Infection Courts of Rice Seedlings with *Pseudomonas plantarii* and *Pseudomonas glumae*

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Bacterial seedling blight of rice grown in nursery boxes has occurred annually in Miyagi, Chiba, Niigata, Kyoto, and Yamaguchi Prefectures, Japan¹⁻⁴). *Pseudomonas plantarii* was reported to be the causal agent for the disease⁵). However, it has not been shown if *P. plantarii* as well as *P. glumae*, which causes seedling rot of rice⁶), actually enters rice seedlings. This paper reports the histological study on the seedling tissues affected with them.

Affected seedlings were prepared in the following manner. Rice seeds, which had been soaked in water at ca. 30 C for 2 days, were placed on 0.5% agar gel and grown at 25 to 30 C in a laboratory. They were inoculated with *Pseudomonas plantarii* NIAES 1723^T (MAFF 03-01723, JCM 5492, ATCC 43733, ICMP 9424, AZ 8201) or *P. glumae* AZ 84431. Some of them were inoculated at the time of or after 2 days of sowing, with 0.1 ml of the inoculum (ca. 10⁸ CFU/ml) per germination. The rest was submerged in the inoculum after 4 days of sowing.

The inoculated seedlings were harvested after 2 to 11 days of inoculation and fixed in formalin-acetic acid-70% alcohol (6.5 : 2.5 : 100). After dehydrated in 70 to 100% alcohol, they were embedded in paraffin and cut into thin section 15 µm thick with a microtome. The tissues were stained by using a standard procedure, the Stoughton's method.

The rice seedlings inoculated with *P. plantarii* at the time of or after 2 days of sowing, showed severe symptoms when harvested after 6 to 11 days of the inoculation. Brown streaks were sometimes observed in coleoptiles (Fig. 1 A, B). Histological study revealed that the bacterium entered the seedlings through stomata in the surfaces of coleoptiles or leaf sheaths and through wounds (Plate I B, C, D, E, F, IIA). The entrance of the bacterium into rice seedlings through such stomata seems to occur easily, since some stomata in the surfaces of coleoptiles and leaf sheaths are always open and they allow nonpathogenic bacteria as well as *Xanthomonas campestris* pv. *oryzae* to enter the seedlings⁷). During the seedling growth, ruptures occur in the surfaces of coleoptiles and roots at the points of emergence of the first leaves or secondary roots. The bacterium entered the seedlings through such wounds (Plate II A). It multiplied and spread intercellularly (Plate I A, C, D, E, II A).

The brown streaks in coleoptiles were due to browning of the parenchyma cells around the vascular bundle sheaths (Plate I F). The brown areas were not always accompanied by the bacterium. The streaks were also observed in the coleoptiles of the seedlings grown on the agar

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Fig. 1. Symptoms on coleoptiles infected with *P. plantarii* and *P. glumae*. (A) and (B) Brown streak in coleoptile infected with *P. plantarii*. (C) Brown lesion on coleoptile infected with *P. glumae*.

gel containing tropolone, a product of the pathogen and toxic to rice seedlings⁸⁾. Therefore, the browning of the cells was considered due to the condensation of tropolone in the cells, which was produced in the basal parts of the seedlings and transported through vessels.

The seedlings, which were submerged in the inoculum after 4 days of sowing and harvested after 2 days of the inoculation, did not show any symptoms. But the bacterium entered them through stomata or wounds (Plate I E).

P. glumae entered rice seedlings, multiplied, and spread in the same manner. It invaded, rotted, and disintegrated the parenchyma of the first or second leaf sheaths (Fig. 1 C, Plate II B, C, D, E, F). The changes of the tissue were not seen with *P. plantarii*. *P. glumae* has a weak ability to rot potato slices, while *P. plantarii* does not⁹⁾. This seems to be a factor to account for the differences in symptoms. Although the coleoptiles were invaded by *P. glumae*, they usually retained their shapes.

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和 文 摘 要

畔上耕児・西山幸司・田部井英夫：*Pseudomonas plantarii* および *P. glumae* のイネ体内への侵入および増殖部位

Pseudomonas plantarii および *P. glumae* を接種したイネ幼苗の連続切片を作成し、これらの細菌の侵入および増殖部位を顕微鏡観察した。その結果、*P. plantarii* は、鞘葉および本葉葉鞘の気孔、鞘葉が裂けた傷痕部、および出根時の傷痕部から侵入し、柔組織の細胞間隙および細胞間空隙部で増殖しているのが観察された。鞘葉には褐色条斑が見られたが、それは維管束鞘外側の柔組織細胞の褐変によるものであった。この褐変部には細菌は必ずしも存在していなかった。*P. glumae* は同様にイネ体内に侵入して、本葉葉鞘の組織を腐敗・崩壊させたが、*P. plantarii* ではそれらは観察されなかった。鞘葉の形態はいずれの細菌を接種した場合にも崩れていなかった。

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Plate I

Plate II

Explanation of plates**Plate I.** Cross sections of rice seedlings inoculated with *P. plantarii*.

- A General view. Co, Coleoptile; 1, 2. and 3, first, second, and third leaf sheaths, respectively.
 - B Infection of coleoptile with *P. plantarii* through stoma.
 - C and D Infection of coleoptile with *P. plantarii* through stoma and multiplication of the pathogen in intercellular spaces of parenchyma.
 - E Infection of the second leaf sheath through stoma with and multiplication of *P. plantarii*.
 - F Browning of parenchyma cells around vascular bundle sheath.
- Arrows indicate bacterium.

Plate II. Cross sections of rice seedlings.

- A Entrance of *P. plantarii* into coleoptile through wound and multiplication in intercellular spaces of coleoptile.
 - B Infection of coleoptile with *P. glumae* through stoma.
 - C Multiplication of *P. glumae* in coleoptile.
 - D, E, and F Disintegration of the first leaf sheath infected with *P. glumae*.
- Arrows indicate bacterium.