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Influence of *Pyricularia oryzae* on Leaf Gowth in Rice Plants

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Summary

The morphological and anatomical characters of rice plants inoculated with the rice blast fungus (*Pyricularia oryzae* Cav.) were examined. When the susceptible conidia of *P. oryzae* were inoculated on the 6th leaf blades (N) of rice seedlings, the elongation of the successive upper leaves began earlier than that in the healthy plants, and was inhibited at later stages. The growth inhibition of the leaf blades of the inoculated plants depended on the leaf stage at inoculation, and there was a high negative correlation between the leaf stage at inoculation and growth inhibition of the leaves. Many immature stomata were observed on the surface of the stunted leaf blades (N+2). The lengths of stomata and epidermal cells on the stunted leaves were shorter than that on the healthy plants. The number of stomata and epidermal cells on the stunted leaves decreased significantly.

There are two types of symptoms on rice plants infected with the rice blast fungus (*Pyricularia oryzae* Cav.). One type is the local symptom which appears on various parts of rice plants, *e.g.* leaves, panicles, and nodes. The other type is the systemic symptom of stunting. Stunting of plants caused by the blast fungus is the so-called "Zurikomi-imochi" symptom.

Some morphological and physiological characters are described for plants stunted by the blast disease, and it has been suggested that the stunting of plants may be caused by a toxic material produced by either the blast fungus or by the infected plants, as leaf growth inhibition is influenced by the number and type of lesions (3).

The plants treated with ethrel, spotted on leaves, showed a symptom quite similar to the stunting of blast-diseased plants. In susceptible cultivars inoculated with the blast fungus, ethylene was evolved immediately after infection and increased considerably toward 11 days (2). Thus, ethylene may be a major factor responsible in the stunting of rice plants infected with the blast fungus.

However, more research is required to elucidate the mechanisms of stunting of infected plants by the rice blast fungus. In this paper, we report the morphological aspects of rice plants infected by the blast fungus, with special reference to the affected parts of plants and the initiation time for growth inhibition of leaves.

Materials and Methods

Plant material and cultivation

The rice cultivar Aichi-asahi (which carries true resistance gene Pi-a) was used. Disinfected seeds were germinated at 25°C and routinely raised at 25–26°C in a phytotron.

The blast fungus and inoculation procedures

The isolate F67-54 (race 047) of *Pyricuralia oryzae* Cav. was used. The isolate was cultured on oat-meal-agar in petri dishes at 25°C for 14 days. After the aerial hyphae were removed by a brush and water, the dishes remained uncovered and were exposed to fluorescent light for 3 days to procedure conidia. Conidial suspensions collected from those cultures were adjusted to a concentration of 2×10^4–2×10^5/ml with distilled water containing 0.02% Tween 20.

Rice seedlings were inoculated with a conidial suspension at four places on the sixth leaf blade by using a punch method soon after full expansion of the sixth leaf. The inoculated plants were incubated in a moist chamber for 24 h at 25°C, and returned to the phytotron at 25°C for 35 days.

Growth inhibition

When the inoculated leaf (N) and five successive upper leaves were fully expanded, the length of leaf blades and sheaths in both inoculated and healthy plants was measured. The percentage of growth inhibition was calculated for the inoculated leaf balde and sheath (N) and successive upper leaf blades and sheaths (N+1, N+2, *etc*) by the following formula:

$$\text{Growth inhibition (\%)} = \left(1-\frac{\text{Length of the stunted leaf blade or sheath}}{\text{Length of the healthy leaf blade or sheath}}\right)\times100$$

Time course of leaf elongation

The length of the inoculated and successive upper leaf blades and sheaths was measured during 10 days after inoculation, and compared with that of corresponding leaf blades and sheaths in the healthy plants.

Results

Leaf elongation of blast infected plants

Significant growth inhibition was observed in the N+1 leaf sheath, the N+2 leaf blade and sheath, and the N+3 leaf blade (Table 1). Growth inhibition

TABLE 1. *Percentage growth inhibition of the inoculated leaf (N) and successive upper leaves (N+1 to N+5) emerged after inoculation with P. oryzae*

	N		N+1		N+2		N+3		N+4		N+5	
	LB[c]	LS[c]	LB	LS	LB	LS	LB	LS	LB	LS	LB	LS
Growth inhibiton[a] (%)	1[b]	−2	−2	14	44	18	57	8	10	−9	−16	−3

[a] Growth inhibition was measured at 35 days after inoculation.
[b] Each value is the mean of 20 plants.
[c] LB : leaf blade, LS : leaf sheath.

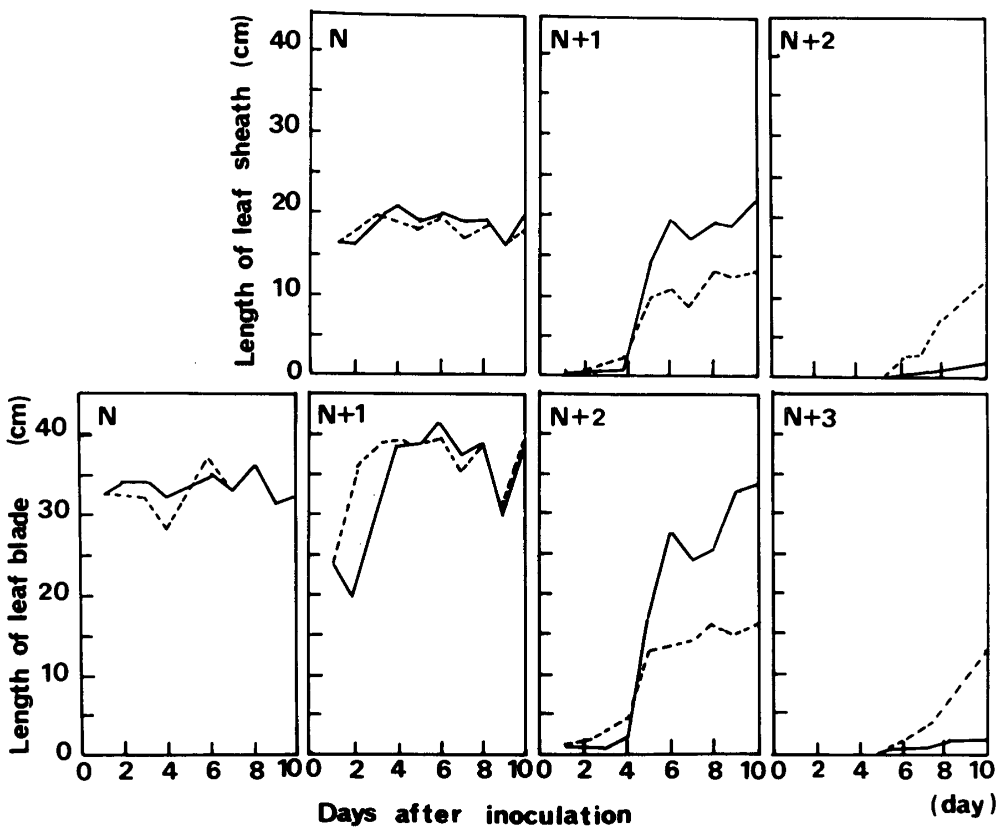

FIG. 1. Changes of length of leaf baldes and leaf sheaths in the inoculated and healthy rice plants.
- - - : Inoculated plant, ——: Healthy plant.

was slight in the N+3 leaf sheath and N+4 leaf blade. Statistically nonsignificant stimulation of growth was observed in the N+4 leaf sheath and the N+5 leaf blade and sheath.

Time course of leaf elongation in blast infected plants

As shown in Fig. 1, the growth of a certain leaf blade was roughly corresponded to that of the sheath of the next immediate lower leaf. No significant elongation of the inoculated leaf blade and sheath (N) was observed during the period tested. The elongation of the N+1 leaf sheath and N+2 leaf blade in the inoculation plants began on the second day after inoculation and reached plateaus after 5 days. Elongation of the corresponding leaf sheath and leaf blade in the healthy plants began on the fourth days and continued growth to significantly higher levels after 6 days. The elongation of the N+2 leaf sheath and the N+3 leaf blade in inoculated plants began on the fifth day, and was stimulated significantly comparing with that of the corresponding in the healthy plant on the same day.

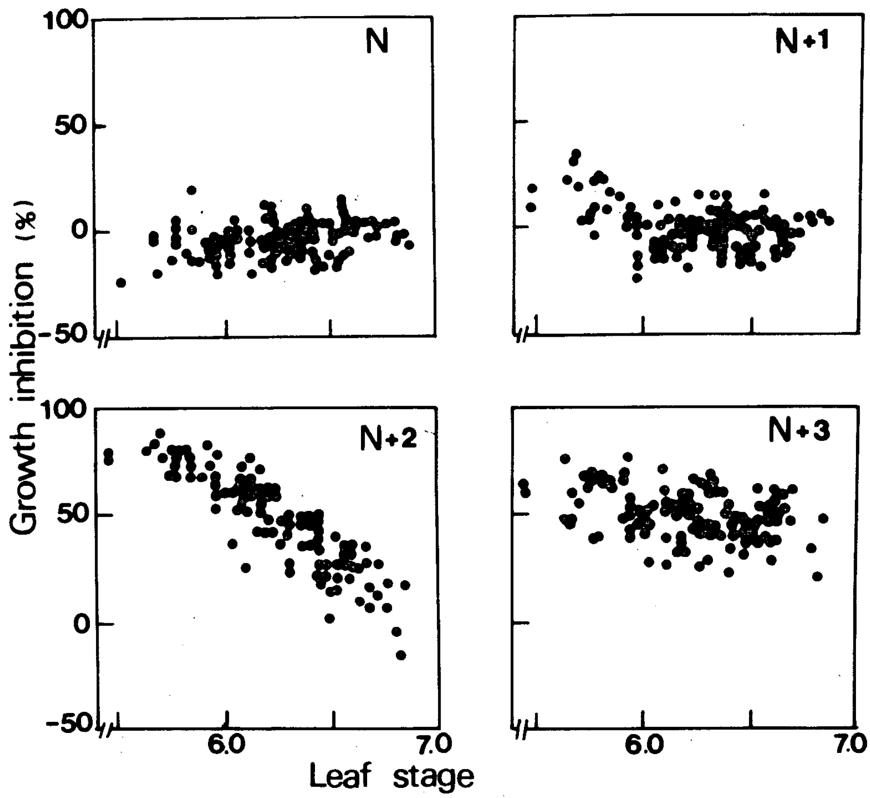

FIG. 2. Relation between inoculated leaf stage at inoculation time and growth inhibition of leaf blades at 14 days after inoculation.

Relation between the leaf stage at inoculating and growth inhibition

The sixth leaf on the main culm was inoculated with conidia at the 5.5-7.0-leafed stage, and growth inhibition was observed on the inoculated and successive upper leaves emerged after inoculation. The N leaf inoculated at the 5.5-7.0-leafed stage and N+1 leaf blade of the plant inoculated at the 6.0-7.0-leafed stage fully expanded without any inhibition of growth. On the contrary, the elongation of the N+2 leaf blade was strongly inhibited when the plant was inoculated at the 5.5-7.0-leafed stage, and there was a high negative correlation between the leaf stage at inoculation and the growth inhibition (Fig. 2). The same tendency was also observed in the N+1 leaf blade when the plant was inoculated at the 5.5-6.0-leafed stage. The growth of the N+3 leaf blades was inhibited irrespective of the leaf stage at inoculation.

Morphological observation on the blast-infected rice plants

The tissues of the inoculated plant, including the apical meristem, were observed by preparing longitudinal sections of the base of rice culm detached 4

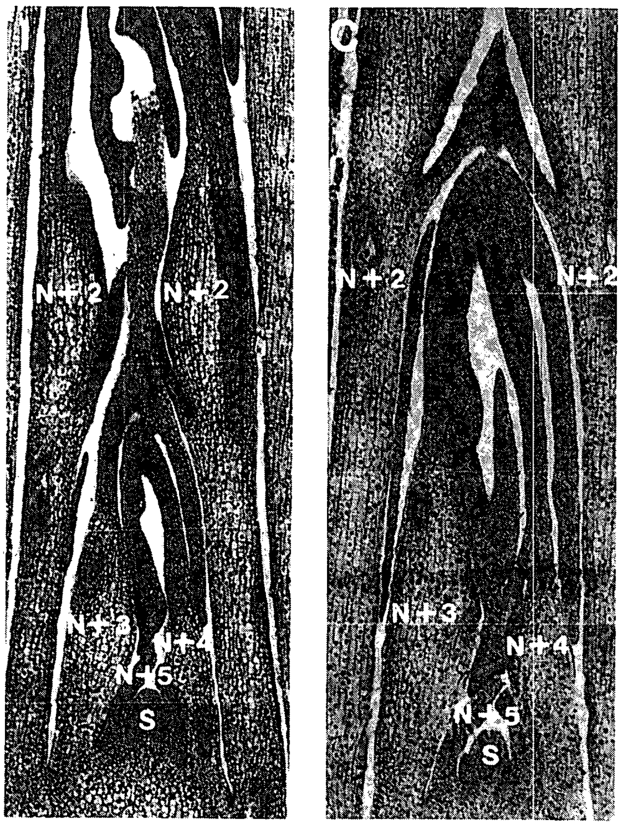

Fig. 3. The longitudinal section of the immature leaves and leaf primodia in the inoculated and healthy rice plants. I: Inoculated plant, C: Healthy plant, S: Shoot apex.

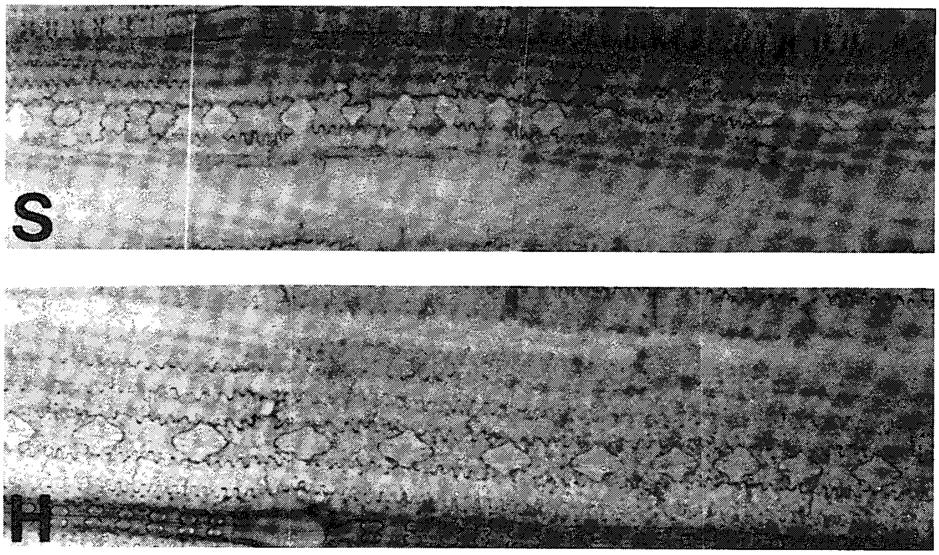

FIG. 4. Surface structure of the stunted and healthy leaf blade (N+2). S: Stunted leaf blade, H: Healthy leaf blade.

TABLE 2. *Differences in surface structures between stunted and healthy leaf blades*

	Average length (μm)	Number of stomata and epidermal cell per stoma line of leaf blade
Stoma		
Stunted	17±3.4	2617±965
Healthy	27±5.9	5805±316
Epidermal cell		
Stunted	39±13	2200±615
Healthy	44±13	4283±460

days after inoculation. There was no distinct difference in the morphology of the immature leaves and leaf primodia between the infected plant and healthy one (Fig. 3). However, many immature stomata were observed on the surface of the N+2 leaf blade in the inoculated plant (Fig. 4). The length and the number of both stomata and epidermal cells arranged by the stomatal line were determined on the N+2 leaf blade (Table 2). The length of stomata and epidermal cells on the stunted leaves was shorter than that on the healthy leaves. The percentage reduction of the number of stomata and epidermal cells was 45% and 51%, respectively. These percentage of reduction approximated to the percentage of growth inhibition (53%) of the stunted leaves.

Discussion

In this study on stunting of rice plants caused by the blast fungus, we recognized not only the inhibition of leaf growth but also the acceleration of the initiation time of leaf elongation. These results confirm the previous report indicating that the interval of leaf emergence in the stunted plants became shorter than that in the healthy plants (3). The initiation of leaf elongation in the stunted plants was earlier than that in healthy plants, and the time for maturing was shorter.

The growth inhibition was observed in the particular leaves, from the N+1 leaf sheath to the N+3 leaf blade, when the blast lesions were present only on the inoculated leaf (N). On the leaf formation in rice plant, the N+1 leaf sheath and the N+2 leaf blade were a conspicuous elongation stage of leaves after full expansion of N leaf. The growth inhibition of the N+2 leaf blade depended on the leaf stage at inoculating. There was a high negative correlation between the leaf stage at inoculation and the growth inhibition. Growth inhibition in the blast-infected plants occurred at the conspicuous elongation stage of leaves. The intercalary meristem may play an important role in the elongation of leaf. Many immature stomata were observed on the stunted leaves. The immatureness of stomata may be caused from the change of the actively of the intercalary meristem as suggested by Yamazaki (1963).

All these data obtained coincide with an assumption that the stunting of rice plants infected with the blast fungus is caused by the change of the actively of intercalary meristem. The elongation of internode and leaf sheath of monocotyledones develops through division and expansion of cells at the base of leaf insertion (1). The slight inhibition of epidermal cell elongation was observed previously (3), and in the present study, however, the growth inhibition of the leaf blade and sheath may be mainly caused by the significant reduction of the number of epidermal cells. This indicates that the suppression of cell division is one of the major factors for stunting of infected plants.

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