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Rice seedlings release momilactone B into the environment

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Abstract

Since the growth inhibitor momilactone B was found recently in root exudates of rice (*Oryza sativa* L.), 3-day-old rice seedlings were transferred to hydroponic culture and the level of momilactone B released into the environment from the seedlings was measured. At day 15 after transfer, the level of momilactone B in the culture solution was 1.8 nmol per seedling compared with endogenous levels of 0.32 and 0.63 nmol per root and shoot, respectively, suggesting that rice seedlings actively releases momilactone B into the culture solution. This release must occur from the roots because only rice roots were immersed in the culture solution. Momilactone B inhibited the growth of ten cress (*Lepidium sativum* L.) seedlings at concentrations greater than 3 μM. Ten rice seedlings were incubated with ten cress seeds in a Petri dish containing 1 ml of medium, the medium contained 18 nmol of momilactone B, which came to 18 μM. This level of momilactone B was enough to reveal growth inhibition of the cress seedlings. Release level of momilactone B and its effectiveness as a growth inhibitor suggest that it may play an important role in rice allelopathy.

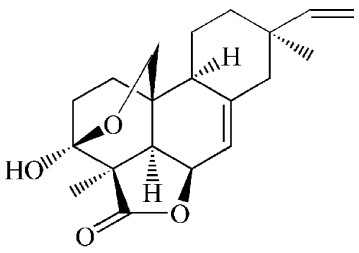
Keywords: *Oryza sativa*; Poaceae; Allelopathy; Growth inhibitor; Momilactone B; Rice; Root exudate

1. Introduction

Rice has been extensively studied with respect to its allelopathic potential and its production of allelochemicals as a part of a strategy for weed control purposes in a variety of agricultural settings (Olofsdotter et al., 1995, 1999; Mattice et al., 1998). In the USA, about 17,000 rice accessions were evaluated for their allelopathic potential and 557 of these were found to inhibit growth of the weed species *Heteranthera limosa* and/or *Ammannia coccinea,* often associated with rice cultivation (Dilday et al., 1994; 1998). In Egypt, 1000 rice accessions were screened for suppressive ability against *Echinochloa crus-galli* and *Cyperus difformis*, and allelopathic activity was found in 45 accessions (Hassan et al., 1998). Similar attempts have been conducted in other several countries, and many rice accessions have been found to possess allelopathic activity (Narwal, 1999). However, the identity of the compounds involved in these allelopathic activities is still unknown.

Several putative allelochemicals, such as *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid and ferulic acid, have been found in extracts of rice leaf and straw, and in soil decomposed straw (Kuwatsuka and Shindo, 1973; Chou and Lin, 1976; Chou and Chiou, 1979). It is not clear, however, whether these compounds are released from living rice plants and act as allelochemicals by inhibiting growth of neighboring plants.

A putative growth inhibitor was recently isolated from rice root exudates and identified as momilactone B (**1**) from its spectral data (**1**; Kato-Noguchi et al., 2002). Momilactone B (**1**) was originally isolated from rice husks as a growth inhibitor involved in seed dormancy (Kato et al., 1973; Takahashi et al., 1976) and was later found in rice leaves and straw as a phytoalexin (Cartwright et al., 1977, 1981; Kodama et al., 1988; Lee et al., 1999). But, this compound has not been reported to be released from living rice plants to the environment.



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In this paper, the endogenous level of momilactone B (**1**) in rice seedlings and release level of momilactone B (**1**) into the environment from the seedlings were determined; its possible involvement in rice allelopathy will be discussed.

2. Results and discussion

2.1. Endogenous level of momilactone B in rice seedlings

Fig. 1 shows the changes in the levels of momilactone B (**1**) in roots and shoots of rice seedlings after transfer to hydroponics culture. Momilactone B (**1**) levels in roots and shoots increased during the experiments, with the level in shoots always greater than that in roots. At day 15, the level in shoots was two-fold greater than that in the roots.

Momilactone A was found in rice leaves and straw and its function as a phytoalexin has been extensively studied (Nojiri et al., 1996; Araki and Kurahashi, 1999; Takahashi et al., 1999; Tamogami and Kodama, 2000). Although the growth inhibitory biological activity of momilactone B (**1**) was much greater than that of momilactone A (Takahashi et al., 1976), the function of momilactone B is obscure.

2.2. Release level of momilactone B (*1*) from rice seedlings

Momilactone B (**1**) was found in culture solutions in which rice seedlings were grown hydroponically (Fig. 2). The level of momilactone B (**1**) in the culture solution increased gradually until day 9 and then increased rapidly. The accumulation rate of momilactone B (**1**) was 0.04 and 0.24 nmol seedling^{-1} day^{-1} during days 0–9 and 9–15, respectively. Surprisingly, the levels in the culture solution at day 12 and 15 were much greater than those in shoots and roots (Figs. 1 and 2).

Momilactone B (**1**, 150 mg) was isolated from 200 kg dry weight of rice husks (Kato et al., 1973; Takahashi et al., 1976), which indicates that one husk (1.2 mg dry weight) contains 0.9 ng (2.7 pmol) of momilactone B (**1**). This value is negligible in comparison with the level of momilactone B (**1**) found in the culture solution in this experiment (Fig. 2). Furthermore, momilactone B (**1**) was found in root exudates from husked rice seedlings (Kato-Noguchi et al., 2002). In the present experiments, only rice roots were immersed in the culture solution as described in the section of Experimental. Thus, the rice seedlings probably release momilactone B (**1**) from their roots into the culture solution. This release may be active because of its high level in the culture solution compared with the level in the seedlings (Figs. 1 and 2).

2.3. Can momilactone B (*1*) act as a growth inhibiting agent?

Effectiveness of monilactone B (**1**) on growth inhibition was determined (Fig. 3). At concentrations greater than 3 μM, momilactone B (**1**) inhibited root and hypocotyl growth of cress seedlings. The concentrations required for 30% inhibition in the assay were 12 and 16 μM for cress roots and hypocotyls, respectively, as interpolated from the concentration–response curves.

At day 15 after transfer to hydroponic culture, the level of momilactone B (**1**) in culture solution was 1.8 nmol per rice seedling (Fig. 2), which indicates that one rice seedling may release 1.8 nmol of momilactone B to the culture solution over this period. If 10 rice seedlings

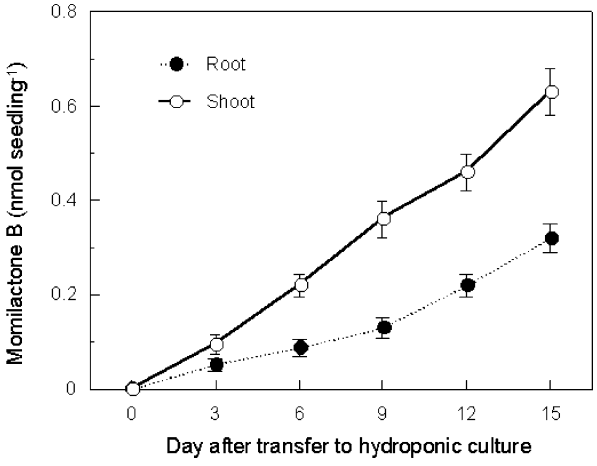

Fig. 1. Changes in levels of momilactone B (**1**) in roots and shoots of rice seedlings. One-hundred rice seedlings were grown hydroponically and the levels of momilactone B in the roots and shoots were determined. Means±SE from three independent experiments with three assays for each determination are shown.

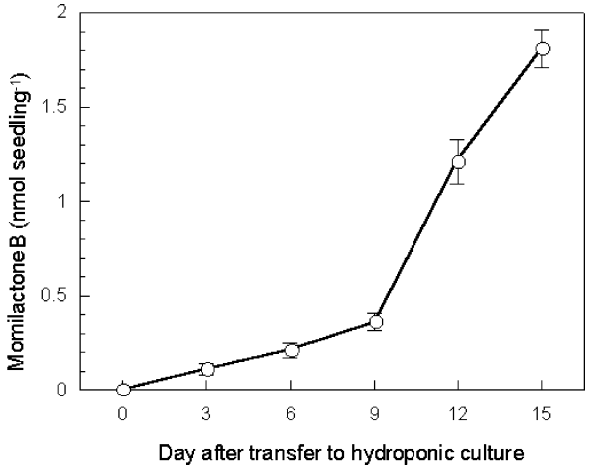

Fig. 2. Changes in level of momilactone B (**1**) in culture solution. One–hundred rice seedlings were grown hydroponically and the level of momilactone B (**1**) in the culture solution was determined. Means ± SE from three independent experiments with three assays for each determination are shown.

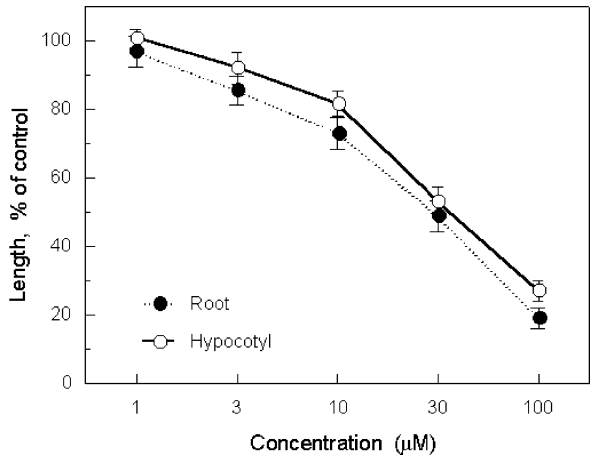

Fig. 3. Effects of momilactone B (**1**) on the growth of hypocotyls and roots of cress seedlings. Means±SE from three independent experiments with 10 plants for each determination are shown. Root and shoot length of control plants were 14.3±1.4 and 7.3±0.7 mm, respectively.

were incubated with 10 cress seeds in one Petri dish containing 1 ml of medium, the medium was found to contain 18 nmol of momilactone B (**1**), which came to 18 μM. This amount of momilactone B (**1**) is able to induce more than 30% growth inhibition in the roots and hypocotyls of cress seedlings (Fig. 3).

In the present research, momilactone B (**1**) was found in rice roots and shoots (Fig. 1) and in the culture solution of the rice seedlings (Fig. 2). Together, the release level of momilactone B (**1**) (Fig. 2) and its effect on growth (Fig. 3) suggest that rice seedlings may produce and release momilactone B (**1**) into the environment, and momilactone B (**1**) may act as an allelochemical that inhibits growth of neighboring plants. Thus, momilactone B (**1**) may play an important role in rice allelopathy. This is the first report that the potent growth inhibitor momilactone B (**1**) may be released from living rice roots to the environment in sufficient quantities to inhibit the growth of neighboring plants.

3. Experimental

3.1. Plant materials and hydroponics

Seeds of rice (*Oryza sativa* L. cv. Koshihikari) were surface sterilized in 70% (v/v) aqueous ethanol for 15 min, rinsed five times with distilled water and allowed to germinate 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, 2.9 W m^{-2} at plant level; FL40SBR, National, Tokyo, Japan). All further manipulations were carried out under sterile conditions.

After 3 days, uniform seedlings, in groups of 100, were transferred onto a sheet of plastic mesh (9×15 cm) that was floated on distilled water (300 ml) in plastic container (12×16×6 (height) cm), and grown at 25 °C with a 12-h photoperiod. The water in the plastic container was kept at the same level by adding distilled water at 24 h intervals and only roots of the seedlings were immersed in the water during the incubation. For determination of momilactone B (**1**), rice seedlings were harvested and the water in the container was collected at day 0, 3, 6, 9, 12 and 15 after being transferred to hydroponics.

3.2. Determination of momilactone B (*1*) in culture solution

The water in the container was filtered through filter paper (No 2; Toyo Ltd). The filtrate was loaded onto a column (2 cm i.d.×15 cm) of synthetic polystyrene adsorbent (35 g, Diaion HP20; Mitsubishi Chemical, Tokyo, Japan), and eluted with distilled water (100 ml), 20 and 80% (v/v) aqueous methanol (100 ml each), and methanol (150 ml). After evaporation, the methanol fraction was dissolved in 50% aqueous methanol (2 ml, v/v) and loaded onto a reversed-phase C_{18} Sep-Pak cartridge (Waters). The cartridge was first eluted with 50% aqueous methanol (15 ml) to remove impurities, and then with methanol (20 ml) to release momilactone B (**1**). The fraction of momilactone B was chromatographed by HPLC (1.0 i.d.×50 cm, ODS AQ-325; YMC Ltd, Kyoto, Japan; eluted at a flow rate of 2 ml min^{-1} with 70% aqueous methanol, detected at 220 nm). Quantification of momilactone B (**1**) was performed by interpolating the peak height on the chromatograms of HPLC to a standard curve made by the peak height of pure momilactone B (**1**) isolated from culture solution of rice seedlings (Kato-Noguchi et al., 2002). The overall recovery of momilactone B added to the culture solution before filtration was 85±7% (mean±SE) as calculated from five replications.

3.3. Determination of momilactone B in rice seedlings

Rice shoots or roots (ca. 10 g fr. wt.) were homogenized with 100 ml of 80% (v/v) aqueous methanol and the homogenate was filtered through filter paper (No. 2). The residue was homogenized again with 100 ml of methanol and filtered. The two filtrates were combined and evaporated in vacuo at 35 °C to give an aqueous residue. Then, the aqueous residue was loaded onto a column of synthetic polystyrene adsorbent and purified, and momilactone B (**1**) was quantified as described earlier.

3.4. Bioassay

Momilactone B (**1**) was dissolved in a small volume of methanol and added to a sheet of filter paper (No. 2) in a 2.8-cm Petri dish and dried. Then, the filter paper in

the Petri dishes was moistened with 0.6 ml of a 0.05% (v/v) aqueous solution of Tween 20, and 10 cress seeds were arranged on the filter paper and grown in the dark at 25 °C. The lengths of the hypocotyls and roots of cress seedlings were measured after 36 h, and the percentage length of the seedlings was determined by reference to the length of control seedlings.

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