Role of Ethylene in Stunting of Rice Infected with Rice Blast Fungus, Pyricularia oryzae Cav.

Tohoku Journal of Agricultural Research
Vol. 43 No. 1-2, September 1992
Printed in Japan

Role of Ethylene in Stunting of Rice Infected with Rice Blast Fungus, *Pyricularia oryzae* Cav.

Hozumi YOSHIDA*, Reiichi YOSHINO**, Shigeru SATOH***,
Teruyoshi HASHIBA* and Yoshio EHARA*

**Faculty of Agriculture, Tohoku University, Sendai 981, Japan*
***Tohoku National Experiment Station, Morioka 020, Japan*
****College of General Education, Tohoku University, Sendai 980, Japan*

(Received, May 22, 1992)

Summary

The relatinoship between stunting of plant and ethylene production in blast-diseased rice was examined. In the compatible combination of rice cv. Aichi-asahi (which carries the true resistence gene Pi-a), and fungal isolate F67-54 (race 047), no differences were found in ethylene production between the inoculated plants and press-injured ones. However, blast infection affected leaf elongation on the second day after inoculation. In the incompatible combination of cv. Aichi-asahi and isolate 1813-2 (race 001), blast infection did not affect leaf elongation in the inoculated plants during six days after inoculation and no differences were found in ethylene production between the inoculated and the healthy plants. These results suggest that net production of ethylene is not related to the stunting of rice infected with blast fungus.

When infected with rice blast fungus (*Pyricularia oryzae* Cav.), the rice plant shows the systemic symptom called "zurikomi-imochi" which means the stunting of the plants.

The mechanism of stunting in blast diseased plants is unknown. Tamari and Kaji (4-6) have suggested that piricularin, isolated from the culture broth of blast fungus and also the diseased rice plant, caused induction and accumulation of coumarin in the plant tissues resulting in stunting. However, Sato and Sato and Kozaka (2, 3) reported that they could detect neither coumarin in the diseased plants nor piricularin in the culture broth of the blast fungus. After that, Kozaka and Teraoka (1) suggsted that ethylene is a major factor for stunting of the infected rice plant. They found that a rice plant treated with spotting ethrel on leaf showed symptoms quite similar to the stunting of a blast-diseased plant. Also, the evolution of ethylene increased considerably till 11 days afterwards in the fungus-inoculated leaves of a susceptible cultivar.

On the other hand, we recognized that blast fungus caused not only the inhibition of leaf growth but also hastened the initiation of leaf elongation (8). These influence cause the progressive type lesions to occur at the conspicuous elongation stage of leaves (8). In this paper, we report on the relations between ethylene production, leaf elongation and blast infection in rice plants.

Materials and Methods

Rice plant

Rice cultivar Aichi-asahi (which carries the true resistance gene Pi-a) was used. The disinfected seeds were germinated at 25°C and grown normally at 25-26°C in the phytotron.

The blast fungus and inoculation procedures

Two isolates of *Pyricularia oryzae* Cav., F67-54 (race 047) and 1813-2 (race 001), were used. The isolates were cultured on oat-meal-agar petri dishes at 25°C for 14 days. After removing the aerial hyphae by a moist brush, the dishes were exposed to fluorescent light for 3 days to induce conidiation. An inoculum paste containing spores, cellulose powder and carboxymethyl cellulose sodium salt (CMC) was used for inoculation.

The rice seedling was inoculated by the punch method with 0.02 g inoculum paste at four places on the sixth leaf blade after full expansion of the leaf. The inoculated plants were incubated in a moist chamber for 24 hr at 25°C and then kept in the phytotron.

Leaf length

The length of the inoculated leaf (N) and two successive upper leaves (N+1 and N+2) was measured for six days after inoculation. Nine leaves from different plants were measured for each treatment.

Ethylene production

Ethylene production was determined from 10 cm-long segment cut from the sixth leaf blade with four inoculation sites and from 10 cm-long segments of the two successive upper leaves emerging after inoculation. Each segment as put in a 85 ml test-tube with 2 ml of distilled water and sealed with a double rubber stopper. After incubation for 3 hr at 30°C in the dark, the gas within the test-tube was exchanged for the air with an air compressor. After 6 hr further incubation the gas within the test-tube was analyzed. Ethylene accumulated in the test-tubes was measured by gas chromatography (Hitachi, Model 063-5050) using an activated alumina column and a flame ionization detector. Each amount of ethylene production is mean of 3 test-tube samples. The press-injured

rice plant without inoculation was used as control.

Results

The length of leaf blade

In compatible combination, there was no difference in the length of N and N+1 leaf blade between the inoculated and the healthy plants (Fig. 1a). However, the length of N+2 leaf blade in the inoculated plant was longer ($p < 0.05$, t-test) than in the healthy plant on the 2nd to 4th day after inoculation. On the other hand, in the incompatible combination, no such differences were found (Fig. 1b).

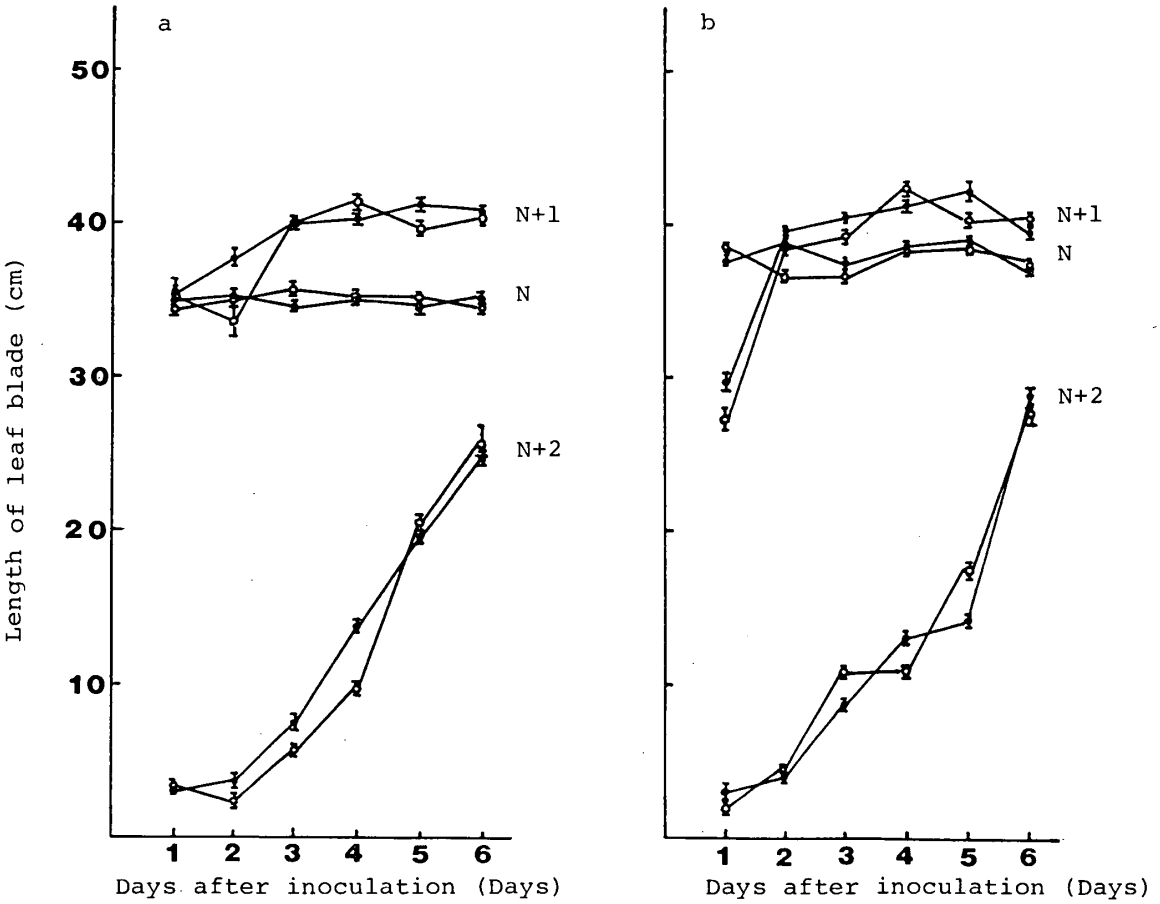

FIG. 1. Length of inoculated leaf blade and successive upper leaf blades emerged after inoculation with rice blast fungus, *Pyricuralia orizae*.
● : Inoculation, ○ : Control = press-injured
a : Compatible combination ; Aichi-asahi-F67-54
b : Incompatible combination ; Aichi-asahi-1813-2

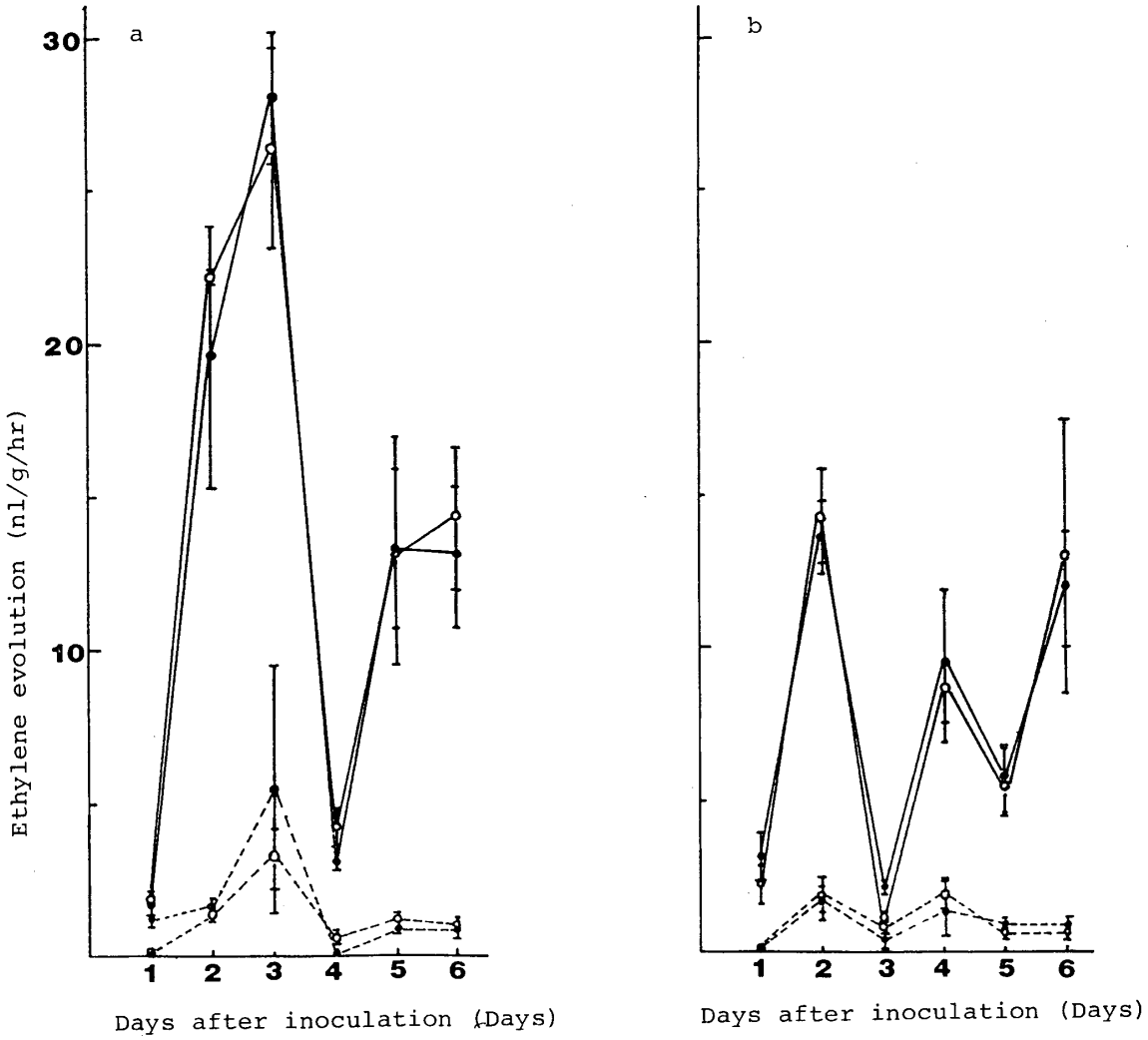

FIG. 2. Ethylene evolution from the inoculated leaf and successive upper leaves (=N+1 and N+2) emerged after inoculation.
●: Inoculation, ○: Control=press-injured
——: Inoculated leaf, - - -: Successive upper leaves emerged after inoculated leaf
a: Compatible combination, Aichi-asahi-F67-54
b: Incompatible combination, Aichi-asahi-1813-2

Ethylene production

There was no difference in the rate of ethylene production in the excised leaf samples and intact leaves (data not shown). Therefore excised leaves were used for measurements.

In the inoculated leaf, the ethylene production on the first day after inoculation was low. On the second day, the ethylene production increased markedly, with the peak occurring on the 3rd day in the compatible combination (Fig. 2a) and on the 2nd day in the incompatible combination (Fig. 2b). Later on, ethyl-

ene production decreased rapidly. No differences in ethylene production between inoculated plants and healthy ones, either in the inoculated leaves or the successive upper leaves. The rate of ethylene production was lower in the upper leaves than in the infected leaf.

Discussion

Kozaka *et al.* concluded that ethylene may be a major factor for stunting of rice plants infected with blast fungus (1). However, they indicated no details of the mechanism of stunting. On the other hand, we have found previously that the first effect of blast infection on leaf growth is an earlier initiation of leaf elongation (8). Therefore we examined whether ethylene was correlated with this effect.

In a compatible combination which resulted in the stunting of rice plant, leaf elongation started on the second day after inoculation, and was much faster than in the press-injured control. However, the ethylene production was similar in infected and control leaves. This accords with i.e., Urushizaki *et al.*, who reported that an inoculation of compatible conidia did not cause the evolution of ethylene when compared to press injury alone (7) and Kozaka *et al.* also reported that the ethylene production was low in inoculated leaves on the second day after inoculation (1). In the incompatible combination which did not result in the stunting of plants, leaf elongation was not accelerated and the ethylene production pattern was similar to that in the compatible combination. Data from Urushizaki *et al.* and Kozaka *et al.* indicate that ethylene production was only slightly higher in infected vs. healthy plants on the second day after inoculation (1, 7).

We think that our ethylene mesurements reflect well the internal ethylene concentrations in the leaf tissues. We therefore conclude that ethylene is not a major factor related to the stunting of rice plants infected with rice blast fungus.

Acknowledgement

The authors are grateful to Dr. H. Yaegashi of National Institute of Agro-Environmental Sciences, Dr. H. Saka of Hokkaido National Agricultural Experiment Station, and Dr. Y. Esashi of College of General Education, Tohoku University for their encouragement of this work.

References

1) Kozaka, T. and Teraoka, T., *Ann. Phytopathol. Soc. Japan* **43**, 549 (1977)
2) Sato, Z. and Kozaka, T., *Ann. Phytopathol. Soc. Japan* **37**, 162 (1971)

3) Sato, Z., *Proc. Assoc. Pl. Prot. Hokuriku* **26**, 83 (1978)
4) Tamari, K. and Kaji, J., *Nippon Nogeikagaku Kaishi* **28**, 254 (1954)
5) Tamari, K. and Kaji, J., *Nippon Nogeikagaku Kaishi* **33**, 178 (1959)
6) Tamari, K. and Kaji, J., *Nippon Nogeikagaku Kaishi* **33**, 181 (1959)
7) Urushizaki, S., Ota, Y., Haruyama, T., Kano, H., Matsumoto, K., Haga, M. and Sekizawa, Y., *Agric. Biol. Chem.* **52**, 3159 (1988)
8) Yoshida, H., Yoshino, R., Hashiba, T. and Ehara, Y., *Tohoku J., Agric. Res.* **43**, 1 (1992)