

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

III. Effect of Percent Penetration and Hyphal Growth on Lesion Formation under Different Temperature Regimes

CHANG-KYU KIM¹ AND SHIZUO MOGI²

金章圭・茂木静夫：氣温變動이 벼 稻熱病菌의 葉身에의 侵入과 發病에 미치는 影響 III. 溫度處理에 따른 侵入率과 菌糸伸展度가 病斑形成에 미치는 影響

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ABSTRACT Effect of percent penetration and hyphal growth on lesion formation was evaluated at 23/15C (day/night), 29/21C regimes and greenhouse condition (33-23C) using three rice cultivars, Aichi-asahi, Toyotama and Yamabiko with 6 isolates of *Pyricularia oryzae*. Percent penetration at 72hr and 96hr after inoculation was highly significant with lesion formation at any temperature regime. At the same time, number of appressoria that exceeded hyphal growth value 11 was also highly significant with lesion formation. However, number of appressoria that exceeded hyphal growth value 30 was significant only at 72hr after inoculation at 29/21C regime. In general, statistical fitness of the model was higher at 23/15C regime than at 29/21C regime or a greenhouse condition. These series of phenomenon were more apparent in a susceptible cultivar Aichi-asahi.

INTRODUCTION

The penetration phase of pathogenesis by *Pyricularia oryzae* was one of the deficient areas of knowledge in regard to rice blast disease until Yoshino¹⁰⁾ developed a method for detection of *P. oryzae* in the rice leaf epidermis. Since Yoshino's work, significant achievements have been reported^{1-7, 11-15)}. However, a little information is available for the effect of percent penetration and hyphal growth value on lesion formation especially under different pre- and postdisposing temperature regimes. In our previous studies^{3, 4)}, it was found that percent penetration and hyphal growth were suppressed by low temperature regime of 23/15C and thus it resulted in delayed symptom

development. In the present study, the above phenomenon was statistically analyzed and discussed.

MATERIALS AND METHODS

Seedlings of Aichi-asahi, Toyotama and Yamabiko were grown in the 25C greenhouse for 17 days and then transferred to growth cabinets controlled at 23/15C (daytime temperature from 9 AM to 5 PM/night temperature from 5 PM to 9 AM) and 29/21C regime for three days prior to inoculation and three days after inoculation. The 20-day-old seedlings were inoculated with conidia suspension (30 spores per 150× microscopic field) of six isolates and kept in an inoculation chamber at 25 C for 20hr. As a control, one set of seedlings was treated in the greenhouse of 33-23C.

Five new fully expanded leaves of each cultivar were collected and fixed in FAA No. 2 solution at 72 and 96hr after inoculation. For microscopic observation and determination of

¹ Department of Plant Pathology, Institute of Agricultural Sciences, Suweon, Korea (農業技術研究所病理科, 水原, 大韓民國)

² Kyushu National Agricultural Experiment Station, Chikugo, Fukuoka, Japan (九州農業試驗場病害 第1研究室, 筑後, 福岡, 日本國)

percent penetration in the leaf epidermis, 300~500 appressoria per treatment were examined by Yoshino's method¹⁰⁾. While percent penetration was checked, hyphal growth value per appressorium was checked by Takahashi's method⁹⁾. Number of lesions produced on 10 seedlings were counted at 7 and 10 days after inoculation and the mean value was used. Simple linear regressions were derived from the original data to clarify the relationship between percent penetration/hyphal growth value and lesion formation.

RESULTS

Percent penetration was highly significant with lesion formation in most combination of temperature regimes and sampling time after inoculation (Table 1). But, percent penetration at 96hr after inoculation in the greenhouse was significantly different at 5% level. This was mainly due to the negligible increase of percent penetration between 72hr to 96hr after inoculation except Toyotama by isolate Ken 54-04.

Effect of hyphal growth value on lesion formation is shown in Table 2. Number of appressoria that exceeded hyphal growth value 11 was significantly different at 1% level

with number of lesions at any temperature regimes and sampling time combination. On the contrary, number of appressoria that exceeded hyphal growth value 30 was not significant with number of lesions except 72hr after inoculation at 29/21C regime. It was impossible to calculate 72hr's regression equation both at 7 days and 10 days after inoculation in 23/15C regime due to no appressorium was available that exceeded hyphal growth value 30. Even though there were cases that mean value of hyphal growth exceeded 30 at 96hr after inoculation in the greenhouse or 29/21C regime, number of appressoria that exceeded hyphal growth value 30 was quite limited and thus it resulted in no significant relationship with lesion formation.

As a result, it is clear that percent penetration and number of appressoria that exceeded hyphal growth value 11 from 72hr to 96hr after inoculation directly affected the number of lesions produced 7 or 10 days after inoculation. This tendency was more apparent in a susceptible cultivar Aichi-asahi.

DISCUSSION

Effect of temperature in a disease cycle of *Pyricularia oryzae* has been widely studied by

Table 1. Simple linear regression between percent penetration of *Pyricularia oryzae* and lesion formation under different pre- and postdisposing temperature regimes

| Temperature | Time after inoculation | Coefficient of determination (R^2) | Regression equation |
|---------------------------------|------------------------|--|---------------------|
| 23/15C ^a (7 DAI) | 72hr | .7556** | Y=0.966x+1.65 |
| | 96hr | .5549** | Y=1.009x-2.00 |
| 23/15C ^b (10 DAI) | 72hr | .5663** | Y=0.882x+6.50 |
| | 96hr | .5282** | Y=1.046x+1.46 |
| 29/21C | 72hr | .5703** | Y=0.849x+1. |
| | 96hr | .5194** | Y=0.899x-1.39 |
| Greenhouse (33-23C) | 72hr | .5265** | Y=0.703x+4.02 |
| | 96hr | .2473* | Y=0.370x+8.31 |

^a Correlation derived from lesion number at 7 days after inoculation.

^b Correlation derived from lesion number at 10 days after inoculation.

** : Significant at 1% level.

* : Significant at 5% level.

Table 2. Simple linear regression between hyphal growth value of *Pyricularia oryzae* in the leaf epidermis and lesion formation under different pre- and postdisposing temperature regimes.

| Temperature | Time after inoculation | Coefficient of determination (R^2) | Regression equation |
|---------------------------------|------------------------|--|---------------------|
| HGV over 11 ^a | | | |
| 23/15C ^b (7 DAI) | 72hr | .6971** ^c | Y=3.481x+4.83 |
| | 96hr | .6836** | Y=1.606x-0.32 |
| 23/15C ^c (10 DAI) | 72hr | .8113** | Y=3.989x+7.75 |
| | 96hr | .6033** | Y=1.602x+3.67 |
| 29/21C | 72hr | .3973** | Y=0.664x+8.31 |
| | 96hr | .4767** | Y=1.018x+2.07 |
| Greenhouse (33-23C) | 72hr | .3552** | Y=0.474x+8.31 |
| | 96hr | .5045** | Y=0.753x+4.65 |
| HGV over 30 ^d | | | |
| 23/15C ^b (7 DAI) | 72hr | — ^f | |
| | 96hr | ns | |
| 23/15C ^c (10 DAI) | 72hr | — | |
| | 96hr | ns | |
| 29/21C | 72hr | .3581** | Y=2.133x+8.40 |
| | 96hr | ns | |
| Greenhouse (33-23C) | 72hr | ns | |
| | 96hr | ns | |

^a Hyphal growth value from one appressorium which exceeded 11.

^b Correlation derived from lesion number at 7 days after inoculation.

^c Correlation derived from lesion number at 10 days after inoculation.

^d Hyphal growth value from one appressorium which exceeded 30.

^e **: Significant at 1% level.

ns : Not significant.

^f No appressorium observed which exceeded hyphal growth value 30 at 72hr after inoculation.

many workers and it is known that the range of temperature requirement for each step is quite different. In the previous reports by the authors^{3,13}, it was found that percent penetration and hyphal growth were favored at 29/21C regime and suppressed by 23/15C regime. In the present study, the relationship between percent penetration/hyphal growth value and lesion formation was clarified using rice seedlings predisposed and postdisposed at different temperature regimes. It was revealed that regardless of pre- and postdisposition temperature regimes, percent penetration at 72hr and 96hr after inoculation was highly significant with number of lesions. Yoshino¹⁴ reported that penetration activity of *P. oryzae* was greatly affected by inoculation temperature

and it occurred most rapidly at near 24C, and this was coincided with lesion number. Kim *et al.*²⁾ also found that there was a highly significant relationship between percent penetration and total number of lesions under different predisposing temperature regimes.

Since Sakamoto⁹⁾ had developed a sheath inoculation technique, Takahashi⁹⁾ improved the method and it was applied in the rice leaf epidermis by Yoshino¹¹⁾ and Koga *et al.*⁷⁾. However, very few information is available concerning the relationship between hyphal growth value and number of lesions produced on the seedlings under pre- and postdisposing temperature regimes. In the present study, number of appressoria that exceeded hyphal growth value 11 and 30 was respectively eva-

luated its effect on the number of lesions. As a result, number of lesions was more affected by the number of appressoria that exceeded hyphal growth value 11 than that of 30. This fact indicates that a leaf blast lesion can be produced when hyphal growth value from a single appressorium is over 11. Yoshino¹³⁾ documented that number of lesions was more affected by the number of microscopic view field observed with the presence of appressoria than percent penetration. Considering that those penetrated appressoria could not all succeed in lesion formation, number of appressoria showing a certain value of hyphal growth might be more closely related with lesion formation.

In most variety by isolate combinations, more susceptible lesions were produced in 29/21C regime or greenhouse condition than 23/15C regime. On the seedlings disposed at 23/15C regime, newly developing lesions were observed at 10 days after inoculation and the number approached to that of 29/21C or greenhouse condition of 7 days after inoculation. It strongly suggests that lesion formation is delayed under low temperature conditions which is mainly due to the suppression of hyphal growth. In terms of statistical fitness, hyphal growth value 11 at 23/15C revealed higher goodness of fit of the model than at 29/21C regime or in the greenhouse.

摘 要

稻熱病病斑形成에 미치는 侵入率과 菌糸伸展度の 效果를 알기 爲하여 23/15C(晝/夜), 29/21C 및 溫室條件(33~23C)에서 處理한 Aichi-asahi, Toyotama, Yamabiko에 6개의 稻熱病菌菌株을 接種한 結果, 接種後 72, 96時間째의 侵入率은 어느 溫度條件에서도 病斑形成과 高度의 有意성이 있었다. 또한 菌糸伸展度 11以上을 나타내는 附着器數도 病斑數와 高度의 有意성이 認定되었다. 그러나 菌糸伸展度 30以上을 나타

내는 附着器數는 29/21C條件의 接種後 72時間째에만 有意성이 認定되었다. 이들 相關關係의 統計的인 中度는 29/21C나 溫室보다는 23/15C에서 더 높았으며 이런 現象은 罹病性品種인 Aichi-asahi에서 더욱 뚜렷하였다.

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