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Races of *Bipolaris maydis* Occurring in Japan and Their Pathogenicity to the *rhm* Resistant Corn Line

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Abstract

The 76 isolates of *Bipolaris* maydis were obtained from diseased corn leaves collected at 35 sites in Japan and their mating type were determined by pairing with tester isokates, as in the case of the 4 isolates obtained from *Panicum* spp. and *Bothriochloa* sp. All the isolates were equally pathogenic to the differential lines of corn with T-cms, C-cms and N-c type cytoplasms in spore-spraying tests. They did not cause wilting of leaves in any type of cytoplasms in toxin tests and no host-specific toxins were detected. As a result, all the Japanese isolates were assigned to race O. Although the 75 isolates from corn produced only small and chlorotic lesions in the *rhm* resistant corn line, one isolate caused severe symptoms with typical and large lesions. The increased ability to cause lesion enlargement of the isolate in the *rhm* resistant line was attributed to the increase in the number of appressoria formed at the time of spore germination and faster hyphal extension in the host cells. This is the first report on a *B. maydis* isolate virulent to the *rhm* corn line in the world.

Discipline: Plant disease
Additional key words: *Cochliobolus heterostrophus*, fungus

Introduction

Southern corn leaf blight caused by the fungus, *Bipolaris maydis* (Nisikado et Miyake) Shoem., is the most severe and destructive disease for corn production in Japan. The acquisition of resistance to the disease is the major target in breeding work for disease resistance. The teleomorph, *Cochliobolus heterostrophus* (Drechs.) Drechs., is heterothallic with two mating types (MATA and MATa)[7]. Little is known about the mating types of the isolates collected in Japan.

B. maydis consists of three races, T, C and O. Races T and C are specifically pathogenic to corn lines with T-cms (cytoplasmic male sterile) and C-cms by producing T-toxin and C-toxin, respectively[6,9,16]. Since race O does not produce any host-specific toxin, it has no specificity for plant cytoplasms and is highly virulent to corn lines with normal cytoplasms (N-c)[13]. Races T and O occur worldwide, while the occurrence of race C has been restricted to China[16]. In Japan, a few cases of race T occurrence were reported in the 1970s[4], and the main race has been considered to be race O. However, race O has never been characterized and the present distribution of the races of *B. maydis* is not known in Japan.

The recessive gene, *rhm*, which is a resistance gene in corn, is used for breeding for resistance. The effect of the gene is distinct and lesion enlargement is clearly suppressed[11,12]. There are no reports on the occurrence of a new race specifically virulent to the *rhm* resistant corn lines.

The objectives of the study were to identify the races of *B. maydis* present in Japan, to clarify the mating types, and to confirm the presence of virulent isolates to the *rhm* resistant line.

Materials and methods

1) Preparation of B. maydis *isolates and crossing*

From 1981 to 1991, infected leaf samples of corn were collected at 35 sites in 7 districts. Single-spore

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isolates of *B. maydis* were obtained and cultured on silica gel with MM liquid media and stocked under dried conditions to prevent changes of pathogenicity[10]. Four isolates from other gramineous plants, supplied by Dr. M. Tsuda, Kyoto Univ., were used in the same way.

Mating type of all the isolates tested was determined by pairing each Japanese isolate with the tester isolates of known mating types, BM8135 (MATA) and HITO7711 (MATa) according to the method described by Nelson[7].

2) Characterization of races of B. maydis

(1) Spore-spraying test

Spore-spraying tests for characterization of races of all the isolates were conducted in a greenhouse using young plants. Some sets of differential lines of corn which had the same nuclear genome background except for the type of cytoplasms were prepared as follows: WF9T × R2040 (T-cms) and WF9 × R2040 (N-c) for the characterization of race T, and Mo17*Ht*C (C-cms) and Mo17*Ht* (N-c) for the characterization of race C. All the plants were grown until they reached the 4–5 leaf stage in a greenhouse at 25°C.

Spore suspensions (10^5 spores per ml) of each isolate were prepared and a 2 ml aliquot was sprayed per plant. The inoculated plants were kept in a moist chamber (25°C) for 16 h and transferred to the greenhouse. One week after inoculation, the 4th leaf of each plant was examined for disease severity, rated on the 0 to 8 scale shown in Fig. 1[3]. Isolate of race T, C1C2, obtained from USA, was used for comparison.

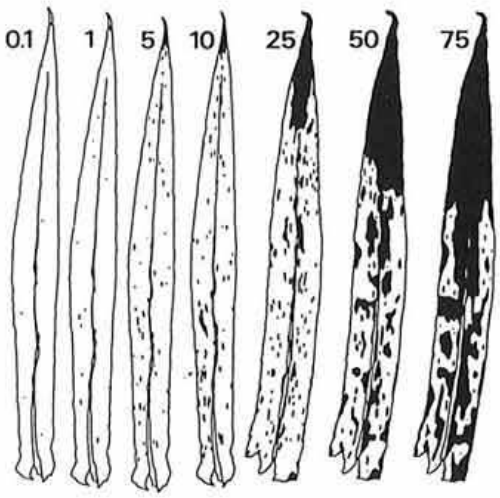

Fig. 1. Standard corn leaf diagrams illustrating various percentages of infection with *B. maydis*[3]
Disease rate: 0; no symptoms, 1; 0.1% of leaf area diseased, 2; 1%, 3; 5%, 4; 10%, 5; 25%, 6; 50%, 7; 75%, 8; whole leaf diseased and killed.

(2) Toxin test

Toxin tests were performed to confirm the production of host-specific toxins of the tested isolates. Each isolate was cultured on CM liquid media at 25°C in darkness for 14 days. The culture filtrates were diluted × 100. The 4th leaves of the differential lines were cut and immersed in 10 ml of the diluted filtrate in glass test tubes. They were incubated at 25°C under continuous illumination for 48 h and wilting of the leaves was checked.

3) Determination of virulence to rhm *resistant line*

Spores of all the isolates were primarily sprayed on the corn inbred lines, A632*rhm* (*rhm* line) and A632*Ht* (non-*rhm* line) as described above and the isolate virulent to A632*rhm* was selected. The number of appressoria formed, the rate of cell invasion, the number of invaded cells, and the lesion area on A632*rhm* and A632*Ht* were examined for the selected isolate, using the single spore inoculation method described previously[14].

Results

1) Isolates collected in Japan and their mating types

Seventy-six isolates of *B. maydis* were obtained from the infected samples (Table 1). All the isolates were crossed with either a tester isolate and they formed fertile pseudothecia. Forty-three isolates belonged to the MATA type and 33 isolates to the MATa type, and MATA was more frequent. Both types were distributed uniformly regardless of their geographical origin (Fig. 2).

Four isolates from the other gramineous plants were also crossed with tester isolates of *B. maydis*. Both MATA and MATa mating types were represented among the isolates.

2) Pathogenicity to differential lines

Based on the spore-spraying test of the isolates from corn, the scores of disease severity ranged from 4.4 to 5.1 in WF9T × R2040 (T-cms), and from 4.5 to 5.0 in WF9 × R2040 (N-c), as shown in Table 1. There were no significant differences between them. The race T isolate, C1C2, induced severe symptoms in T-cms lines and the disease severity in T-cms lines (5.0) was significantly different from that in N-c lines

Table 1. Results of spore-spraying and toxin test in different corn cytoplasms of the *B. maydis* isolates and characterization of races

Isolates	No. of isolates	Pathogenicity in[1]				Toxicity in[2]			Race
		T[3]	N	C	M	T	C	N	
Isolates collected in									
Hokkaido and Tohoku dists.	14	5.0	5.0	4.1	4.2	−	−	−	O
Kanto dist.	17	4.6	4.5	3.6	4.1	−	−	−	O
Chubu and Kansai dists.	13	4.4	4.5	3.3	3.9	−	−	−	O
Kyushu dist.	32	5.1	5.0	3.9	4.2	−	−	−	O
Isolates obtained from									
Panicum maximum	1	n. t.	n. t.	n. t.	n. t.	−	−	−	O
P. dichotomiflorum	1	3.3	3.0	2.7	2.3	−	−	−	O
P. miliaceum	1	n. t.	n. t.	n. t.	n. t.	−	−	−	O
Bothriochloa sp.	1	5.0	4.7	3.7	4.0	−	−	−	O
Isolate of race T									
C1C2	1	5.0*	2.7	1.5	2.0	+	−	−	T

1): Results of spore spraying test (0: no symptoms, 8: whole leaf diseased).
2): Results of toxin test (+: severe wilting, −: no wilting).
3): Differential lines (T: WF9T × R2040 (T-cms), N: WF9 × R2040 (N-c), C: Mo17*HtC* (C-cms), M: Mo17*Ht* (N-c)).
n. t.: Not tested because of the loss of sporulation capacity.
* Significant differences were detected among the differential lines ($P<0.05$).

Fig. 2. Distribution of mating types, MATA and MATa, of the *B. maydis* isolates in Japan

(2.7). When Mo17*HtC* (C-cms) and Mo17*Ht* (N-c) were inoculated, the disease severity ranged from 3.3 to 4.1 in C-cms lines and from 3.9 to 4.2 in N-c lines and there were also no significant differences between them. The averaged data of the isolates collected in each district are shown in Table 1 and the data of each isolate were reported previously[15].

Although race T caused severe wilting in the corn lines with T-cms in the toxin tests, no Japanese isolates obtained from corn caused wilting in the T-cms lines. The race T and the Japanese isolates did not cause wilting in the C-cms and N-c lines.

The isolates obtained from *Panicum* spp. and *Bothriochloa* sp. also showed a similar virulence to the differential lines and did not cause wilting in any type of cytoplasms.

3) *Characterization of the isolate virulent to the* rhm *line*

Although the 75 isolates obtained from corn produced only small and chlorotic lesions on A632*rhm* in the preliminary inoculation test, one isolate caused severe symptoms. The isolate, BM8376, collected in Kyushu district, produced typical and large lesions on the leaves of A632*rhm* (Plate 1). Based on the single spore inoculation test, BM8376 produced lesions approximately 20 fold as large as those of the check isolate, BM8380, 7 days after inoculation in A632*rhm* (Table 2). In A632*Ht*, the isolate also produced large lesions 2–3 fold as large as those of the check. An increased number of appressoria was observed for the conidia of BM8376 inoculated in A632*rhm* and the difference from the check isolate was significant. Although the rates of

host-cell invasion were not different among treatments, the number of cells invaded by infection of hyphae of BM8376 increased faster in A632*rhm* than in the check, but the difference was not significant.

Discussion

All the isolates tested were assigned to *B. maydis*, since they could be crossed with the tester isolates. The ratio of appearance of mating types was 4:3 for MATA and MATa, and the ratio was fairly close to 1:1, mainly due to the repeated crossing of the fungi for a long period of time in Japan, as reported by Leonard for race O in USA[5].

There were no significant differences in the pathogenicity of all the Japanese isolates from corn to the differential lines in the spore-spraying test. On the other hand, race T showed a strong pathogenicity in T-cms, but not in N-c. Based on the toxin test, no wilting of leaves of the corn lines with T-cms, C-cms and N-c lines, was observed when the Japanese isolates were tested. It was, therefore, concluded that the host-specific toxins, T- and C-toxins, were not produced by the Japanese isolates. Severe wilting was observed in T-cms by race T and the difference in the toxicity in T-cms between the

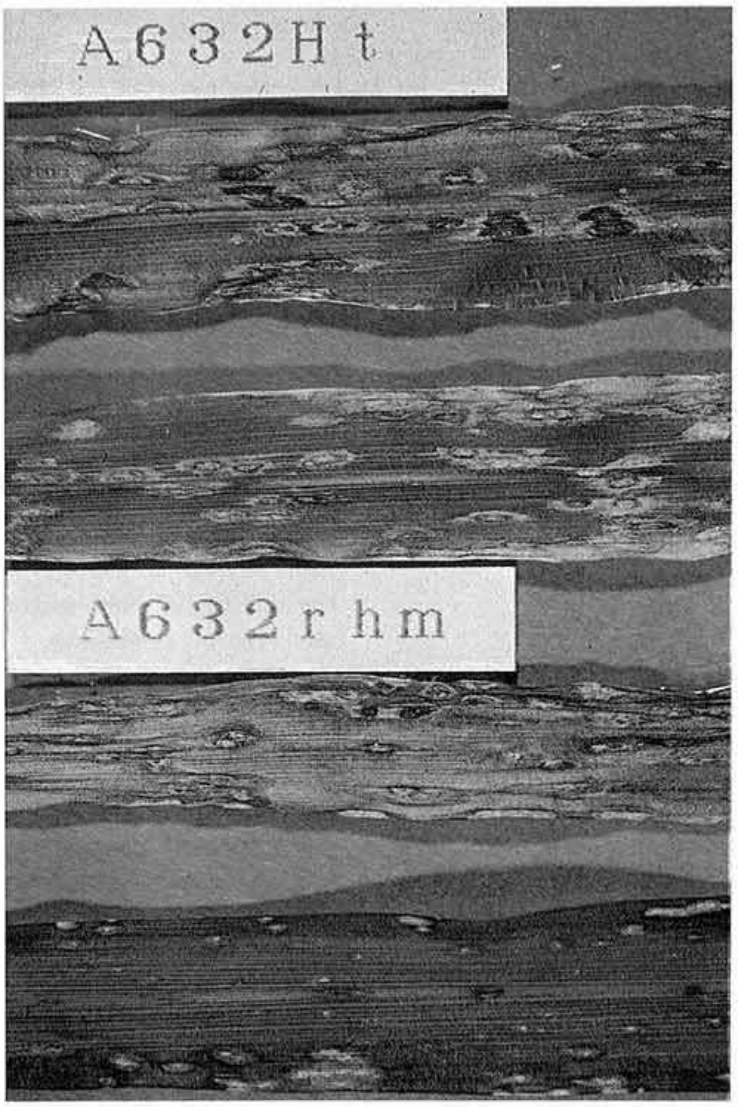

Plate 1. Symptoms of the *rhm*-virulent (BM8376) and normal (BM8380) isolates in the corn inbred line, A632*rhm* and A632*Ht*

A632*Ht* and A632*rhm* were inoculated with BM8376 (upper) and BM8380 (lower), respectively.

Table 2. Histopathological characteristics of *rhm*-virulent (BM8376) and normal (BM8380) isolates on leaves of corn inbred lines, A632*rhm* and A632*Ht*

Isolates	Inoculated corn lines	No. of appressoria[1]	Rate of cell invasion[2] (%)	No. of invaded cells[3]			Lesion area (mm^2)[4]		
				5 h	7 h	9 h	3 days	5 days	7 days
BM8380	A632*rhm*	1.1a*	84.3	1.6a	1.9a	2.2a	0.2a	0.3a	0.3a
	A632*Ht*	1.3a	85.2	1.6a	2.0a	2.6ab	1.3b	3.2b	6.0b
BM8376	A632*rhm*	1.8b	90.7	1.4a	2.0a	2.7ab	1.5b	3.0b	5.6b
	A632*Ht*	2.1b	88.2	1.6a	2.3a	2.9b	3.0c	7.9c	14.2c

1): Number of appressoria produced from 1 conidium.
2): Data were collected 7 h after inoculation.
3): Number of cells invaded by hyphae from 1 appressorium.
4): Lesions derived from a single conidium were measured 3, 5 and 7 days after inoculation.
* Means within the same row with different letters were significantly different ($P<0.05$).

Japanese isolates and race T was distinct. Since it is known that race O shows a similar pathogenicity to all the types of cytoplasms of corn and does not produce any host-specific toxins[13], all the Japanese isolates were assigned to race O. Since race O shows a strong pathogenicity also in N-c[2], severe outbreaks could occur in corn with N-c which is mainly cultivated presently in Japan under favorable climatic conditions. Since no race T isolates were discovered among the isolates tested, the population of race T was considered to have decreased in Japan. However, race T may possibly occur again if varieties with T-cms are cultivated on a large scale. Race C also was not detected in these studies. The invasion of race C must be monitored, since corn lines with C-cms are often used for breeding in Japan.

B. maydis has often been isolated from many gramineous hosts besides corn, for example, *Cynodon, Digitaria, Oryza, Panicum, Saccharum*, etc.[1,8]. The isolates obtained from *Panicum* spp. and *Bothriochloa* sp. in Japan were also assigned to *B. maydis*. On the basis of their pathogenicity to the differential lines and the results of toxin tests, they can be characterized as race O, as in the case of the races from corn. The gramineous plants infected with *B. maydis* are considered to perform a role of primary inocula for the spread of the disease in corn.

Although there have been no reports on the isolates virulent to the *rhm* resistant line in the world, one virulent isolate was discovered in this study. The isolate produced typical and large lesions in the *rhm* resistant line, in contrast to the normal *B. maydis* isolates which produced only chlorotic lesions. The isolate produced a larger number of appressoria at the stage of spore germination and invaded a larger number of host-cells after inoculation, compared with the check isolate. The ability to produce large lesions in the *rhm* line was attributed to these characteristics in the host-cells. Since the rate of host-cell invasion did not differ from the check, the invasion ability was not considered to increase in the isolate.

It is significant for the breeding and cultivation of corn that the types of races of *B. maydis* and their distribution could be determined for the first time in Japan. Besides, the existence of the *rhm*-virulent isolate may affect breeding, since the *rhm* gene has been a useful source of resistance to the disease. Although the isolate may be possibly classified into a new pathogenic race because of its host-specific virulence to the *rhm* line, the production of host-specific toxins and the genes controlling the virulence must be determined to designate it as a new race.

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