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Efficacy of plant extracts against the rice blast fungus *Magnaporthe oryzae*

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Abstract Rice blast disease caused by *Magnaporthe oryzae* is the most destructive fungal disease affecting rice production. Overcoming fungicide-resistance to *M. oryzae* is critical for chemical control. Accordingly, in this study, the fungicidal activity of extracts of *Houttuynia cordata* and *Artemisia indica* against rice blast caused by *M. oryzae* was evaluated for new fungicide development. When spores of *M. oryzae* were suspended in an extract of *H. cordata* or *A. indica* and transferred onto glass slides, spore germination and appressorium formation of *M. oryzae* were significantly inhibited. Furthermore, blast lesion formation by *M. oryzae* was significantly inhibited in the presence of both extracts, compared to distilled water. These results suggest that antifungal substance (s) effective against rice blast fungus *M. oryzae* are contained in extracts of *H. cordata* and *A. indica*. Thus, extracts of *H. cordata* and *A. indica* may be candidate control agents for plant protection against diseases such as rice blast disease.

Keywords : Antifungal activity, Plant extract, *Houttuynia cordata*, *Artemisia indica*, Rice blast.

Introduction

It is well known that rice blast disease caused by *Magnaporthe oryzae* (Hebert) Barr is one of the most destructive diseases of agricultural crops. Although resistant cultivars and chemical fungicides play important roles in the control of *M. oryzae,* the durability of genetic resistance in improved rice cultivars is often short lived in the field because of the pathogen's ability to rapidly evolve and overcome resistance (Ahn 1994). The key to the chemical control of plant diseases is to overcome fungicide-resistant strains of the pathogen (So et al., 2002; Yamaguchi et al., 2002). Therefore, a search for antifungal compounds is required to develop new fungicides. Antifungal compounds of microbial and plant origin play an important role in the biological and chemical control of plant diseases (Fravel 1998; Shimizu et al., 2000; Uddin and Viji 2002; Chaijuckam and Davis 2010).

Recently, control of plant disease by natural products has been achieved. Many plant diseases caused by fungi have been inhibited by plant extracts (Onyeke and Ugwuoke 2011, Bowers and Locke 2000, Wang et al., 2004). Furthermore, the mycelial growth, conidial germination, and appressorium formation of rice blast were significantly inhibited and leaf blast was reduced by rue (*Ruta graveolens*) extract (Reis et al., 2015). Previously, it has been reported that compounds from *Houttuynia cordata* and *Artemisia indica* have important properties, including antimutagenic, anticancer, antiviral, antibacterial, antimicrobial, cytotoxic, and antioxidant activities (Kumar et al., 2014; Rashid et al., 2013). However, to the best of our knowledge, there have been no reports regarding the use of *H. cordata* and *A. indica* extracts to control plant pathogens. The objective of this study was to investigate the antifungal activity of extracts from *H. cordata* and *A. indica* against rice blast fungus, *M. oryzae*.

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Materials and Methods

Microorganism and Plant extracts

The leaves of *H. cordata* and *A. indica* were collected in Shimane University. The leaves (10g) were cut into 1-cm segments and placed into flask to which 200mL distilled water was added, followed by treatment at 100℃ for 20min. After cooling, these extracts were filtered through filter paper (Toyo Roshi Kaisha, Ltd, Japan) (1-fold extract), and a rotary evaporator was used to concentrate the extract. The volume of aqueous solution was adjusted according to the initial leaf weight. For a 40-fold concentrated extract, the aqueous volume of samples extracted from 10g fresh weight of leaves was adjusted to 5mL. Original (1-fold extract) and 40-fold concentrated extracts were used to assess spore germination and lesion formation.

Magnaporthe oryzae (strain Naga 69-150, race 007) was grown on rice bran agar at 26℃ for 14 days, washed with running distilled water to remove aerial hyphae, and maintained at 26℃ under near-ultraviolet radiation (FL20s BL-B; Panasonic, Osaka, Japan) for 2 days to induce sporulation.

Rice plants (*Oryza sativa* 'Koshihikari') were prepared as described previously (Ohba 2002; Ashizawa and Zenbayashi 2005).

Influence of the plant extract on the spore germination of *M. oryzae*

M. oryzae spores (1×10^5 spores/mL) suspended in 1-fold and 40-fold concentrated extract of *H. cordata* and *A. indica* or distilled water were dropped onto glass slides and maintained in a moist chamber at 26℃. After 24h incubation, the percentage of spore germination and appressorium formation was determined by light microscopy.

Plant extract treatment in rice leaves

The spores of *M. oryzae* and original extracts of *H. cordata* and *A. indica* were prepared as described above. To investigate the inhibitory effects on lesion formation, rice at the 3-leaf stage was inoculated with *M. oryzae* spores (1×10^5 spores/mL) in the presence of extracts of *H. cordata* or *A. indica*. Distilled water was used as a control. The inoculated rice plants were incubated in a moist chamber at 26℃. The disease incidence and disease severity were investigated 5 days after inoculation. Disease incidence = (the number of diseased rice plants/the number of investigated rice plants) $\times100$. Disease severity = $\{(4A+3B+2C+D)/(4\times15)\}\times100$, A: second leaf was blighted (lesion area percentage 50%) B: appreciable number of lesions were formed (lesion area percentage 20%) C: appreciable number of lesions were formed (lesion area percentage 10%) D: few lesions were formed (lesion area percentage 0.5%) E: No lesion.

Statistical analysis

Data have been reported in terms of the mean ± standard deviation (SD) values. Statistically significant differences were determined using Scheffe's test.

Results and Discussion

To determine the antifungal activity *H. cordata* or *A. indica* extracts against the infectious behavior of *M. oryzae*, spore germination and appressorium formation of *M. oryzae* spores in the presence of the extracts were investigated. The percentages of *M. oryzae* spore germination in 1-fold and 40-fold concentrated extracts of *H. cordata* were 5.2±10.9% and 0.0±0.0%, respectively (Fig. 1A), whereas in the *A. indica* extract, the percentages were 12.6±21.7% and 0.2±0.6%, respectively (Fig. 1A). In contrast, in the control, the percentage of spore germination in distilled water was 99.1±1.6% (Fig. 1A).

Furthermore, the percentages of *M. oryzae* appressorium formation in 1-fold and 40-fold concentrated extracts of *H. cordata* were 0.3±1.0% and 0.0±0.0%, respectively (Fig. 1B), whereas in *A. indica* extract, the percentages were 0.3±1.0%, and 0.0±0.0%, respectively (Fig. 1B). In the control, the percentage of appressorium formation in distilled water was 98.1±2.7% (Fig. 1B). These results indicated that spore germination and appressorium formation in *M. oryzae* were significantly inhibited by extracts of *H. cordata* and *A. indica*.

To elucidate the effect *H. cordata* or *A. indica* extracts, rice leaves were inoculated with *M. oryzae* spores in the presence of extracts of *H. cordata* and *A. indica* and blast lesion formation was assessed. Disease incidence and

disease severity of the control were 100% and 90±6.8%, respectively (Fig. 2). However, in leaves treated with extracts of *H. cordata* and *A. indica*, disease incidence was 35.6±10.2% and 22.2±10.2%, respectively (Fig. 2). Moreover, disease severity was 23.3±6.0% and 11.1 ±3.5%, respectively (Fig. 2). These results indicated that blast lesion formation was significantly inhibited by extracts of *H. cordata* and *A. indica*.

To the best of the authors' knowledge, this study is the first report of the application of extracts from *H. cordata* and *A. indica* to control *M. oryzae* on rice leaves. It has been reported that *H. cordata* and *A. indica* extracts produce various types of biochemical compounds, including antimutagenic, anticancer, antiviral, antibacterial, anti-allergic, and antioxidant activities (Kumar et al., 2014; Rashid et al., 2013). In the current study, we observed that extracts from *H. cordata* and *A. indica* control *M. oryzae*. Furthermore, because we used heated water extract in this study, it is conceivable that the rice blast inhibitory compound (s) is soluble in water. Additional studies are required to identify the active antifungal compound (s) in the extracts of *H. cordata* and *A. indica*.

Interestingly, we observed that an extract of bamboo inhibited the infection behavior of *M. oryzae* and blast lesion formation in rice, and identified 2,6-dimethoxy-1,4-benzoquinone as an antifungal compound that induced resistance against *M. oryzae* in rice (Ueno and Yoshikiyo 2014). It is therefore necessary to investigate whether the inhibitory compound (s) induce resistance, and to

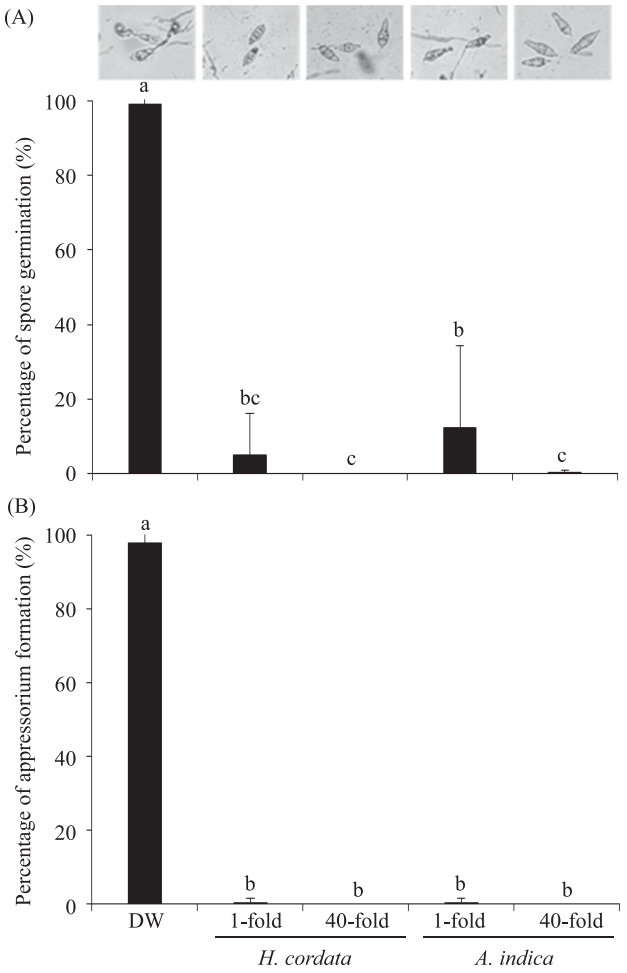

Fig. 1 Effect of plant extracts (*H. cordata* or *A. indica*) on the infection behaviors of *Magnaporthe oryzae*. Spore suspension of *M. oryzae* was dropped onto glass slides in the presence extract of *H. cordata* or *A. indica* of different concentration and incubated in a moist chamber at 26℃. After 24 h, (A) the percentage of spore germination (A) and appressorium formation (B) were determined by light microscopy. The data are the means of the results of three experiments with six replications. Bars represent ± SD. Means followed by different letters are significantly different according to the (Scheffe's test, $P < 0.05$).

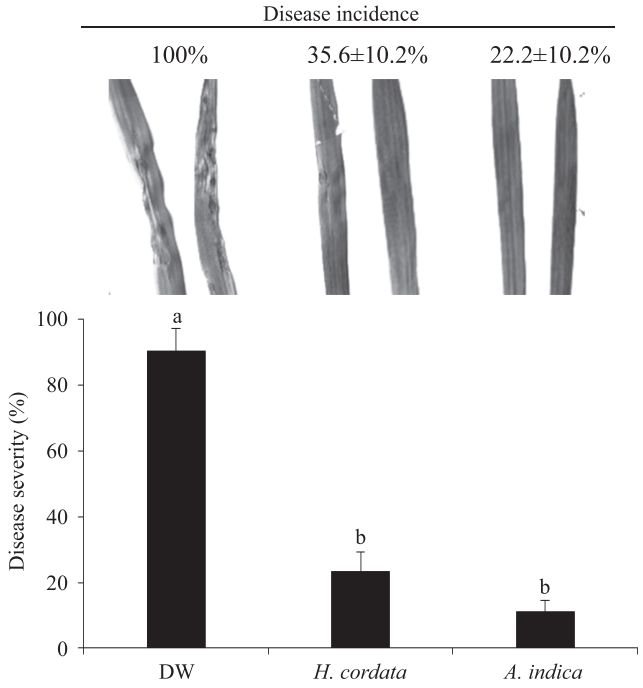

Fig. 2 Effect of plant extracts (*H. cordata* or *A. indica*) on lesion formation of *Magnaporthe oryzae* in rice leaves. Rice leaves were inoculated with *M. oryzae* in the presence of extracts of *H. cordata, A. indica,* or distilled water. After 5 days, disease incidence and disease severity were investigated. The data are the means of the results of three experiments with 15 replications. Bars represent ± SD. Means followed by different letters are significantly different according to the (Scheffe's test, $P < 0.05$).

determine the inhibitory mechanism of the extracts.

In conclusion, studies on extracts of *H. cordata* and *A. indica* may yield a candidate control agent that affords plants protection against diseases such as rice blast disease.

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References

Ahn, W.W. (1994) International collaboration on breeding for resistance to rice blast. In: Rice blast disease. RS Zeigler, SA Leong, PS Teng, eds. CAB International, Wallingford, UK, pp.137-153.

Ashizawa, T. and Zenbayashi, K. S. (2005) Improved efficiency in differentiating race of rice blast fungus using a multi-cell tray and plant growth regulator. Japanese Journal of Phytopathology, 71: 11-15.

Bowers, J. H. and Locke, J. C. (2000) Effect of botanical extracts on the population density of *Fusarium oxysporum* in soil and control of *Fusarium wilt* in the greenhouse. Plant Disease. 84: 300-305.

Chaijuckam, P. and Davis, R. M. (2010) Efficacy of natural plant products on the control of aggregate sheath spot of rice. Plant Disease. 94: 986-992.

Fravel, D. R. (1988) Role of antibiotics in the biocontrol of plant diseases. Annual Review of Phytopathology. 26: 75-92.

Kumar, M., Prasad, S. K. and Hemalatha, S. (2014) A current update on the phytopharm acological aspects of *Houttuynia cordata* Thunb. Pharmacognosy Review. 8(15): 22-35.

Ohba, A. (2002) Adaptability of cultivation of differential rice varieties using plant growth retardant for identification of pathogenic races of blast fungus in Miyagi prefecture. Annual Report of the Society of agricultural chemicals of North Japan, 53: 37-40.

Onyeke, C.C. and Ugwuoke, K.I. (2011) Effects of Botanical Extracts on the Mycelial Growth of Seed-Borne Fungi of The African Yam Bean, *Sphenostylis stenocarpa* (Hochst ex a. Rich) Harms. Nigeria Journal. Biotechonogy, 22: 1- 7.

Rashid, S., Rather, M. A., Shah, W. A., Bhat, B. A. (2013) Chemical composition, antimicrobial, cytotoxic and antioxidant activities of the essential oil of *Artemisia indica* Willd. Food Chemistry, 138: 693-700.

Reis, K. B., Côrtes, M. V. de C. B., Martins, F. S., Filippi, M. C. C. de, Paula, J. R. de and Conceição, E. C. da (2015) Characterization of rue extract and its potential for controlling rice blast. Pesquisa Agropecuária Brasileira, 50: 1121-1130.

Shimizu, M., Nakagawa, Y., Sato, Y., Furumai, T., Igaroshi, Y., Onaka, H., Yoshida, R. and Kunoh, H. (2000) Studies on endophytic actinomycetes and *Streptomyces* sp. isolated from Rododendron and its antifungal activity. Journal of General Plant Pathology, 66: 360-366.

So, K., Fuji, M., Iwabuchi, H., Kanayama, M. and Yamaguchi, J. (2002) Effects of various fungicides against less carpropamid-sensitive rice blast fungus isolated from the northwest area in Saga prefecture. Japanese Journal of Phytopathology, 68:262 (Abstract, in Japanese).

Uddin, W. and Viji, G. (2002) Biological control of turfgrass disease. In: Gnanamanickam, SS eds. Biological control of crop disease. Marcel Dekker, New York, pp. 313-337.

Ueno, M. and Yoshikiyo, K. (2014) 2,6-Dimethoxy-1,4-Benzoquinone enhances resistance against the rice blast fungus *Magnaporthe oryzae*. Journal of Phytopathology, 162: 731-736.

Wang, W. Q., Ben-Daniel, B. H., and Cohen, Y. (2004) Control of plant diseases by extracts of *Inula viscosa*. Phytopathology, 94:1042-1047.

Yamaguchi, J., Kuchiki, F., Hirayae, K. and So, K. (2002) Decreased effect of carpropamid for rice blast control in the west north area of Saga prefecture in 2001. Japanese Journal of Phytopathology, 68: 261 (Abstract, in Japanese).