiospore infection. Each gall caused by single basidiospore infections was separated by ditches of approximately 1 cm depth. Spermogonia in each part of the gall were used independently for mating experiments as clones originated from a single infection. Since galls may sporulate for years, they could also be used for mating experiments every year. The advantage of obtaining clones of spermogonia derived from a single infection made it possible to determine the mating system of *C. quercuum*. However, making many clones to devide the leaf segment of the host plant with spermogonia derived from a single basidiospore is difficult in

most rust fungi because the spermogonia are small (e.g., *P. graminis* Pers.: Pers. have spermogonia originating from a single basidiospore of 6–12 mm diam; Craigie, 1927) and spermogonia can only be used once.

Axenic cultures derived from single basidiospores are considered to be suitable for making many clones to investigate the mating type of rust fungi. Mating experiments of rust fungi using axenic cultures derived from single basidiospores were reported for *Gymnosporangium asiaticum* (Tetsuka and Katsuya, 1984). They suggested that *G. asiaticum* has a two-allelomorphic bipolar mating system. The successful establishment of axenic cultures derived from single basidiospores has been reported (Tetsuka and Katsuya, 1981; Hu and Amerson, 1991; Narisawa et al., 1992). Further progress in methods to establish single-basidiospore cultures is necessary in order to use the cultures in the study of the mating type of other rust fungi.

Tetsuka and Katsuya (1984) suggested that the colony type of haploid axenic cultures of *G. asiaticum* (fluffy and water-soaked type) was related to mating type. However, all 15 cultres of *P. coronata* var. *coronata* seem to have the same colony type (rough, glossy surface). Thus, the colony type of the fungus was not related to mating type.

Many basidiospores were deposited in a spot on the medium to establish axenic cultures of *C. fusiforme* Hedgc. et N. Hunt ex Cumm. (=*C. quercuum* f. sp. *fusiforme*) (Hare, 1978), *C. quercuum* (Yamazaki and Katsuya, 1990) and *P. coronata* var. *coronata* (Narisawa et al., 1993). The dikaryotic hyphae were expected to be produced as a result of anastomosis between monokaryotic hyphae originating from basidiospores with different mating types. However, Hare (1978) reported that mycelial cells in the colonies from basidiospores of *C. quercuum* f. sp. *fusiforme* were monokaryotic. Yamazaki and Katsuya (1990) stated that axenic cultures from basidiospores of *C. quercuum* had been consistently monokaryotic for 3 years. Dikaryotic hyphae were only recognized in *P. coronata* var. *coronata* (Narisawa et al., 1993). In the present study, dikaryotic hyphae were also observed in 90.5% of crossing combinations between different cultures. These results suggest that anastomosis between hyphae of *P. coronata* var. *coronata* occurred rather easily under the cultural condition compared with the other rust fungi.

In the present study, we considered that two cultures are compatible if dikaryotic hyphae are present as a result of hyphal confrontation but incompatible if they are absent. However, production of spores, i.e., aeciospores or urediniospores, from dikaryotic hyphae could not be induced, nor could pathogenicity of dikaryotic hyphae against the host plant, *Calamagrostis arundinacea* Roth, be demonstrated. Dikaryotic cultures of the fungus from leaf segments with urediniospores could not produce spores under the same cultural conditions used in the present study (unpublished data). Unsuitable cultural conditions were considered to be mainly responsible for this lack of spore production. Further studies are required to examine suitable conditions for the cultures to produce spores and to be pathogenic on the host plants.

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