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Short communication

# Variation in sensitivity of *Magnaporthe oryzae* isolates from Korea to edifenphos and iprobenfos

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ABSTRACT

In this study, the sensitivity of *Magnaporthe oryzae* isolates from different geographic areas in Korea to two phosphorothiolate (PTL) fungicides, edifenphos and iprobenfos, was determined. A total of 1080 *M. oryzae* isolates were collected from rice-cultivating fields in 11 locations throughout Korea in 1997 and 1998. The minimum inhibitory concentrations (MICs) of edifenphos (20 μg a.i. $ml^{-1}$) and iprobenfos (55 μg a.i. $ml^{-1}$) against seven representative sensitive isolates of *M. oryzae* were determined from the dose-response curves, and the MICs were then used as single discriminatory concentrations for the detection of fungicide resistance. Of the 1080 tested isolates, the isolates less sensitive to edifenphos and iprobenfos were 57 and 84%, respectively, and 53% of the isolates were less sensitive to both fungicides. Approximately 11% of all tested isolates showed no growth at the MICs of the tested fungicides. Isolates with a relative mycelial growth of 0.05 to edifenphos occurred at the greatest frequency (33%); isolates with a relative growth of 0.3 to iprobenfos occurred at the greatest frequency (23%). The frequency distribution of isolates sensitive to either edifenphos or iprobenfos varied with geographic location. The two-sample median test yielded high medians in the Kwangju and Chonju isolates for edifenphos, and in the Kwangju, Donghae, and Yangyang isolates for iprobenfos, but low medians in the Kangnung isolates for edifenphos and in the Kangnung, Gwangju, and Chunchon isolates for iprobenfos, respectively, as compared to the isolates from the other locations. Analysis of Spearman's rank correlation test showed a significantly positive relationship ($r = 0.490$, $P < 0.001$) between edifenphos and iprobenfos sensitivities. Therefore, these results indicate that differences in sensitivity to the PTL-fungicides, edifenphos and iprobenfos, exist among *M. oryzae* populations collected from different geographic areas in Korea, and isolates less sensitive to these fungicides exhibit cross-resistance with each other.



## 1. Introduction

Recent increase in rice (*Oryza sativa* L.) yield in the major rice-producing Asian countries has been mainly attributed to the effective control of rice blast caused by *Magnaporthe oryzae* B. Couch [previously known as *Magnaporthe grisea* (Hebert) (Barr)] (Couch and Kohn, 2002) (teleomorph of *Pyricularia oryzae* Cavara), one of the most destructive rice diseases (Savary et al., 2000). In general, measures for the control of rice blast disease include planting of resistant cultivars, application of fungicides, and manipulation of planting time, fertilizers, and irrigations. Despite availability of diverse methods to control rice blast, it has been most effectively managed via the application of synthetic fungicides, including tricyclazole, probenazole, edifenphos, and iprobenfos. These fungicides have been widely used for controlling rice blast in Korea.

Although fungicides are very effective in controlling plant diseases, there have been a number of studies reporting the resistance of plant pathogens such as the grey mold pathogen, *Botrytis cinerea* to benzimidazole and the dicarboximide fungicides (Lennox and Spotts, 2003), as well as the resistance of the apple scab pathogen, *Venturia inaequalis* and brown rot pathogen, *Monilinia fructicola* to sterol demethylation inhibitors and anilinopyrimidine fungicides (Köller and Wilcox, 2001; Köller et al., 2005; Holb and Schnabel, 2007). The sterol demethylation inhibitors have been suggested to interfere with the fungicidal effects of phosphorothiolates (PTLs) including edifenphos and iprobenfos (Uesugi, 2001). However, the fungicides edifenphos and iprobenfos, which exert their fungitoxic activity via the inhibition of choline biosynthesis, provided the potential possibility of resistance emergence, as observed in *Bipolaris oryzae* against edifenphos (Kodama et al., 1979, 1980; Annamalai and Lalithakumari, 1992; Uesugi, 2001).

Resistance to the PTL-fungicides, edifenphos and iprobenfos, in rice blast fungal populations has proven to be an important

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problem as well as an interesting physiological phenomenon. Fungal isolates resistant to the fungicides could be selected *in vitro* from a large number of conidia on agar media without the need for any mutagenic treatment (Uesugi, 1978). The PTL resistance of field isolates has been attributed to reduced activation of the P–S bond cleavage of the phosphorothiolates and increased activation of S–C bond cleavage (Uesugi, 2001). An analogy of similar antifungal activities of edifenphos and iprobenfos revealed the existence of cross-resistance to both fungicides (Uesugi and Tomizawa, 1971; Tomizawa and Uesugi, 1972). In addition, cross-resistance between edifenphos or iprobenfos and isoprothiolane (Katagiri and Uesugi, 1977) and negative cross-resistance between edifenphos or iprobenfos and phosphoramiate compounds (Uesugi et al., 1974) have been reported, as has negative cross-resistance to the sterol biosynthesis inhibitors, prochloraz and fenpropimorph (Leroux et al., 2000). These reports revealed a potential relationship in sensitivity between edifenphos or iprobenfos and other agro-fungicides. The principal objectives of this study were to assess differences in sensitivity to the PTL-fungicides, edifenphos and iprobenfos, of the *M. oryzae* isolates from different geographical regions in Korea, and to determine whether cross-resistance between edifenphos and iprobenfos occurs in these field isolates.

## 2. Materials and methods

### 2.1. Isolation and maintenance of the fungus

A total of 1080 isolates of *M. oryzae* were collected from the necks and leaves of blasted rice plants grown in the fields at 11 locations in Korea, in 1997 and 1998 (Fig. 1 and Table 1). To isolate the fungus from the neck or leaf lesions, small fragments (5 mm long) of the lesions were surface-sterilized for 1 min with 1% NaOCl, rinsed three times with sterile water, and then dried on sterile filter paper. These sterilized fragments were then placed on water agar containing 50 μg ml$^{-1}$ streptomycin sulphate to suppress bacterial growth. After 24–36 h of incubation at 28 °C in darkness, the conidia of *M. oryzae* generated on the fragments were transferred to potato dextrose agar (PDA, Difco, Detroit, USA), and hyphal tip cultures were then made for the mycelial growth assays. However, monoconidial isolation using selected isolates to determine the minimum inhibitory concentration (MIC) was conducted under a light microscope.

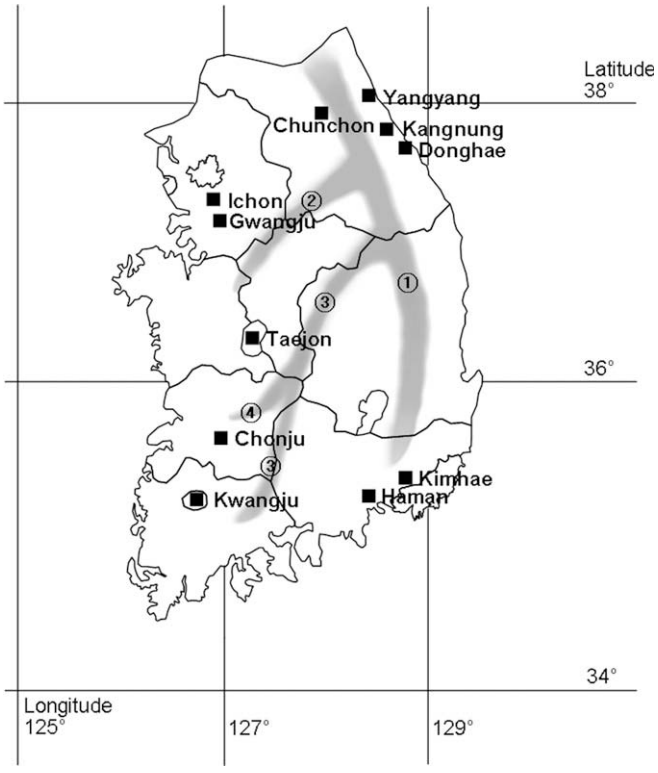


**Fig. 1.** Sampling locations for collecting *Magnaporthe oryzae* isolates from necks or leaves of blasted rice plants in Korea, in 1997 and 1998. ①: Taebaek Mountains, ②: Charyung Mountains, ③: Sobaek Mountains, ④: Noryung Mountains.

**Table 1**
Sampling locations, sources and numbers of *Magnaporthe oryzae* isolates collected from rice plants grown in the fields of 11 locations in Korea, 1997 and 1998

| Location | Plant part | Year collected | No. isolates |
|---|---|---|---|
| Chonju | Neck | 1997, 1998 | 61 (29)[a] |
| Chunchon | Neck | 1997, 1998 | 69 (39) |
| Donghae | Neck | 1998 | 96 |
| Gwangju | Neck | 1997, 1998 | 71 (19) |
| Haman | Neck | 1997, 1998 | 36 (65) |
| Ichon | Neck | 1997, 1998 | 75 (54) |
| Kangnung | Neck | 1997, 1998 | 11 (87) |
| Kimhae | Neck | 1998 | 90 |
| Kwangju | Neck/leaf | 1998 | 92 |
| Taejon | Neck | 1997, 1998 | 83 (23) |
| Yangyang | Neck | 1998 | 80 |
| Combined | | | 1080 |

[a] Numbers in parentheses indicate numbers of *M. oryzae* isolates collected in 1998.

To maintain the cultures used in this study, mycelial agar plugs of *M. oryzae* were stored at 4 °C in vials containing sterile distilled water (Lee et al., 1994). A modified long-term storage of the collected isolates was also conducted as described by Latterell and Rossi (1986). Rice nodes (1 cm long) collected at harvest were cut and autoclaved twice at 121 °C for 1 h, over a period of 2 d. The sterilized rice nodes were then positioned on the margins of the mycelia of *M. oryzae* cultured on PDA for 5 d. These were incubated until the mycelia covered the nodes, and were then air-dried completely at 28 °C. The dried nodes were stored at room temperature in sterilized microtubes.

### 2.2. Fungicides

Edifenphos (*O*-ethyl *S*,*S*-diphenyl phosphorodithioate) and iprobenfos (*S*-benzyl *O*,*O*-di-isopropyl phosphorothioate) were utilized in this study. A technically produced edifenphos (85% a.i.) and a commercially formulated iprobenfos (48% a.i., EC) were provided by Dongbu Hannong Chemicals Co., Seoul, Korea and Yung-Il Chemicals Co., Seoul, Korea, respectively.

### 2.3. Mycelial growth assay for fungicide resistance

Stock solutions of the fungicides were prepared by dissolving the technical-grade edifenphos (85% a.i.) and the commercial iprobenfos (48% a.i.) in an acetone:water mixture (80:20, v/v). The final acetone concentration in all media including the fungicide-unamended controls was adjusted to less than 1% of total medium volume. The solutions were stored at 4 °C in darkness and used within a week to ensure proper fungicide activity.

To determine the fungicide baseline sensitivity, seven isolates of *M. oryzae* sensitive to edifenphos (isolates CC015, CJ056, DH147, GJ004, KH109, KJ152, and TJ040) and iprobenfos (isolates CC079, CJ056, GJ004, KH156, KJ152, KN024, and TJ029) were selected, respectively, from seven locations divided by mountain ranges in