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# Efficacy of buckwheat straw extracts against the rice blast fungus *Magnaporthe oryzae*

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**Abstract** Rice is one of the most important crops in the world. Rice blast disease caused by *Magnaporthe oryzae* is one of three major destructive diseases of rice. Chemical fungicides are required for the control of rice blast. However, overcoming fungicide resistance of *M. oryzae* is critical for successful chemical control. In this study, the inhibitory activity of hot water extracts of buckwheat straw against *M. oryzae* was evaluated for new fungicide development. Germination of *M. oryzae* conidia was significantly inhibited after incubation for 24 h in an extract of buckwheat straw. Significantly fewer blast lesions were formed in *M. oryzae* inoculated seedlings treated with the extract compared to seedlings treated with distilled water. These results indicate that inhibitory substances effective against *M. oryzae* may be present in hot water extracts of buckwheat straw.

**Keywords :** Buckwheat straw extract, Antifungal activity, *Magnaporthe oryzae,* Rice blast, *Oryza sativa, Fagopyrum esculentum* Moench

## Introduction

Rice blast caused by *Magnaporthe oryzae,* bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae,* and sheath blight caused by *Thanatephorus cucumeris* are three major diseases of rice (Zou et al. 2000; Hu et al. 2008; Li et al. 1999). It is well known that 50–90% of the expected crop is lost to rice blast (Agrios 2005). Resistant cultivars play an important role in the control of *M. oryzae.* However, the durability of genetic resistance in improved rice cultivars is often short lived in the field (Ahn 1994). Consequently, chemical fungicides are required in strategies for controlling rice blast. *M. oryzae* resistance to these chemicals has been reported in cases of extensive use (So et al. 2002; Yamaguchi et al. 2002). Therefore, a search for inhibitory 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 1988; Shimizu et al. 2000; Uddin and Viji 2002; Chaijuckam and Davis 2010).

Buckwheat (*Fagopyrum esculentum* Moench) is a commonly grown food crop in Japan. The classic Japanese work on agriculture, *Nihon Nousho Zenshu,* mentions that extracts of buckwheat straw were used to control rice blast in the Edo Period. However, inhibitory activity of buckwheat straw extracts against *M. oryzae* has not been clearly elucidated experimentally to date. This study assessed the effects of buckwheat straw extract on *M. oryzae* conidia germination in vitro and blast lesion formation in rice seedlings.

## Materials and Methods

### Rice plant and pathogen

Seedlings of rice cultivar *Oryza sativa* L. 'Koshihikari' were grown to five– to six–leaf stage in a glasshouse as described previously (Fujita et al. 1994). *M. oryzae* (strain Naga 69–150, race 007) was grown on rice bran agar medium (50 g rice bran, 20 g sucrose, and 20 g agar dissolved to a final volume of 1 L with distilled water) at 26–28 ℃ for 10–14 days. The growth plates were kept at 25–26 ℃ for approximately 48 h under near–UV light. The synchronously formed conidia were then used in the

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following experiments.

**Preparation of buckwheat straw extract**

Hot water extract was prepared from the straw of buckwheat cultivar *Fagopyrum esculentum* Moench 'cv. Shinshuoosoba'. Buckwheat plants were grown for 2–3 months in an experimental field. After harvest, the plants were air dried to make straw. The extract was prepared by boiling 5 g of shredded straw in distilled water in a flask at 100 ℃ for 30 min. After cooling, the extract was filtered through gauze and concentrated in a rotary evaporator. For the following experiments, the volume of extracted samples (5g) was adjusted to 1 mL.

**Infection inhibiting activity of buckwheat straw extract**

*M. oryzae* conidia ($1 \times 10^5$ conidia/mL), suspended in hot water extract of buckwheat straw or distilled water, were placed on glass slides and maintained in a moist chamber at 26–28 ℃. After incubation for 24 h, the percentage of conidia germinating was determined by light microscopy.

**Inoculation test using rice plants**

To investigate the suppression of blast lesion formation, rice seedlings at five– to six–leaf stage were inoculated with *M. oryzae* conidia ($1 \times 10^5$ conidia/mL) in the presence of buckwheat straw extract. Distilled water was used as a control. The inoculated seedlings were incubated in a moist chamber at 26–28 ℃ and then maintained under natural light conditions. The number of blast lesions on rice leaves were counted 7 days after inoculation.

**Statistical analysis**

Data are reported as mean ± standard deviation (SD). Significant differences between treatments were determined using t–tests.

**Results and Discussion**

Conidia germination of *M. oryzae* was significantly inhibited by extracts of buckwheat straw (Fig. 1A). The percentage of *M. oryzae* conidia germinating in buckwheat straw extract was 0.3 ± 0.9 (Fig. 1B). In contrast, in the distilled water control, the percentage of conidia germinating was 99.9 ± 0.4 (Fig. 1B). This inhibitory activity was confirmed not only for the buckwheat cultivar Shinshuoosoba but also for two other cultivars, Kitawasesoba and Dattansoba (data not shown).

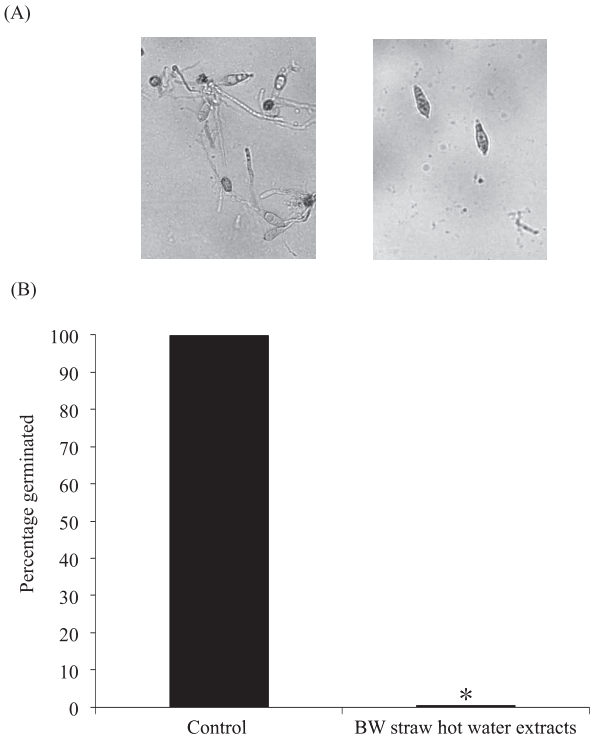


**Fig. 1** Inhibitory activity of buckwheat (BW) straw hot water extracts on conida germination of *Magnaporthe oryzae*. The conidia of *M. oryzae* were suspended in straw hot water extracts and dropped onto glass slides. After 24 h of incubation in a moist chamber at 26–28℃, conidia germination was observed by light microscopy. (A) Conidia germination of *M. oryzae* observed under a light microscope. (B) The percentage of conidia germination. The experiments were conducted in triplicates. A total of 300 conidia per experiment were examined. Asterisk indicates significant difference compared with the control (t-test, $P < 0.05$).

The development of blast lesion formation was significantly inhibited in leaves sprayed with hot water extracts of buckwheat straw (Fig. 2A). The number of blast lesions in the control was 21.2 ± 21.9 compared with only 6.6 ± 5.6 lesions in leaves treated with buckwheat straw extract (Fig. 2B).

Buckwheat has more rutin (an antioxidant flavonoid) than a number of other plant species (Dietrych–Szostak and Oleszek 1999). We considered whether rutin was the compound responsible for the inhibition of *M. oryzae*. Infection behavior of *M. oryzae* was not inhibited by rutin

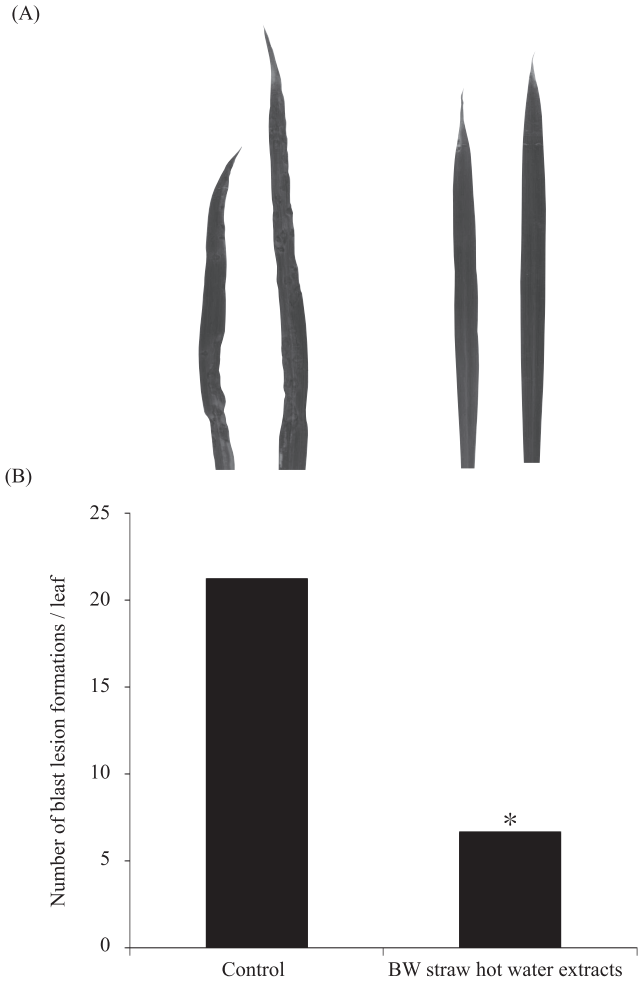


**Fig. 2** Suppression of rice blast lesion formation by buckwheat (BW) straw hot water extracts. Rice plants were inoculated with *M. oryzae* in the presence or absence of BW straw hot water extracts. After 7 days, disease development (A) and the number of blast lesions (B) was investigated. Experiments were conducted in triplicates. A total of thirty-five rice plants per experiment were examined. Asterisk indicates significant difference compared with the control (*t*-test, $P < 0.05$).

at 0–400 ppm (data not shown). Furthermore, buckwheat straw extract was prepared in hot water in this study, and it is known that rutin is degraded at high temperatures. These results suggest that the inhibitory substances are heat stable and water soluble. Further studies are required to identify these active substances in hot water extracts of buckwheat straw.

The present study may contribute to the development of a new fungicide against rice blast disease.

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## References

Agrios, G.N. (2005) Plant pathology, 5th ed. Elsevier Academic Press, San Diego, California. pp. 463.

Ahn, W.W. (1994) International collaboration on breeding for resistance to rice blast. In: Rice blast disease. R.S. Zeigler, S.A. Leong, P.S. Teng, (eds.) CAB International, Wallingford, UK. pp. 137–153.

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.

Dietrych–Szostak, D., and Oleszek, W. (1999) Effect of processing on the flavonoid content in buckwheat (*Fagopyrum esculentum* Moench) grain. Journal of Agricultural and Food Chemistry 47: 4384–4387.

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

Fujita, K., Arase, S., Hiratsuka, H., Honda, Y., and Nozu, M. (1994) The role of toxin (s) produced by germinating conidia of *Pyricularia oryzae* in pathogenesis. Journal of Phytopathology 142: 245–252.

Hu, K.M., Qiu, D.Y., Shen, X.L., Li, X.H., and Wang, S.P. (2008) Isolation and manipulation of quantitative trait loci for disease resistance in rice using a candidate gene approach. Molecular Plant 1: 786–793.

Li, Z.K., Luo, L.J., Mei, H.W., Paterson, A.H., Zhao, X.H., Zhong, D.B., Wang, Y.P., Yu, X.Q., Zhu, L., Tabien, R., Stansel, J.W., and Ying, C.S. (1999) A "defeated" rice resistance gene acts as a QTL against a virulent strain of *Xanthomonas oryzae* pv. *oryzae*. Molecular Genetics and Genomics 261: 58–63.

Shimizu, M., Nakagawa, Y., Sato, Y., Furumai, T., Igarashi, Y., Onaka, H., Yoshida, R., and Kunoh, H. (2000) Studies on endophytic actinomycetes (Ⅰ) *Streptomyces* sp. isolated from Rhododendron 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.

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).

Zou, J.H., Pan, X.B., Chen, Z.X., Xu, J.Y., Lu, J.F., Zhai, W.X., and Zhu, L.H. (2000) Mapping quantitative trait loci controlling sheath blight resistance in two rice cultivars (*Oryza sativa* L.) Theoretical and Applied Genetics 101: 569–573.