Examination of Rice Hydathode Water Pores Exposed to *Xanthomonas campestris* pv. *oryzae*

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ABSTRACT

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Compatible and incompatible interactions between *Xanthomonas campestris* pv. *oryzae* and the hydathode water pores of rice (*Oryza sativa*) were studied by scanning electron microscopy. Leaves of the rice cultivars Cas 209 and IR20, spray-inoculated with isolates of two *X. c. oryzae* races or water, were sampled for examination by scanning electron microscopy at 24 and 48 hr after inoculation. Individual water pores were examined for the presence or absence of plugging exudate. Water pore plugs were present following all treatments, and no significant differences among treatments were observed in the percentages of pores plugged. Water pores with exudate were also observed in untreated Cas 209 leaf samples. Therefore, plugging of water pores is not specifically induced in incompatible interactions between *X. c. oryzae* and rice.

The rice bacterial blight pathogen, *Xanthomonas campestris* pv. *oryzae* (Ishiyama) Dye, is primarily a vascular pathogen (8,9). The pathogen does not actively or directly penetrate host barriers but enters the plant through hydathode water pores or wounds (8,9). Hydathodes (1–5 mm in length and 10–15 per leaf) are located near the edge of each leaf (6). Each hydathode contains 10 to 20 water pores, which are similar in appearance to stomata but are about two to four times larger (6).

Once bacteria have entered a water pore, they multiply in the epithem and invade the vessels through the vascular pass (9). Horino (1,2) used transmission electron microscopy to observe compatible and incompatible interactions between *X. c. oryzae* isolates and rice leaf vessels. Three days after inoculation, bacterial cells within vessels in incompatible interactions were irregular in shape and enveloped by abundant host-produced fibrillar material. In contrast, bacterial cells in compatible combinations appeared normal and were not surrounded by the fibrillar material until 20 days after inoculation. On the basis of transmission electron microscopy in these studies (1,2) and those of Tsuno and Wakimoto (11), it was suggested that the production of fibrillar material in vessels might be involved in host resistance to *X. c. oryzae*.

Mew and co-workers (6) used scanning electron microscopy (SEM) to investigate the interactions between *X. c. oryzae* isolates and hydathodes of resistant and susceptible rice cultivars. In incompatible combinations, bacteria were embedded in an exudate, which appeared to emanate from the rice hydathode water pores by 24–48 hr after inoculation. The composition of the exudate, which often sealed the pore opening, and its relation to the fibrillar material described by Horino (1,2) are not known. Mew et al (6) did not observe exudates in compatible combinations. They found that bacterial numbers did not increase on the water pore surfaces in incompatible combinations, whereas bacteria multiplied in compatible interactions. They concluded that immobilization and inhibition of multiplication at the infection court are important events in resistance and that these are the result of excretions from host water pores. Because the excretions were reported to occur only in incompatible interactions, it seemed possible that the induction of the excretions might be a race-specific event. However, in these studies the numbers of plugged water pores in incompatible or compatible interactions were not determined, nor were controls included so that background plugging of water pores could be assessed. Therefore, our objective was to determine whether exudate formation at water pores is part of the host response in race-specific resistance. To do so, we quantified water pore plugging in compatible and incompatible interactions between the rice cultivars Cas 209 and IR20 and isolates of *X. c. oryzae* races 1 and 2.

MATERIALS AND METHODS

Bacterial cultures. *X. c. oryzae* isolates PXO61 (race 1) and PXO86 (race 2) were obtained from T. W. Mew at the International Rice Research Institute, Los Baños, the Philippines. The bacteria were maintained on peptone-sucrose agar (10) at 28 C for routine use and stored at −80 C in 15% glycerol (4) or lyophilized for long-term storage. For experiments, peptone-sucrose broth was inoculated with bacteria from 2-day-old cultures on peptone-sucrose agar and incubated overnight at 28 C with shaking. The cells were washed twice with sterile distilled water by centrifuging at 6,750*g* for 10 min and then adjusted to 10^8 colony-forming units per milliliter in sterile distilled water.

Rice cultivars. Seeds of the rice cultivars Cas 209 and IR20, which contain, respectively, the *Xa-10* and *Xa-4* genes for resistance to bacterial blight were obtained from T. W. Mew at the International Rice Research Institute. Cas 209 is resistant to isolates of race 2 but susceptible to isolates of race 1 (5). IR20 is resistant to isolates of race 1 but susceptible to isolates of race 2 (5). Two seeds were planted in 8.9-cm-square pots containing Bacto potting soil (Michigan Peat Co., Houston, TX) supplemented with fertilizer (Peter's 20-20-20, W. R. Grace, Cambridge, MA). The plants were grown in a greenhouse at 28–32 C during the day and 22–26 C at night. At 2 wk past sowing, the pots containing rice seedlings were placed in trays with 5 cm of water.

Inoculation procedure. Inoculation techniques and plant incubation conditions were as described by Mew et al (6). Prior to inoculation, 55-day-old rice plants were placed in large plastic bags and incubated in the dark overnight in a growth chamber at 22 C. Leaves were sprayed with bacterial suspensions from an atomizer (No. 152, The DeVilbiss Co., Somerset, PA) until fine droplets uniformly covered the leaf blades (6). The atomizer was held 50 to 60 cm away from the leaves during spraying to avoid damage to leaf surface structures. Plants sprayed with distilled water served as controls. All plants were returned to bags and incubated for 1 hr at 28 C with light (277–292 μE, from a combination of cool white fluorescent tubes and tungsten incandescent bulbs) in a growth chamber. The plants were then removed from the bags and placed in a greenhouse (28–32 C during the day and 22–26 C at night) until sampling.

Sampling and preparation for SEM. For each treatment, one leaf was taken from each of two different pots at 24 or 48 hr after inoculation or at both times. Six sections (1 × 8 mm) containing hydathodes were cut from the leaf margin of each leaf with a razor blade. The 12 sections from each treatment were combined, fixed in 0.025 M potassium phosphate buffer (pH 7.0) containing 3% glutaraldehyde for 24 hr at 4 C, washed three times with buffer, and then dehydrated at room temperature in a graded ethanol series of 30, 50, 75, 80, 85, 90, 95, and 100%. The sections were critical-point-dried with liquid carbon dioxide, coated with platinum, and then examined with SEM (ETEC Autoscan, Perkin-Elmer Electron Beam Technology, Hayward, CA). Individual water pores (totaling 28–358) were counted and scored for the presence or absence of plugs.

Because sections within a treatment were pooled and not scored individually, treatments within an experiment were not replicated and could not be compared statistically. Therefore, in order to compare treatments, experiments (blocks) were replicated over time in a randomized complete block design (7).

To determine if the presence of bacteria on the guard cells of water pores correlated with exudate formation, sections of Cas 209 leaves were treated with PXO86 cells and sampled at 48 hr for SEM examination as described above. Individual water pores with and without plugs were counted and were scored for the presence or absence of bacteria on the surface of the guard cells. This experiment was repeated four times.

RESULTS AND DISCUSSION

Figure 1 shows scanning electron micrographs of Cas 209 leaf surfaces. A portion of a hydathode containing several water pores is visible in Figure 1A. An example of a water pore plugged with exudate is shown in Figure 1B. This means of visualizing the water pores was used to quantify water pore plugging. The data are reported in Tables 1 and 2.

Exudate was observed in some water pores in all treatments of Cas 209, including compatible interactions (with PXO61), incompatible interactions (with PXO86), and water inoculation (Table 1). The mean percentages of pores plugged on Cas 209 in incompatible interactions (with PXO86) were not greater than those observed in compatible interactions (with PXO61) or after treatment with water. Analysis of the data (percentage of water pores with exudate) from leaves sampled 24 and 48 hr after inoculation indicated no significant difference ($P = 0.05$) on Cas 209 among any of the treatments at either time. Thus, although the percentages varied between experiments, the numbers of water pores with exudate were not correlated with the interactions between the rice cultivar Cas 209 and any treatment (with bacterial isolate PXO86 or PXO61 or with water). The exudate was not induced by the inoculation procedure, because in separate experiments, water pore plugs were also present on healthy, untreated Cas 209 leaves (mean 18%, with a standard error of ±6%).

In incompatible combinations with Cas 209, the percentage of plugged water pores on which bacterial cells were observed (mean 6%, with a standard error of ±1%) was not significantly different ($P = 0.05$) from the percentage of plugged water pores on which no bacteria were observed (7 ± 5%). Bacterial cells were also observed on water pores that were not plugged (33 ± 10%) in the incompatible combination. Bacteria may have been present in these experiments but not observed if they were inside the water pore. However, the percentage of plugged water pores with bacteria present (6 ± 1%) was much lower than that of pores without exudate and with bacteria present (33 ± 10%). Further, water pore plugging in bacterial-treated tissues was not significantly different from that in water-treated tissues (Table 1). Therefore, it is unlikely that in Cas 209 water pore exudate was induced by bacteria.

Results similar to those shown for interactions with the cultivar Cas 209 were observed following treatment of the cultivar IR20 with *X. c. oryzae* isolate PXO86 (compatible) or PXO61 (incompatible) or with water (Table 2) and in an unreplicated survey of compatible and incompatible interactions with other rice cultivars (DV85 and IR1545-339, data not shown). Again, although the numbers varied between experiments, the percentage of water pores with exudate was not correlated with interaction type (compatible or incompatible) for any rice cultivar and treatment (bacteria or water). Thus, water pore plugging is not specifically induced in incompatible interactions between these rice cultivars and bacteria.

To determine if differential plugging was dependent on experimental conditions other than those described by Mew et al (6), variables such as plant age (4–5 versus 7–8 wk), bacterial

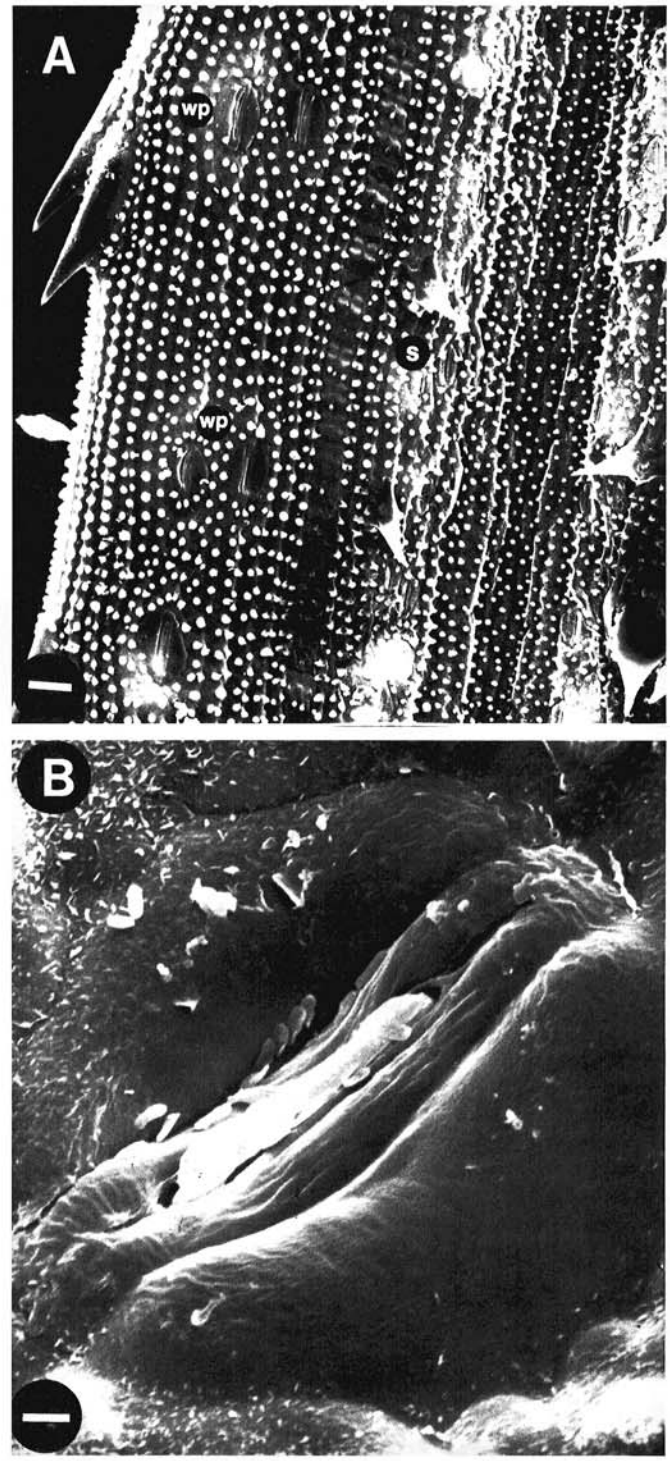

Fig. 1. Scanning electron micrographs of Cas 209 rice leaf surfaces. **A,** Leaf section showing stomata (s) and a portion of a hydathode with several water pores (wp). Bar = 20 μm. **B,** Leaf section 24 hr after inoculation with *Xanthomonas campestris* pv. *oryzae* isolate PXO86. Bacteria can be seen on the surface of the water pore. An exudate plugs the water pore opening. Bar = 2 μm.

TABLE 1. Percentage of water pores with exudate at 24 and 48 hr after treatment of the rice cultivar Cas 209 with *Xanthomonas campestris* pv. *oryzae* isolate PXO86 or PXO61 or with water

	Percentage of water pores with exudate[a]					
	24 hr			48 hr		
	PXO86 (I)[b]	PXO61 (C)[c]	Water	PXO86 (I)	PXO61 (C)	Water
Block 1	18 (12/66)	13 (30/244)	16 (18/116)	27 (27/110)	28 (60/211)	40 (44/111)
Block 2	6 (6/97)	6 (7/119)	11 (9/80)	10 (23/240)	14 (23/165)	13 (45/358)
Block 3	16 (10/61)	ND[d]	15 (10/65)	14 (28/199)	ND	11 (23/203)
Block 4	ND	ND	ND	19 (8/43)	36 (28/77)	33 (13/39)
Mean and standard error	13.3 ± 6.4	9.5 ± 4.9	14 ± 2.6	17.5 ± 3.0	23.2 ± 3.7	24.3 ± 3.0

Analysis of variance

	Mean square		*F* value[e]	
Source of variation	24 hr	48 hr	24 hr	48 hr
Blocks	53.0417	285.07	10.55*	7.90*
Treatments	7.3750	51.0069	1.47	1.41
Error	5.0278	36.0972	—	—

[a]Individual water pores with and without exudate were counted. The data in parentheses are the number of water pores with exudate and the total water pores counted, respectively.
[b]I = incompatible interaction.
[c]C = compatible interaction.
[d]ND = not determined.
[e]Asterisk (*) denotes statistically significant difference between blocks at $P = 0.05$. There was no significant difference between treatments at $P = 0.05$.

TABLE 2. Percentage of water pores with exudate at 48 hr after treatment of the rice cultivar IR20 with *Xanthomonas campestris* pv. *oryzae* isolate PXO86 or PXO61 or with water

	Percentage of water pores with exudate[a]		
	PXO86 (C)[b]	PXO61 (I)[c]	Water
Block 1	22 (7/31)	16 (5/31)	11 (3/28)
Block 2	16 (6/38)	10 (5/51)	12 (7/58)
Mean and standard error	19.0 ± 4.3	13.0 ± 4.3	11.5 ± 0.7

Analysis of variance

Source of variation	Mean square	*F* value[d]
Blocks	20.1667	2.47
Treatments	31.5000	3.86
Error	8.1667	—

[a]Individual water pores with and without exudate were counted. The data in parentheses are the number of water pores with exudate and the total water pores counted, respectively.
[b]C = compatible interaction.
[c]I = incompatible interaction.
[d]Neither blocks nor treatments were significantly different at $P = 0.05$.

concentrations (10^6 versus 10^9 colony-forming units per milliliter), humidity levels (70 versus 100% RH), and tissue dehydration procedures (critical-point drying versus freeze-drying) (3) were tested in unreplicated experiments (data not shown). Although the percentage of water pores with exudate varied between experiments, again no difference that would indicate specific induction by any treatment was observed. For example, when Cas 209 leaves were treated with PXO86 and then incubated at 100 or 70% RH for 24 hr, 13 and 11% plugged water pores were observed, respectively.

The SEM study by Mew et al (6) indicated that incompatible interactions between *X. c. oryzae* isolates (race 0, avirulent, isolate PXO40; race 1, isolate PXO61; and race 2, isolate PXO86) and rice cultivars (Cas 209, resistant to race 2, and TN-1, susceptible to races 1 and 2) were characterized by induction of a host exudate that emanated from and sealed off water pores. Water pore plugs were not observed in compatible interactions. These studies suggested that exudate formation is involved in resistance and is specifically induced in an incompatible combination. We used the same experimental conditions and isolate combinations (PXO86 and PXO61) with cultivar Cas 209 as reported by Mew et al (6) but, in addition, replicated our experiments over time and sampled water-treated controls. Our results differ from those of Mew et al (6) in that we observed plugging in both compatible and incompatible interactions with cultivar Cas 209. Further, the percentage of plugged water pores in the water-treated control leaves was not significantly different from that observed in either the compatible or the incompatible interactions. Although they did not indicate the numbers of plugged water pores, Mew et al (6) estimated the numbers of bacteria on 50 water pores per treatment and indicated exudate formation occurred only in incompatible combinations. Water-treated or untreated controls were not included, and it is not clear whether the experiments were replicated (the 50 water pores may have been from one inoculated leaf per plant). We observed that plugged water pores were not evenly distributed within hydathodes (data not shown). Thus, if only a few hydathodes were examined, differences that suggest race-specific induction of plugging might have been observed.

In summary, our results indicate that the induction of water pore exudate is not specific to incompatible interactions between *X. c. oryzae* and rice, as previously suggested. Further, because the percentage of water pores with exudate in bacterial-treated tissues was similar to that in water-treated tissues, water pore plugging is not induced by bacteria, nor is it a general resistance mechanism in rice to *X. c. oryzae*.

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