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Sectors from *Pyricularia grisea* Isolates on Edifenphos and Iprobenfos-Amended Media

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Sectors of rice blast fungus, *Pyricularia grisea*, frequently appeared on potato dextrose agar amended with edifenphos and iprobenfos. Thus, we assessed the sector-forming frequency of isolates of *P. grisea* and compared the fungicide sensitivity between wild types and sectors against the fungicides. The 905 isolates of the fungus were obtained from rice-growing locations in Korea from 1997-1998. When the isolates were grown on potato dextrose agar amended with minimal inhibitory concentrations of edifenphos (20 μg a.i./ml) and iprobenfos (55 μg a.i./ml), they produced sectors that overcame the effect of the fungicides. Among the 905 isolates tested, 9.0% produced sectors against edifenphos and 5.6%, against iprobenfos. Different sector-forming frequencies were also observed among the 11 locations of Korea. Sectors obtained from the fungicide-amended media generally grew more than their counterpart wild types grown on the media with either edifenphos or iprobenfos, regardless of their origins. In this study, greater relative growth of sectors over wild types of tested isolates can support the resistant characteristic of the fungus to survive against the fungicides. Therefore, the results indicate that the sectoring in rice blast fungus, *P. grisea*, may play a role in the occurrence of fungicide resistance.

Keywords : edifenphos, fungicide resistance, iprobenfos, *Magnaporthe grisea, Pyricularia grisea*, rice blast, sector

Pyricularia grisea (Cooke) Sacc. [anamorph of *Magnaporthe grisea* (T.T. Hebert) Yaegashi & Udagawa], the causal agent of rice (*Oryzae sativa* L.) blast, is the most serious disease in rice. Control of the rice blast has been dependent widely on the application of fungicides with different modes of action, such as tricyclazole, probenazole, edifenphos, and iprobenfos. The fungicides, edifenphos and iprobenfos, lipid biosynthesis inhibitors, have been used widely and effectively to control the disease in Korea since 1969. However, these have been considered to be a possible occurrence of resistant isolates against edifenphos and iprobenfos. These fungicides in particular have the potential to produce resistant isolates because resistant isolates are easily obtained from media containing the fungicides (Uesugi et al., 1978).

Since, we have frequently observed spontaneous sectors in the *P. grisea* isolates in media amended with the fungicides, these sectors may be one of the factors that contribute to the occurrence of fungicide resistance. Sectors frequently seem to differ from their wild origins. For example, they grow faster on cultures amended with fungicides and have somewhat different morphology. Therefore, in this study, we assessed sector-forming frequency of *P. grisea* isolates obtained from 11 rice-growing locations in 1997 and 1998, Korea; and compared their fungicide sensitivity between wild types and sectors against edifenphos and iprobenfos.

P. grisea isolates (Kim, 2000) were obtained from necks and leaves of blasted rice plants from 11 different locations (Table 1) in Korea in 1997 and 1998. All isolates were stored at room temperature by using a long-term storage method described by Latterell and Rossi (1986). Edifenphos (*O*-ethyl *S, S*-diphenyl phosphorodithioate, Hinosan®) and iprobenfos (*S*-benzyl *O, O*-di-isopropyl phosphorothioate, IBP® or Kitazin P®) were used in this experiment. A technical product of edifenphos (85% a.i.) provided by Dongbu Hannong Chemicals Co., Ltd., Seoul, Korea and commercial formulation of iprobenfos (48% a.i.) produced by Yung-Il Chemicals Co., Ltd., Seoul, Korea were used. Stock solutions of the fungicides were made by dissolving them in the mixtures of acetone:water (80:20, v/v). The final concentration of acetone in the plates was not over 1% of medium volume. Stock solutions were stored at 4°C in the dark and used within a week to preserve fungicide activity.

P. grisea isolates from the long-term storage cultures were initially grown on potato dextrose agar (PDA) at 28°C for 7 days. The actively growing mycelial plugs (5 mm in diameter) from the cultures were transferred into PDA amended with edifenphos (20 μg a.i./ml) and iprobenfos (55 μg a.i./ml) to generate sectors of the fungus. These

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Table 1. Number and percentage of sector[a]-forming isolates of *Pyricularia grisea* collected from 11 rice-growing locations of Korea in 1997 and 1998

Location	Number of Isolates observed	Number (%) of sector-forming isolates	
		Edifenphos	Iprobenfos
Chonju	80	14 (17.5)[a]	7 (8.8)
Chunchon	67	7 (10.4)	1 (1.5)
Donghae	103	9 (8.7)	3 (2.9)
Gwanju	73	9 (12.3)	11 (15.1)
Haman	85	9 (10.6)	5 (5.9)
Ichon	104	10 (9.6)	5 (4.8)
Kangnung	80	2 (2.5)	2 (2.5)
Kimhae	73	5 (6.8)	2 (2.7)
Kwangju	82	9 (11.0)	5 (6.1)
Taejon	85	5 (6.7)	4 (5.3)
Yangyang	83	2 (2.4)	6 (7.2)
Total	905	81 (9.0)	51 (5.6)

[a]Sectors were observed on potato dextrose agar amended with edifenphos and iprobenfos at minimal inhibitory concentrations of 20 μg a.i./ml and 55 μg a.i./ml, respectively.

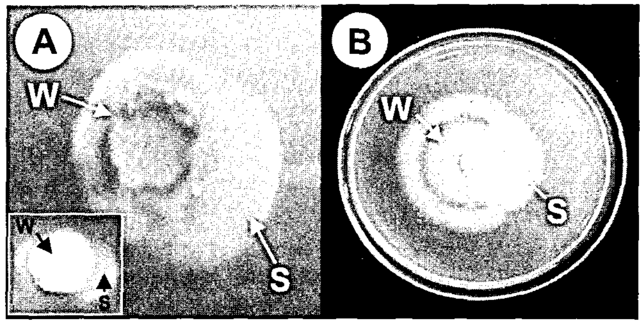

Fig. 1. Appearance of sectors (S) in the colonies (W) of *Pyricularia grisea* on potato dextrose agar amended with (**A**) iprobenfos and (**B**) edifenphos at minimal inhibitory concentrations of 55 μg a.i./ml and 20 μg a.i./ml, respectively. Small box of the Fig. 1A shows the initial appearance of morphologically distinct sectors of a colony of *P. grisea* on the iprobenfos-amended media.

fungicide concentrations were determined from our previous research (Kim and Kim, 1999) as the minimal inhibitory concentrations (MICs) of the fungicides. For each isolate, four mycelial plugs were placed on a PDA plate containing each fungicide. The plates for production of spontaneous sectors were examined for 7 days.

Mycelial growth of wild types and sectors of *P. grisea* on PDA amended with MICs of edifenphos and iprobenfos, respectively, were compared. Nine isolates (Fig. 2) producing sectors to the fungicide each were randomly selected and examined in their mycelial growth on the fungicide-amended PDA as described above. PDA amended with acetone served as controls. Mycelial diameters were measured 7 days after inoculation at 28°C in the dark. A relative mycelial growth was calculated as a diameter on a medium amended with a fungicide divided by a diameter

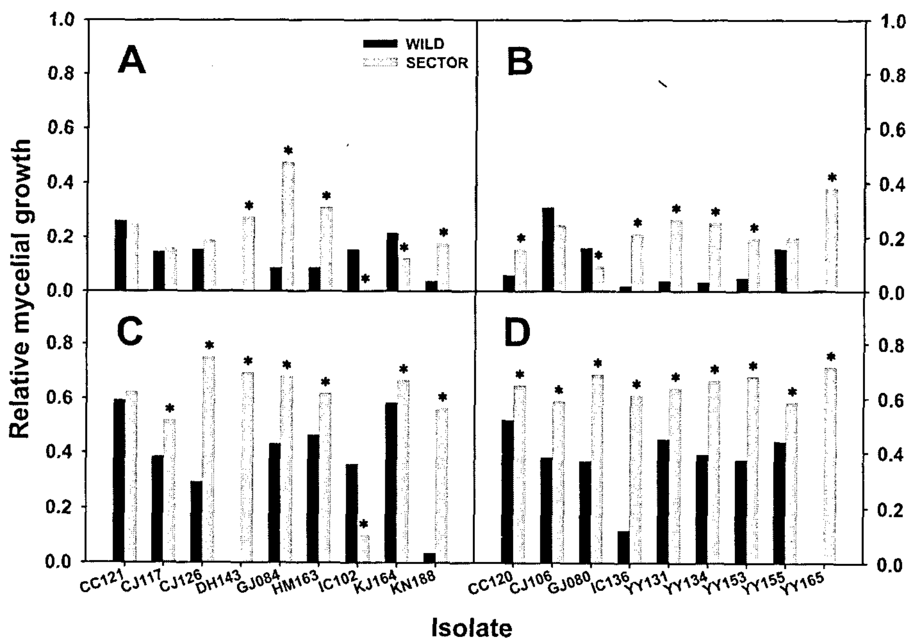

Fig. 2. Comparisons of relative mycelial growth between wild types (black bars) and sectors (grey bars) of nine isolates of *Pyricularia grisea* against (A, B) edifenphos and (C, D) iprobenfos, respectively. The sectors of isolates of *P. grisea* were originally obtained from media amended with (A, C) edifenphos and (B, D) iprobenfos, respectivley. This experiment was conducted twice with three replications each and statistical analysis was conducted with the combined data. An asterisk indicates significant difference between the wild type and the sector of each isolate at $P = 0.05$.

on control medium amended with acetone. This experiment was conducted twice with three replications each. Statistical analysis of data was conducted by using the Statistical Analysis System (SAS institute, 1998). Data for relative mycelial diameter were transformed to arcsine before analysis. Analysis of variance was determined by using the general linear model procedure and means were separated with the least significance difference at $P = 0.05$.

The isolates of *P. grisea* from 11 rice-growing locations of Korea produced sectors on PDA amended with MICs of edifenphos and iprobenfos (Table 1 and Fig. 1). Among 905 isolates tested, 9.0% produced sectors against edifenphos and 5.6% against iprobenfos. Different sector-forming frequencies were also observed among the 11 locations of Korea. Isolates from Chonju, Gawngju, and Kwangju showed highest sector-forming frequencies; while those from Kangnung, Yangyang, and Chunchon showed the lowest against either edifenphos or iprobenfos (Table 1). The frequency of sector occurrence in each location may be related with the amounts of fungicides being used, since Chonju and Kwangju are major rice-growing areas in Korea.

When sectors, obtained from media amended with edifenphos or iprobenfos, were compared with their wild types of the isolates, the sectors generally grew more than their wild types on media with either edifenphos or iprobenfos, regardless of their origins (Fig. 2).

As observed in this study, fungal isolate-producing sectors that were reported in some genus on different media contained different chemicals or high temperatures (Correll and Leslie, 1987; Vannacci and Cristani, 1998). These sectors exhibited different strengths in overcoming toxicity of chemicals, abnormal temperatures or utilizing the chemical compounds on media when compared with their wild parents. This sectoring could be the result of anastomosis or genetic change that possibly could be significantly influenced by fungicide treatment. Thus, sectoring may be a natural means of survival and adaptation under fungicide application (Xia et al., 2000). This study shows that with greater relative growth, compared with wild types, tested isolates can support the resistant characteristic of the fungus to survive against the fungicide stress, resulting from spontaneous mutations. The results, therefore, indicate that the sectoring in rice blast fungus *P. grisea* may play a role in the occurrence of fungicide resistance.

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