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BRIEF COMMUNICATION

Rice seedlings release allelopathic substances

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Abstract

Rice (*Oryza sativa* L.) seedlings inhibited the growth of hypocotyls and roots of cress (*Lepidium sativum* L.) seedlings when both seedlings were grown together. Two growth inhibiting substances were found in the culture solution in which rice seedlings were hydroponically grown for 14 d. One growth inhibitor was further purified. This suggests that the rice seedlings may produce growth inhibiting substances, acting as allelochemicals to other plants, and release them from their roots into the environment.

Additional key words: growth inhibition, hydroponics, *Lepidium sativum*, *Oryza sativa*, root exudates.

Allelopathy is the direct influence of a chemical released from one living plant on the development and growth of other plants (*e.g.*, Rice 1984, Putnam and Tang 1986, Gross and Parthier 1994, Inderjit 1996). In sustainable agricultural system, the use of allelopathy to control weeds could contribute towards increasing yield of agricultural crop plants. In most integrated weed management systems, synthetic chemical herbicides may continue to be a key component, but controlling weeds through allelopathy is one strategy to reduce herbicide dependency (Duke 1986, Putnam 1988, Einhellig 1996, Seigler 1996).

Chou and Lin (1976) reported that aqueous extracts of decomposing rice residues in waterlogged soil inhibited root growth of lettuce seedlings. Common putative allelochemicals, such as *p*-hydroxybenzoic, vanillic, ferulic, *p*-coumaric, and *o*-hydroxyphenylacetic acids, were found in aqueous extracts of rice residues or straws (Kuwatsuka and Shindo 1973, Chou *et al.* 1991). It is not clear, however, whether these compounds are released from roots of living rice plants. In the present research we examined the allelochemicals in rice root exudates for clarification of the allelopathic potential of rice.

Seeds of rice (*Oryza sativa* L. cv. Koshihikari) were surface sterilized in an aqueous solution of 70 % (v/v) ethanol for 15 min, rinsed five times in distilled water and allowed to germinate on a sheet of moist filter paper (No. 1; *Toyo Ltd*, Tokyo, Japan) at 25 °C in a daily cycle of 12-h photoperiod in a growth chamber. Light was provided from above with a white fluorescent lamp (irradiance 2.9 W m^{-2} at plant level; *FL40SBR*, *National*, Tokyo). After 4 d, uniform rice seedlings were transferred, in groups of six, to 5.5-cm Petri dishes each containing a sheet of filter paper (No. 2) moistened with 3.5 cm^3 of 1 mM phosphate buffer (pH 6.0) as described by Weidenhamer *et al.* (1987), and grown for 3 d. Then, 10 cress (*Lepidium sativum* L.) seeds were arranged on the same filter paper in the Petri dishes, and incubated at 25 °C and 12-h photoperiod. The medium in the Petri dishes was kept at the same level by adding distilled water. After 3 d, the lengths of the hypocotyls and roots of the cress seedlings were measured. Control seedlings were incubated without rice seedlings.

Rice seeds were sterilized and germinated as described above. After 3 d, 100 uniform germinating seeds were transferred onto a sheet of plastic mesh (9 × 15 cm) which was floated on the distilled water (300 cm^3) in plastic container (12 × 16 × 6 (height) cm), and grown at 25 °C and 12-h photoperiod. The water in the plastic container was kept at the same level by adding the distilled water at 24-h interval. After 14 d, the water in the container was filtered through filter paper (No. 2). Then, the filtrate was loaded onto a column (3 × 21 cm) of synthetic adsorbent (100 g, *Diaion HP20*, *Mitsubishi*

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Chemical, Tokyo), and eluted with 600 cm^3 of 20 and 80 % (v/v) aqueous methanol (a total of 300 cm^3 was separately collected), and 450 cm^3 methanol. The biological activity of the fractions was determined using a cress bioassay as described below. After evaporation, the active residue was dissolved in 50 % aqueous methanol (2 cm^3, v/v) and loaded on to reverse-phase C_{18} *Sep-Pak* cartridges (*Waters*, Tokyo, Japan). The cartridge was eluted with 50 % aqueous methanol (15 cm^3) and methanol (20 cm^3). The activity was found in fractions obtained by elution with 50 % aqueous methanol. After evaporation, the active material was purified by HPLC (10 mm × 50 cm, *ODS AQ-325*; *YMC Ltd*, Kyoto, Japan; eluted at a flow rate of 2 cm^3 min^{-1} with 70 % aqueous methanol, detected at 220 nm), and inhibitory activity was found in a peak fraction eluted between 63 - 65 min.

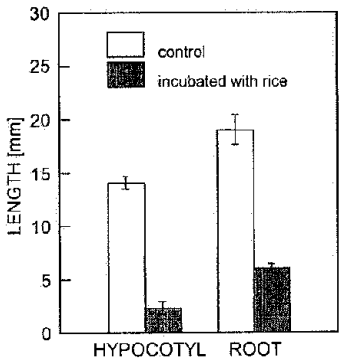

Fig. 1. Effects of rice seedlings on hypocotyl and root growth of cress seedlings. Cress seeds were incubated with 7-d-old rice seedlings at 25 °C in the daily cycle of 12-h photoperiod for 3 d. Then, the hypocotyl and root length of the cress seedlings were measured. Control seedlings were grown without rice seedlings. Means ± SE from three independent experiments with 10 plants for each determination are shown.

Each fraction from the chromatography was evaporated to dryness, dissolved in a small volume of methanol, added to a sheet of filter paper (No. 2) in a 2.8-cm Petri dish and dried. The filter paper in the Petri dishes was moistened with 0.6 cm^3 of a 0.05 % (v/v) aqueous solution of Tween 20, and 10 cress seeds were arranged on each Petri dish and grown in the dark at 25 °C. Control seedlings were treated with plain solution without extracts. After 36 h, the lengths of the hypocotyls and roots of the cress seedlings were measured.

In order to know the allelopathic potential of early developmental rice seedlings, cress seeds were grown with 7-d-old rice seedlings for 3 d. Two species may grow in one Petri dish without interspecies competition for light and nutrients, since young seedlings withdraw nutrients from the seeds and light is unnecessary in this developmental stage (Fuerst and Putnam 1983). The growth of hypocotyls and roots of cress seedlings was inhibited by the presence of rice seedlings (Fig. 1). The hypocotyls and roots length of the cress roots was 16 and 37 % of those of control seedlings. Thus, the rice seedlings inhibited the neighboring plant growth and the inhibitory effect may not be due to the competitive interfering for light and nutrients, suggesting that rice seedlings may produce growth inhibiting substances and release into neighboring environment.

Rice seedlings were hydroponically grown for 14 d and the culture solution was subjected to a column on synthetic adsorbent, and biological activity of eluted fractions was evaluated by cress bioassay (Fig. 2). Two peaks of inhibitory activity were detected in fractions 3 (elution with 80 % aqueous methanol) and 5 (elution with methanol), which indicates that there were two growth inhibiting substances in the culture solution. However, the activity was greater in the fraction 5 than that of fraction 3. The inhibition of the fraction 5 for cress hypocotyls and roots was 1.6- and 1.7-fold greater than

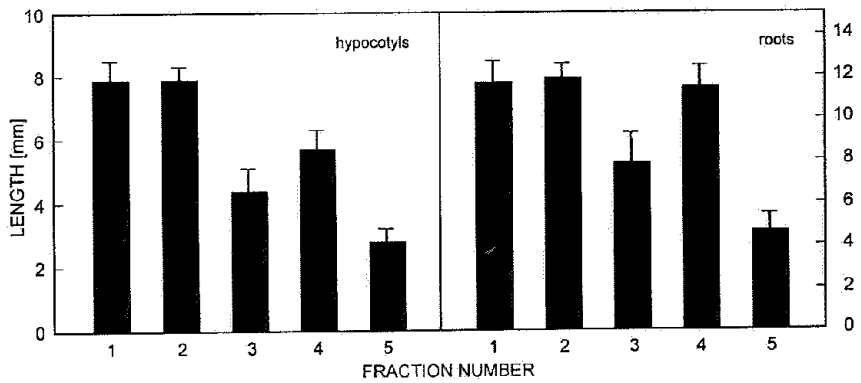

Fig. 2. Cress bioassay of fractions obtained after column chromatography of the culture solution of rice seedlings. All tested samples corresponded to 30 cm^3 equivalent of culture solution. Cress seeds were incubated in the dark at 25 °C for 36 h. Means ± SE from three independent experiments with 10 plants for each determination are shown. Length of hypocotyls and roots of control plants was 7.7 ± 0.5 and 12.2 ± 0.6 mm, respectively.

that of the fraction 3, respectively. After evaporation of the fraction 5 (Fig. 2), the active residue was further purified by a C_{18} *Sep-Pak* cartridge and the activity was detected in the fraction eluted with 40 % aqueous methanol (data not shown). Finally, the active residue was purified by HPLC and the inhibitory activity was found in a peak fraction eluted between 35 to 36 min (Fig. 3).

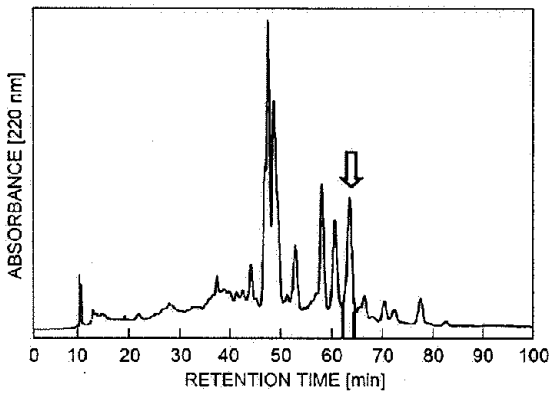

Fig. 3. HPLC chromatogram of active material purified with C_{18} Sep-Pak cartridge. Arrow indicates a peak fraction in which the inhibitory activity was detected.

It was hypothesized that most allelochemicals were released during germination and early developmental stage of plants on which the plants were most competitive with neighboring plants for resources such as light, nutrients and water (Dekker and Meggitt 1983). Seven-d-old rice seedlings inhibited the neighboring plant growth (Fig. 1). Two growth inhibiting substances were found in the culture solution after separation by synthetic adsorbent column chromatography followed by cress bioassay (Fig. 2) and one substance was further purified and isolated by C_{18} *Sep-Pak* cartridge and HPLC. These results suggest that the early developmental rice seedlings may produce growth inhibiting substances and release them into the environment from their roots, and the substances may act as allelochemicals to other plants. In the present research, however, the active components were not identified. Thus, large scale purification of the inhibiting substances is now underway to clarify the chemical basis of the allelopathic system in rice plants.

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