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Research Note

Decreased Symptoms of Rice Blast Disease on Leaves of *bar*-Expressing Transgenic Rice Plants following Treatment with Bialaphos

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Transgenic rice plants harboring the bialaphos-resistant (*bar*) gene expressed from the maize ubiquitin promoter were inoculated with mycelia of the rice blast disease pathogen *Magnaporthe grisea*. Lesions caused by this fungal pathogen were decreased on the leaves of the transgenic rice plants following treatment with bialaphos. Pretreatment of plants with bialaphos 1 day before the inoculation also suppressed disease symptoms to 20% of that in the control. Therefore, it may therefore be possible to control rice blast pathogen by the use of *bar*-transgenic rice plants along with bialaphos treatment.

Transgenic rice plants (*Oryza sativa* L. *japonica* cv. Yamahoshi) containing a maize ubiquitin promoter fused to the bialaphos-resistant (*bar*) gene (Thompson et al. 1987) have been established (Toki et al. 1992; Uchimiya et al. 1993). Such *bar*-transgenic plants have a high level of tolerance to bialaphos and phosphinothricin (De Block et al. 1987; Toki et al. 1992), non-selective herbicides that control grasses and broadleaf weeds (Gallina and Stephenson 1992). Bialaphos also has antibiotic activity against the rice fungal pathogens *Rhizoctonia solani* Kühn (De Datta 1981) and *Magnaporthe grisea* (Hebert) Barr (Kondo et al. 1973), which cause sheath blight and blast disease, respectively. Uchimiya et al. (1993) showed that treatment of *bar*-transgenic rice plants with bialaphos prevented infection by *R. solani*, the sheath blight pathogen. Thus, we were prompted to determine if bialaphos-treated transgenic plants would be tolerant to rice blast disease, one of the most severe fungal diseases of rice.

Both appresorium formation by the fungus on cellophane (Araki and Miyagi 1977) and mycelium growth on agar medium (Uesugi 1981) were completely inhibited by 50 μg of bialaphos per ml, while spore germination (Araki and Miyagi 1977) was not inhibited (Fig. 1). Transgenic rice plants containing the chimeric *bar* gene that were sprayed with more than 100 μg of the herbicide per ml survived, as reported previously (Toki et al. 1992). Seedlings from *bar*-transgenic homozygous plants were grown in an incubation chamber for 2 weeks at 28°C. *M. grisea* (KEN60-19, race 037) was cultured on an oatmeal agar medium at 28°C for 2 to 3 weeks. Spores were harvested from the aerial mycelia and incubated at 28°C for 1 week. Spore formation was then induced by UV irradiation. Rice plants at the two-leaf stage were inoculated by spraying with a spore suspension (10^6 spore/ml) containing 0.01% Tween 20. The plants were placed under saturated humidity at 28°C, kept in the dark for the first day, and then exposed to a 12-h light/12-h dark cycle. One day before, 1 h be-

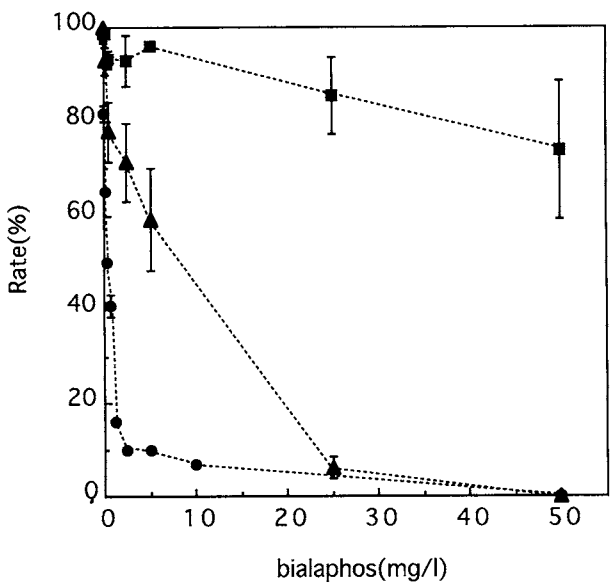

Fig. 1. Effects of bialaphos on *Magnaporthe grisea* spore development. Fungal spore suspensions (10^4 spores/ml) containing different concentrations (0 to 50 μg/ml) of bialaphos were dropped and incubated on cellophane in a petri dish at 28°C for 2 days; spore germination and appressoria formation were observed with a microscope (Araki and Miyagi 1977). (■); Spore germination (%) = [1 – (germinated spores / observed spores)] × 100. (▲); Appressoria formation (%) = [1 – (appresoria / germinated spores)] × 100. Fungus block, punched with corkborer, was placed on agar medium and inhibitive rate assessed from mycelium growth measured in terms of radius (Uesugi 1981). (●); Hypha growth rate (%) = (test hypha radius / control hypha radius) × 100. Results are average of three replicates. Error bars = standard errors of the mean.

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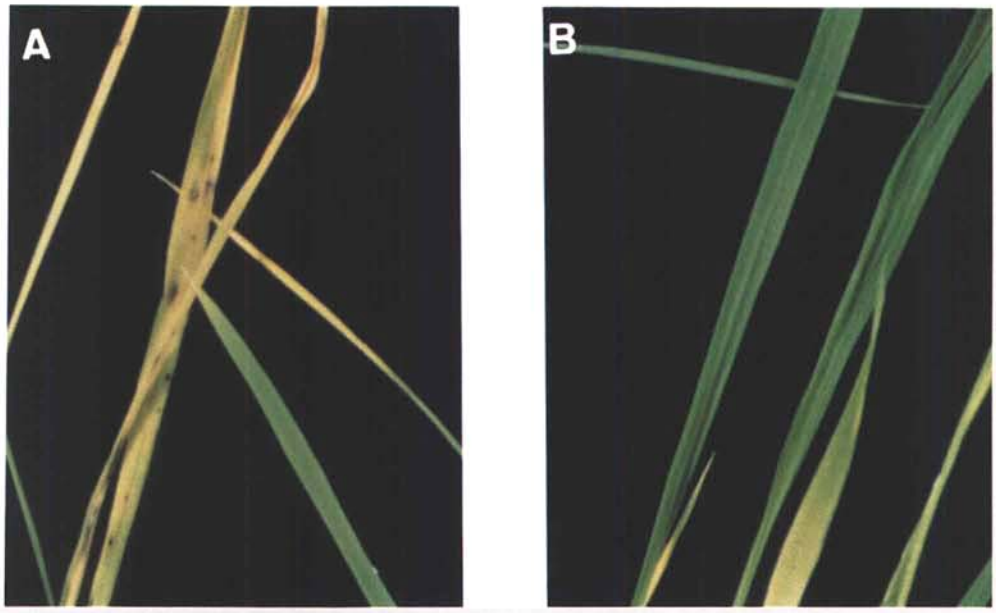

Fig. 2. Rice blast disease symptoms on leaves of *bar*-transgenic rice plants treated with and without bialaphos. Infected area and lesions were observed at 7 days after inoculation. **A,** Inoculated transgenic plants not treated with bialaphos. **B,** Transgenic plants treated with 100 µg of bialaphos per ml, 1 day before inoculation.

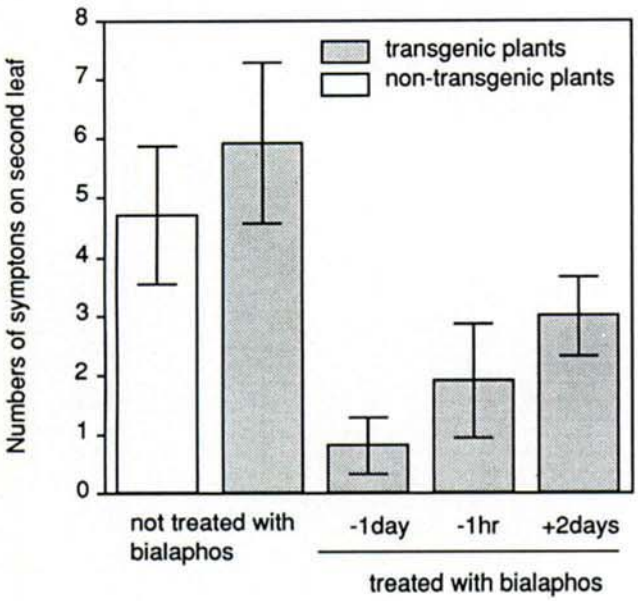

Fig. 3. Number of lesions caused by blast fungus on bialaphos-treated *bar*-transgenic or nontransgenic rice plants. Plants were inoculated by blast pathogen; in the meantime, 100 µg of aqueous bialaphos solution per ml was sprayed 1 day before, 1 h before, or 2 days after inoculation. Plants were placed under saturated humidity at 28°C, kept in the dark for the first day, and then exposed to a 12-h light/12-h dark cycle. Number of lesions on second leaves of rice plants counted 7 days after inoculation. Bars = standard errors of the mean.

fore, or 2 days after inoculation, the plants were sprayed with an aqueous solution containing 100 µg of bialaphos per ml. Number of lesions on the second leaves of the rice plants was counted 7 days after inoculation. The *bar*-transgenic plants that had been treated with the herbicide had fewer lesions (Fig. 2B) than untreated transgenic plants (Fig. 2A) and non-transgenic plants were killed by the herbicide (not shown).

Treatment with bialaphos 1 day before the fungus inoculation reduced the number of lesions to 20% of that in the control (Fig. 3). Even 2 days after inoculation, herbicide treatment was effective in suppressing the number of disease lesions to about 50% of that in the control. Recently, Ahmad et al. (1995) reported that bialaphos has toxic effects on soil microorganisms (many species of *Bacillus*, *Pseudomonas,* and *Tricoderma*) and on phytopathogenic fungi such as *R. solani* and *Sclerotinia sclerotiorum*. Therefore, by carefully selecting the bialaphos concentration and the time of application, it may be possible to control infections by the rice blast pathogen, weed infestation, and soil microorganisms simultaneously in fields of *bar*-transgenic rice cultivars. The same strategy may also be useful for other agronomic plants.

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