

Effect of Temperature Treatments on the Penetration and Disease Development in the Leaf Epidermis by the Rice Blast Fungus, *Pyricularia oryzae* Cavara

II. Difference in Percent Penetration, Hyphal Growth and Lesion Formation by Pre- and Postdisposing Temperatures

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氣温變動이 벼 稻熱病菌의 葉身에의 侵入과 發病에 미치는 影響

II. 接種前 및 接種時의 溫度處理에 依한 侵入· 菌糸伸展·病斑形成 差異

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ABSTRACT

Three rice cultivars, Aichi-asahi, Toyotama and Yamabiko, possessing a resistance gene *Pi-a* were evaluated for penetration, hyphal growth in the leaf epidermis and lesion formation using 6 isolates of *Pyricularia oryzae* by treating pre- and postdisposing temperatures of 23/15°C and 29/21°C (day/night) regimes, respectively. Percent penetration of the fungus was higher on the seedlings disposed at 29/21°C regime and more lesions were formed at 7 days after inoculation than at 23/15°C regime. Degree of hyphal growth and number of host cells with hyphal growth were remarkably increased from 72 to 96 hr after inoculation at 29/21°C regime. However, lesion formation on the seedlings disposed at 23/15°C regime was delayed, possibly as a result of the suppressed hyphal growth until 96 hr after inoculation.

Key words: *Pyricularia oryzae*, pre- and postdisposing temperature, penetration.

要 約

稻熱病 抵抗性遺傳子 *Pi-a*를 가지고 있는 Aichi-asahi, Toyotama, Yamabiko를 供試하여 接種前·接種後 23/15°C, 29/21°C에 各各 3日間 處理하였다. 稻熱病菌 6個 菌株을 接種한 後, 稻熱病菌의 侵入·菌糸伸展·病斑數를 調査한 結果, 29/21°C에 處理한 幼菌에서 侵入率이 높고 形成 病斑數도 많았다. 菌糸伸展度와 被侵入細胞數는 29/21°C의 境遇 接種後 72時間에서 96時間 사이에 顯著히 增加하였다. 그러나 23/15°C에 處理한 幼苗에서는 侵入·菌糸伸展이 抑制되어 病斑形成이 遲延되었다.

INTRODUCTION

Delay of symptom development due to the suppressed activities of blast fungus under the low temperature conditions was documented by Kim and Mogi(3) in the previous study. Percent penetration and hyphal growth of *Pyricularia oryzae* were favored at 29/21°C regime after inoculation. Percent penetration in the rice leaf epidermis was found to increase during 28 to 72 hr after inoculation (6, 10), and degree of hyphal growth was increased since 96 hr after inoculation (5, 10). In relation to low temperature and lesion formation, Kimura *et al.*(4) and Ohata *et al.*(7) reported that seedlings treated at about 19°C became very susceptible and produced many susceptible lesions two weeks after the treatment. However, no information is available for the change of percent penetration and hyphal growth using cultivars possessing a resistance gene *Pi-a*. How the fungus behaviors will be changed by low temperature treatment prior to and after inoculation are also not yet known. Based on the above background, this research was initiated to examine changes in penetration and lesion formation in the leaf epidermis of three rice cultivars by *P. oryzae* at different temperature regimes.

MATERIALS AND METHODS

Three cultivars, Aichi-asahi, Toyotama and Yamabiko, possessing a resistance gene *Pi-a* were evaluated throughout the experiment. Tested isolates of *P. oryzae* were Ken54-04, Hoku 1, TH68-126, Ken60-19, Ken53-33 and P-2b. Seedlings were grown in the 25°C greenhouse for 17 days

and then transferred to growth cabinets controlled at 23/15°C (daytime temperature from 9:00 a.m. to 5:00 p.m. and night temperature from 5:00 p.m. to 9:00 a.m.) and 29/21°C regimes for three days prior to inoculation and three days after inoculation. The 20 day-old seedlings were inoculated with conidia suspension (30 conidia per 150X microscopic field) of each isolate and then kept in an inoculation chamber at 25°C for 20 hr. As a control, seedlings were treated in the greenhouse of 33 to 23°C range.

Five new fully expanded leaves of each of the cultivars tested were collected and fixed in FAA No. 2 solution at 72 and 96 hr after inoculation. For microscopic observation and determination of percent penetration and hyphal growth in the leaf epidermis, 300-500 appressoria for each treatment were examined by using Yoshino's method(9). Numbers of lesions produced on seedlings were counted at 7 or 10 days after inoculation. The values presented were averages of ten seedlings.

RESULTS

Lesion formation. Among 6 isolates tested, isolate P-2b produced more lesions, following by isolates TH68-126, Ken54-04, Ken53-33, Ken60-19 and Hoku 1 (Fig. 1). More lesions were produced on Aichi-asahi in most of the isolates and temperature regimes combination. More susceptible lesions were produced in 29/21°C regime or greenhouse condition than 23/15°C regime except for isolate Ken54-04. On the seedlings disposed at 23/15°C regime, newly developing lesions were observed when recounted on 10 days after inoculation,

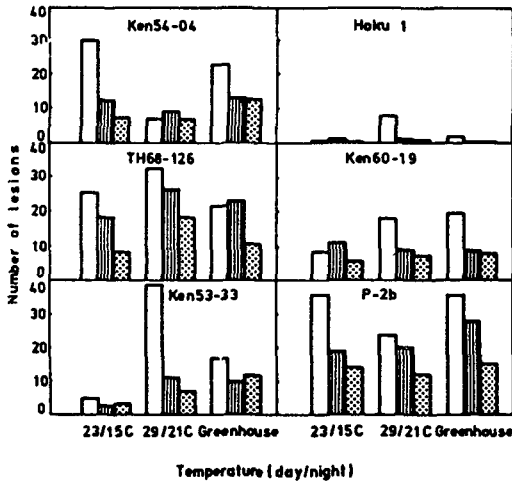


Fig. 1. Number of susceptible lesions produced 7 days after inoculation on three rice cultivars (Aichi-asahi: , Toyotama: , Yamabiko:) with 6 isolates of *Pyricularia oryzae* at different temperature regimes.

indicating delay of lesion formation under low temperature conditions.

Percent penetration. Percent penetration from

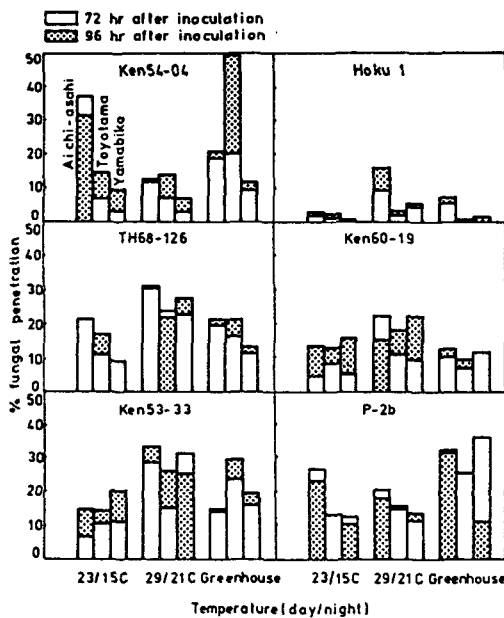


Fig. 2. Percentage of fungal penetration at 72 and 96 hr after inoculation on three rice cultivars with 6 isolates of *Pyricularia oryzae* at different temperature regimes.

72 to 96 hr after inoculation of *P. oryzae* ranged from 0 to 56.9% (Fig. 2). Percent penetration of the fungus greatly varied among isolates, demonstrating less than 10% by isolate Hoku 1 and 10-30% by other isolates. Seedlings disposed at 23/15°C regime generally showed lower percent penetration than at 29/21°C regime or greenhouse condition. In general, percent penetration was higher in Aichi-asahi than in other two cultivars. Increase in percent penetration from 72 to 96 hr after inoculation was observed in most combinations. In particular percent penetration by isolates Ken60-19 and Ken53-33 on the seedlings disposed at 23/15°C regime increased remarkably.

Degree of hyphal growth. Mean values of degree of hyphal growth ranged from 3.7 to 131.0 between 72 to 96 hr after inoculation and the greatest value reached 618 in the cultivar Yamabiko by isolate Ken53-33 at 29/21°C regime. At 23/15°C regime, degree of hyphal growth at 96 hr after inoculation was low and the highest value was only 31.4, but that of at 29/21°C regime or greenhouse condition

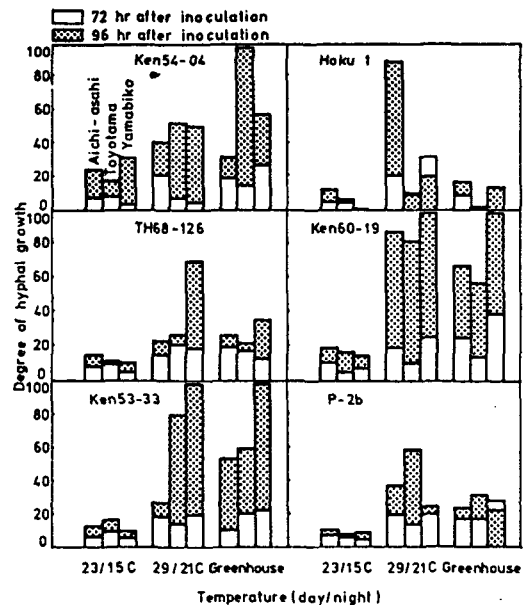


Fig. 3. Degree of hyphal growth in the leaf epidermal cells at 72 and 96 hr after inoculation on three rice cultivars with 6 isolates of *Pyricularia oryzae* at different temperature regimes.

was 131.0 or 111.4, respectively (Fig. 3).

Apparent hyphal growth was observed in the seedlings at 29/21°C regime or greenhouse condition from 72 to 96 hr after inoculation. This tendency was more obvious in all cultivars by isolates Ken60-19 and Ken53-33. The characteristics of rapid hyphal growth at higher temperature conditions made it possible to observe the first symptoms of leaf blast at 29/21°C regime 4 days after inoculation.

Number of host cells with hyphal growth from a single appressorium. While microscopic observation for hyphal growth was done, number of host cells on which hyphal growth was observed from a single appressorium was also examined. The number of cells with hyphal growth was greatly affected by pre- and postdisposing temperature regimes. Mean numbers of cells with hyphal growth ranged from 1.0 to 36.4 during 72 to 96 hr after inoculation. Increase in number of cells with hyphal growth at 23/15°C regime was very limited from 72 to 96 hr after inoculation and the mean number of cells at 96 hr after inoculation was mostly 5-6 cells (Fig. 4). At 29/21°C regime or 33-23°C greenhouse condition, a rapid increase in number of cells with hyphal growth was observed' reaching more or less

10 cells at 72 hr after inoculation. In many cases it was over 30 cells at 96 hr after inoculation.

DISCUSSION

In general, percent penetration and lesion formation by *P. oryzae* was higher in Aichi-asahi than Toyotama or Yamabiko, revealing coincidence of percent penetration and lesion formation with the susceptibility of rice cultivars to blast disease regardless of temperature treatment prior to and after inoculation. However, percent penetration of the fungus was higher in the seedlings disposed at 29/21°C regime or greenhouse condition than those at 23/15°C regime. Number of lesions was also more on the seedlings at higher temperature. Yoshino(11) reported that penetration of *P. oryzae* were favored at around 24°C. The seedlings used in this study were kept at 25°C chamber after inoculation for 40 hr, which might give a condition favorable for penetration of the fungus. Ohata *et al.*(7) demonstrated that rice plants under low air temperature of 19°C for one week were found to be less susceptible than control plants at 26°C until 6 days after treatment, possibly because of decreased content of soluble nitrogen, increased contents of sugar and flavonoids. Thus, his suggestions may well explain our findings that penetration of *P. oryzae* was low on the seedlings disposed at 23/15°C regime for 3 days prior to and after inoculation, respectively.

Although increase in penetration of *P. oryzae* since 72 hr after inoculation was known to be negligible (10, 11), percent penetration increased until 96 or 120 hr after inoculation as shown in our previous study(3). Particularly, the remarkable increase in percent penetration in the three cultivars by isolates Ken60-19 and Ken53-33 at 23/15°C regime may result in a notable increase in lesions from 7 to 10 days after inoculation. Lower increase rate of percent penetration at 23/15°C regime than at 29/21°C regime or greenhouse condition suggested that penetration of *P. oryzae* at the early stage of growth were suppressed by artificial low temperature treatment or under the natural low

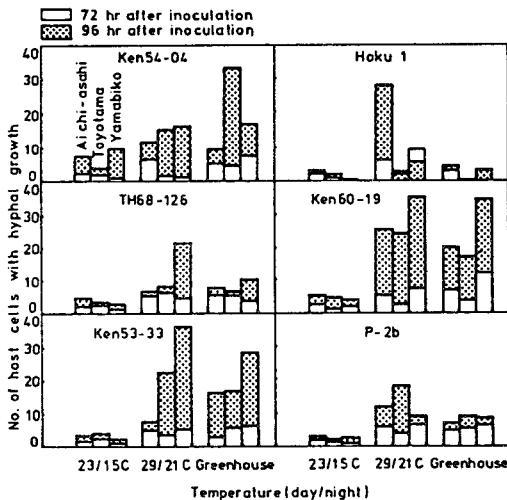


Fig. 4. Number of host cells on which hyphal growth was observed at 72 and 96 hr after inoculation with 6 isolates of *Pyricularia oryzae* at different temperature regimes.

temperature conditions.

Degree of hyphal growth and number of host cells with hyphal growth remarkably increased from 72 to 96 hr after inoculation (Figs. 3 & 4). Previous reports (5, 10) also indicated their increases since 96 hr after inoculation. However, the increase pattern in our study was lower at 23/15°C regime but notable at 29/21°C regime or 33-23°C greenhouse condition, which may be directly related to lesion formation patterns at each temperature regime. Percent penetration was consistent with susceptibility of the tested cultivars to rice blast, but degree of hyphal growth or number of host cells with hyphal growth did not always accord with varietal resistance because of representing better hyphal growth in the resistant cultivars Toyotama and Yamabiko than in the susceptible cultivar Aichi-asahi. Thus, these results suggest that hyphal growth might be affected by temperature and isolate combination rather than varietal resistance.

Blast fungus behaviors during 72 to 96 hr after inoculation were greatly affected by pre- and postdisposing temperature regimes. Strong suppression of hyphal growth was also observed on the seedlings disposed at 23/15°C regime, thus resulting in limited number of host cells with hyphal growth and slow, delayed lesion formation. Kato *et al.* (2) also reported the delay of lesion formation and enlargement of lesion under low temperature conditions. This phenomenon may be explained by prolonged incubation period (1, 8). In our study it was proved mainly due to the suppression of hyphal growth after penetration. Also, the difference in blast fungus behavior during 72 to 96 hr after inoculation under different temperature regimes suggested that lesion formation may be closely related to blast fungus behavior at about 96 hr after inoculation.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. B. K. Hwang, associate professor of Department of Plant Protec-

tion, College of Agriculture, Korea University, for his advice during the preparation of this manuscript.

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