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AN ELONGATED CONIDIUM STRAIN AND MATING TYPE DISTRIBUTION OF *COCHLIOBOLUS MIYABEANUS*[1,2]

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Abstract

A strain of *Cochliobolus miyabeanus* with slender and elongated type conidia was obtained from rice leaf brown spot collected at Lien-Hua-Tsu (Nantou), Sun-Shing (Ilan) and Hualien and also obtained from leaf brown spot of *Zizania latifolia* at Nankang. Conidia usually curved, cylindrical slightly tapering toward the ends; pale to mid golden brown, 100 to 168 (mostly 120 to 140) μm × 17 μm, 8 to 13 pseudoseptate. This strain, like the common strain, is pathogenic to both *Oryza sativa* and *Zizania latifolia*. Elongated strain and common strain are cross-fertile; the single spore culture of both strains mated on rice straw sections which were placed on Sach's medium plates produced perithecia two weeks after incubation. Mating type *A* and *a* are co-existed at the same location. And mating type *A* predominates over mating type *a* in all three locations examined.

Introduction

In previous reports (Chang, 1974, 1975) the writer demonstrated that there were strains existed in *Cochliobolus miyabeanus* in terms of their sporulation and morphological characters. These strains mated each other and formed perithecia. We have gone further to investigate the distribution of mating types in the areas where brown leaf spot of rice occurs and looking for the existence of the other types of strains in the natural population of this fungus. Herein, some of our findings are presented and their significance to our understanding to this fungus are disscussed.

Materials and Methods

Sources of isolates and maintenance of cultures

All isolates were obtained from tissues showing leaf spot which were

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collected at Hualien, Sun-Shing (Ilan) and Lien-Hua-Tsu (Yu-Tsu, Nantou District). The methods of isolation were as follows: Individual spot tissue was cut and surface sterilized by submerging in 30% sodium hypochloride solution for 3 min., rinsed with sterilized water and the excessive water on the surface was absorbed by sterilized filter paper. The tissue of spot lesion was then placed on potato dextrose agar (PDA) plate and incubated at 25°–27°C under cool white fluorescent light. The mycelia emerged from diseased tissues and sporulated. Single spore isolation was made and the cultures were maintained on PDA slants at 15°C for future use.

Mating type identification

In the beginning of the survey of the distribution of mating types, the isolates obtained at Wan-Kuway (Sun-Shing) were mated randomly and found that two isolates WK1A and WK1C were cross-fertile and formed abundant perithecia on autoclaved corn leaf section and thus isolate KW1A was designated as mating type *A* and isolate WK1C as mating type *a*. Those crossed fertile with WK1A were mating type *a* and those crossed fertile with WK1C were mating type *A*. The procedure of mating experiment was as described previously (Chang, 1974) except the corn leaf sections were replaced by rice straw sections, otherwise the rest of the conditions were remained the same.

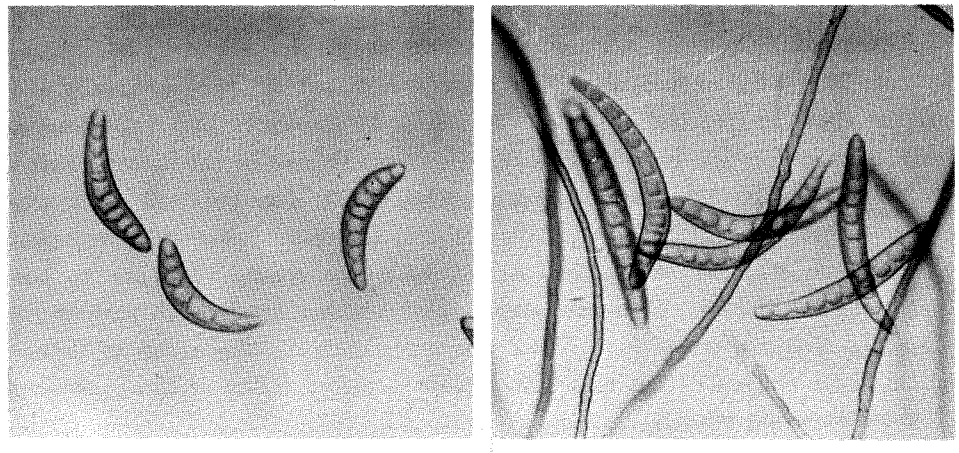
Pathogenicity Test

One month old rice seedlings (variety Tainan 5) grown in pots were used as host plants. Inoculation was made by spraying spore suspension onto the leaves and the inoculated plants were then covered with polyethylene plastic bag for 14 hr at 25°–30°C, after that the inoculated plants were moved to the open greenhouse for later examination the inoculation results.

Results

A new morphological strain with elongated type conidia of ***Cochlioblns miyabeanus*** *(****Helminthosporium oryzae****) new for Taiwan*

During the making isolation and collection of the isolates of *Helminthosporium oryzae* from Wan-Kuway (Sun-Shing), some isolates with elongated type conidia were found. Later the isolates with elongated type conidia were also obtained from the brown leaf spot samples collected at Hualien and Lien-Hua-Tsu. The case occurred at Lien-Hua-Tsu is worth mentioning that the frequence of the appearance of this new strain is relatively high; among 124 isolates, 27 isolates are with elongated type conidia. The shape of conidia of common strain and this new morphological strain is shown in Fig. 1. The shape is distinctly different; the elongated conidia are usually curved, cylindrical, slightly tapering toward the ends, pale to mid golden brown, 100 to



(A) (B)

Fig. 1. Conidia of *Cochliobolus miyabeanus*; (A) common type, (B) elongated type.

168 μm (mostly 120 to 140 μm)×17 μm, 4 to 13 (mostly 8 to 9) pseudoseptate. The main distinct difference between this new strain and common strain, which is the causal organism of brown spot of rice, is that the length of conidia. The dimensions of conidia of these two strains are shown in Table 1. The length of conidia of common strain is from around 56 to 140 μm and mostly, 93 to 112 μm in length and about 17 μm wide. Number of conidial septum of these two strains are most 8 to 9 for the new strain and 7 to 8 for the common strain. There is no difference in width for both strains, it is about 17 μm for both.

Table 1. *Comparison of the length of conidia of two strains of* ***Cochliobolus miyabeanus***

Common type		Elongated type	
Length (μm)	Frequency (per 214)	Length (μm)	Frequency(per 224)
55.92	1	93.20	1
65.24	5	102.52	4
64.56	4	114.84	9
83.88	13	121.16	38
93.20	62	130.48	76
102.52	78	139.80	52
111.84	49	149.12	32
121.16	7	158.44	8
130.48	4	167.76	4
139.80	1		

Pathogenicity of the strain with elongated conidia

Rice seedling leaves inoculated with spore suspension showed small necrotic spots 24 hr after inoculation. The dark brown spot enlarged gradually and final the spot size reached up to 4.5×2.0 mm and surrounded by a yellow zone. There is no difference between the shape, color and size of the spots caused by this new strain and common strain. The result was obtained from the testing on one rice cultivar, i.e., Taiwan 5, and for this reason no conclusion can be made whether these two strains have the same virulence to the other cultivars of rice. This new strain, like common strain, also pathogenic to *Zizania latifolia* when inoculation was made onto it. The result of pathogenicity test suggests that this new isolate is also a strain of *C. miyabeanus* but not a separate species.

Distribution of mating types

So far 324 isolates were mated with isolates WK1A and WK1C, among them 103 isolates obtained from Lien-Hua-Tsu, 85 isolates from Sun-Shing and 136 isolates from Hualien. And in 324 isolates, 24 isolates are the strain with elongated type conidia; 13 from Lien-Hua-Tsue and 11 from Sun-Shing. The results of mating are shown in Table 2. The isolates crossed fertile with isolate KW1C and formed perithecia were predominant over the isolates which crossed fertile with KW1A and formed perithecia, the ratio between them is about 2 to 1, i.e., mating type *A* isolates : mating type *a* isolates = 2:1. Furthermore, in all three locations where the isolates were obtained, mating type *A* isolates are predominant over the mating type *a*.

Table 2. *Distribution of mating type of* ***Cochliobolus miyabeanus*** *in three locations where isolates were obtained and tested*

Mating type	No. of mating type in		
	Lien-Hua-Tsu	Sun-Shing	Hualien
a	32	15	45
A	48	45	70

As regards to relationship between this new strain and common strain, the results of mating test between them shows that among 13 isolates obtained from Lien-Hua-Tsu, 3 isolatts were compatible with isolate KW1A and 3 isolates were compatible with isolate KW1C. And 11 isolates obtained at Sun-Shing, 2 isolates were compatible with isolates WK1A and 5 isolates were compatible with isolate WK1C and the rest formed no perithecia. This preliminary result indicates that the affinity between these two fungus types of isolates is very close and seems to be no reason to claim that they are two

separate species even though some of the isolates did not formed perithecia when mated. Without further confirmation the writer still suspect that the failure to form perithecia is due to the unknown artifact. This negative result is also occurred frequently in the crosses among the isolates of the common strain. This new strain is indeed also the causal fungus of brown leaf spot of rice.

Discussion

Nishikado in 1928 first reported that strains of *H. oryzae* which obtained from Dr. Tisdale (the isolates were isolated in Louisiana) were different from the isolates in Japan on the shape, length, width and color of conidia. The conidia of American strain, which he called, is longer and more slender than the Japanese isolates. In his studies he found that the length of conidia of American and Japanese strains were 103.13 μm and 89.46 μm, respectively. The length of conidia of present new strain, in most of the cases, is from 121.16 to 149.12 μm and could be up to 197.76 μm, while the length of conidia of the common strain is from 93.20 to 111.84 μm. The fact shows that there is, indeed, existed the strain with elongated type conidia in the natural population of *H. oryzae* in Taiwan. The difference of the length of conidia of Taiwan and American isolates might be due to the substractes and other unspecific conditions such as moisture. Nishikado used Hopkin's medium and the present writer used autoclaved corn leaf sections for spore formation. It was proved that there is no difference in pathogenicity between these two morphological strains, both showed highly virulent to *Oryza sativa* and *Zizania latifolia*. Same results were reported by Nishikado almost half a century ago, he demonstrated that Japanese and American strains had the same pathogenicity to *Oryza sativa*. The present new morphological strain is the first record in Taiwan, and the writer believes that the strain has existed in Taiwan for long period of time, because it has been located at various widely separate locations such as Hualien, Sun-Shing, Lien-Hua-Tsu, and even at Nankang from the brown leaf spot of *Z. latifolia*. The reason of this delaying of notification of its existence is that previous workers have not paid much attention to examine the morphological distinction of this very common plant pathogenic fungus.

The predominance of mating type *A* isolates over mating type *a* isolates in all three locations where isolates were collected suggests that the mating type *A* isolates might possess high potential of adaptation to their environments than the mating type *a* isolates. It is feasible to speculate that the combination effects of change of rice cultivars and other environmental factors such fertilizer, fungicide application, may play an important role in determina-

tion of the distribution of mating types of this fungus in Taiwan, the effects of rice cultivars and other factors are remained to be investigated. Tsuda and Ueyama (1976) in Japan reported that in field both mating types were distributed almost equally and moreover, co-existence of these types in the same localities or on the same rice plant was demonstrated.

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稻胡麻葉枯病菌較長型分生胞子菌系及本菌結合型之分佈

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一種較長型分生胞子菌系在花蓮，三星鄉，魚池鄉及南港都發現到，其分生胞子之長度爲 100 至 168μm，多數是 120 至 140μm。寬度爲 17μm，本菌系的所有菌株對水稻和茭白都具有强的病原性，本菌系的菌株和普遍型菌結合產生有性世代。故知其同屬一種。胡麻葉枯病菌的兩種結合型 *A* 和 *a* 在所調查的三個地區都同時存在，但結合型 *A* 菌株在數量均較結合型 *a* 爲多。