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Contribution of momilactone A and B to rice allelopathy

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ABSTRACT

Eight cultivars of rice (*Oryza sativa* L.) inhibited shoot and root growth of *Echinochloa crus-galli* when co-cultured with rice seedlings in a bioassay medium. Momilactone A and B were found in the bioassay medium of all rice cultivars, and concentrations of momilactone A and B in the medium were 0.21 – 1.5 and 0.66 – 3.8 μmol/L, respectively, indicating that all rice cultivars may secrete momilactone A and B into the medium. Exogenously applied momilactone A and B inhibited the growth of shoots and roots of *E. crus-galli* at concentrations greater than 30 and 1 μmol/L, respectively. The concentrations required for 50% growth inhibition of *E. crus-galli* shoots and roots, respectively, were 146 and 91 μmol/L for momilactone A and 6.5 and 6.9 μmol/L for momilactone B. Considering the growth inhibitory activity and concentrations found in the bioassay medium, momilactone A may have caused only 0.8–2.2% of the observed growth inhibition of *E. crus-galli* roots and shoots by rice. However, momilactone B in the medium was estimated to be able to cause 59–82% of the observed growth inhibition of *E. crus-galli* roots and shoots by the rice seedlings. In addition, the concentrations of momilactone B in the medium reflected the observed differences in the growth inhibition of *E. crus-galli* by the eight rice cultivars investigated. This suggests that the allelopathic activity of rice may depend primarily on the secretion level of momilactone B. Therefore, momilactone B may play a very important role in rice allelopathy.

Introduction

The first observation of allelopathy in rice (*Oryza sativa* L.) was made in field examinations in Arkansas, USA in which 191 of 5000 rice accessions inhibited the growth of *Heteranthera limosa* (SW.) Willd./Vahl (Dilday et al., 1989). This finding led to a large field screening program. Since then, more than 16,000 rice accessions from 99 countries in the USDA-ARS germplasm collection have been screened. Of these, 412 accessions inhibited the growth of *H. limosa* and 145 accessions inhibited the growth of *Ammannia coccinea* Rottb. (Dilday et al., 1994, 1998). Similar attempts have been conducted in some other countries, and a large number of rice cultivars have been found to inhibit the growth of several plant species when these rice varieties were grown together with these plants under field and/or laboratory conditions (Kim et al., 1999; Olofsdotter et al., 1999; Azmi et al., 2000; Gealy et al., 2003; Seal et al., 2004a; Kim et al., 2005). These findings suggest that rice may produce and secrete allelochemicals into its neighboring environment.

Several phenolic acids have been found in rice root exudates of allelopathic and non-allelopathic rice cultivars (Olofsdotter et al., 2002; Seal et al., 2004a). However, allelopathic rice cultivars did not secrete significantly greater amounts of phenolic acids than non-allelopathic cultivars. Furthermore, considering the inhibitory activity of phenolic acids, the secretion level of phenolic acids from rice was insufficient to cause growth inhibition of neighboring plants (Olofsdotter et al., 2002; Seal et al., 2004b).

A number of secondary metabolites, Phenolic acids, hydroxamic acids, fatty acids, terpenes and indoles, were identified in extracts and residues of rice plants as candidates for rice allelochemicals (Rimando and Duke, 2003). However, it is not yet clear if these compounds are released from living rice plants. Although most plant tissues contain potential allelochemicals, only compounds released from the plants into the environments can inhibit the germination and growth of neighboring plant species and, thus, act as allelochemicals in natural ecosystems (Putnam and Tang, 1986).

Another potential allelochemical isolated from root exudates of the rice cultivar Koshihikari is momilactone B (Kato-Noguchi et al., 2002). The secretion of momilactone B was also later confirmed for another rice cultivar (Kong et al., 2004). Momilactone B inhibits the growth of typical rice weeds such as *Echinochloa crus-galli* (L.) Beauv. and *E. colonum* (L.) Link at concentrations greater than 1 μmol/L. Furthermore, rice plants secrete momilactone B from the roots into the rhizosphere over their entire life cycle (Kato-Noguchi et al., 2008b). These observations suggest that rice plants may inhibit the growth of the neighboring plants through the secretion of momilactone B into their rhizosphere. In addition, another potential allelochemical, momilactone A, has been found in rice root exudates of cv. Koshihikari (Kato-Noguchi et al., 2008a). However, except for

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the occurrence of both momilactones in rice root exudates, there is no information available regarding the contributions of momilactone A and B to rice allelopathy.

Dekker and Meggitt (1983) hypothesized that most allelochemicals are released during the early developmental stages, when plants are most vulnerable to stress and compete with neighboring plants for resources such as light, nutrients and water. Therefore, in the present study, the allelopathic activity against *E. crus-galli* and secretion levels of momilactone A and B into the growth medium were determined in eight rice cultivars at the seedling stage. The contributions of momilactone A and B to rice allelopathy are discussed as a function of the interrelation between the cultivar-specific growth inhibitory activity and the secretion levels of momilactone A and B.

Materials and methods

Isolation of momilactone A and B

Momilactone A and B were isolated from husks of rice (*Oryza sativa* L. cv. Koshihikari) and identified by ^{1}H- and ^{13}C-NMR spectra as described by Kato-Noguchi et al. (2002, 2008a).

Plant materials

Eight widely cultivated japonica type cultivars of rice, cvs Kinuhikari, Hinohikari, Nipponbare, Sasanishiki, Yukihikari, Norin 8, Kamenoo and Koshihikari were chosen as donor plants for the donor–receiver bioassay described below. *Echinochloa crus-galli* was chosen as a receiver plant because this plant is the most significant biological constraint on rice production (Xuan et al., 2006).

Donor–receiver bioassay

Rice seeds were surface sterilized in 70% (v/v) aqueous ethanol for 15 min, rinsed five times with distilled water and germinated on a sheet of moist filter paper (no. 1; Toyo Ltd., Tokyo, Japan) at 25 °C with a 12 h photoperiod in a growth chamber. Light was provided from above with a white fluorescent tube (irradiance, 120 μmol/m^2/s at plant level; FL40SBR, National, Tokyo, Japan). After four days, six rice seedlings per cultivar with uniform root and shoot length were transferred to 5.5 cm Petri dishes, each containing two sheets of filter paper (no. 2; Toyo Ltd.), moistened with 3 mL of 1 mmol/L MES buffer (pH 6.0) as described by Weidenhamer et al. (1987), and grown for another three days.

Seeds of *E. crus-galli* were sterilized as described and germinated on a sheet of moist filter paper (no. 1) at 25 °C in the darkness for three days. Ten *E. crus-galli* seedlings with uniform root and shoot lengths were then arranged randomly on the filter paper in the Petri dishes and incubated with the six 7-day-old rice seedlings at 25 °C with a 12 h photoperiod as described above. The medium in the Petri dishes was kept constant by adding the evaporated MES buffer at 12 h interval. After three days, the lengths of the shoots and roots of the *E. crus-galli* seedlings were measured. Control seedlings were incubated in the absence of rice seedlings. Percentage inhibition was determined by the formula: [(control plant length − plant length incubated with rice)/control plant length] × 100. There were three replications per cultivar and the experiment was repeated seven times with three Petri dishes for each experiment. Significant differences were examined by Tukey's test. The liquid growth medium and filter paper in the Petri dishes were collected at the end of the bioassays for determination of momilactone A and B concentrations.

Determination of momilactone A and B

The medium of the bioassay and filter paper in the Petri dishes were extracted with 100 mL of 50% aqueous methanol for two days. The extracts were then loaded onto a reverse-phase C_{18} Sep-Pak cartridge (Waters). The cartridge was first eluted with 50% aqueous methanol to remove impurities, and then eluted with methanol to release momilactone A and B. Methanol elates were concentrated to 1 mL and analyzed by liquid chromatography–tandem mass spectrometry (LC–MS/MS) with the positive-ion mode and nitrogen for the collision gas (10 − 50 μL injection volume). Momilactone A and B, respectively, were detected in the multiple-reaction monitoring mode by the combination of m/z 315 and 217, and the combination of m/z 331 and 268. Quantification of momilactone A and B was performed as described by Obara et al. (2002). The determination of momilactones was done for seven bioassays with three replicates per cultivar. Significant differences were examined by Tukey's test. The overall recovery of momilactone A and B was 87% and 85% as calculated from five replications.

Bioassay of momilactone A and B

Momilatone A and B were dissolved in methanol, added to two sheets of filter paper (no. 2) in a 5.5 cm Petri dish. Methanol was subsequently evaporated and the filter paper in the Petri dishes was moistened with 3 mL of 1 mmol/L MES buffer. The final concentrations of momilactone A and B were 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 300, 1000, 3000 and 10,000 μmol/L. Ten uniform seedlings of *E. crus-galli* germinated in the darkness at 25 °C for three days were then arranged on the filter paper in the Petri dishes, and were incubated at 25 °C with a 12 h photoperiod for another three days. The lengths of seedling roots and shoots were measured as response parameters. Control seedlings were grown on the filter paper moistened with 3 mL of 1 mmol/L MES buffer. Percentage inhibition was determined by the formula: [(control plant length − plant length incubated with rice)/control plant length] × 100. There were three replicates per momilactone treatment and the experiment was repeated six times.

Results and discussion

Allelopathic activity of rice cultivars

Allelopathic activities of rice seedlings of eight cultivars against *E. crus-galli* were determined by a "donor–receiver bioassay." All rice cultivars inhibited the growth of shoots and roots of *E. crus-galli* seedlings, but with different levels of inhibitory activity. Cultivar Koshihikari showed the greatest inhibitory effects on both shoot and root growth of *E. crus-galli* (Table 1). Previous studies have also reported variations in the allelopathic activity among rice cultivars (Dilday et al., 1998; Kim et al., 1999; Olofsdotter et al., 1999; Azmi et al., 2000; Gealy et al., 2003; Seal et al., 2004a; Kim et al., 2005).

The *E. crus-galli* seedlings grew with the rice seedlings without competition for nutrients, because no nutrients were added in the bioassay medium. Competition for light can also be excluded, as photosynthesis is considered to be unnecessary during the early developmental stages of these seedlings, when most nutrients are withdrawn from seed reserves (Fuerst and Putnam, 1983). In addition, no significant pH changes occurred in the medium