0971-4693/94 US $ 5.00
Allelopathy Journal 24 (2): 397-404 (2009) *International Allelopathy Foundation* 2009
Table: -, Figs : 3

SHORT COMMUNICATION

Inhibitory effects of rice seedling extracts and root exudates of Japanese traditional rice varieties

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(Received in revised form: July 14, 2009)

ABSTRACT

The extracts of eight Japanese traditional rice seedlings, including varieties of Soujaakamai, Tanegashimaakamai and Tushimaakamai which have been observed in the literature for more than several hundreds years, inhibited root and shoot growth of cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa L.*) and barnyardgrass (*Echinochloa crus-galli* L. Beauv.). Tushimaakamai had the greatest inhibitory activity. All root exudates of rice varieties also inhibited the root and shoot growth of test plant species and Awaakamai recorded the greatest inhibitory activity. Therefore, Japanese traditional varieties may produce allelochemicals and release them into rice rhizosphere as root exudates. However, these rice varieties showed different range of the inhibitory activity by the extracts and exudates, and test plants also show different sensitivity. Significant correlation between the inhibitory activity of rice extracts and rice root exudates were only found on alfalfa shoots and lettuce roots. Thus, except for alfalfa shoots and lettuce roots, the responses of these test plants to rice extracts are probably different from those to rice root exudates regarding the growth inhibition, which indicates that inhibitory substances in rice extracts and/or rice plants may not necessarily release into rice rhizosphere.

Key words: Alfalfa, allelopathy, barnyardgrass, cress, donor-receiver bioassay, *Echinochloa crus-galli,* growth inhibitor, *Lactuca sativa*, *Lepidium sativum*, lettuce, *Medicago sativa, Oryza sativa*, rice, root exudates.

INTRODUCTION

Rice has been extensively studied for its allelopathy as part of strategy for sustainable weed management (2,13,14,16,17). Many rice varieties inhibit the growth of several plant species when these varieties were grown together with these plants under field and/or laboratory conditions (1,4,6,11,17). These findings suggest that rice may produce and release allelochemical(s) into neighboring environment. Numerous secondary compounds have been isolated from the rice plants by organic or aqueous solvents as putative rice allelochemcals (12,19,20). However, it is not clear whether these compounds are released from the rice plants or from the decomposition of plant residues in sufficient quantities to inhibit the growth of neighbouring plants, and thereafter act as rice allelochemicals (18,21,22).

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Rice was introduced to Japan more than 2000 years ago from Asian Continent and was improved unremittingly. However, a few rice varieties retained their traits for more than several hundreds years in Shinto-shrine owing to the religious ceremony, such as varieties of Soujaakamai, Tanegashimaakamai and Tushimaakamai (7,15). The allelopathic potential of these traditional rice varieties has not been tested. In this research, inhibitory activities of seedling extracts and root residues of Japanese traditional rice varieties including Soujaakamai, Tanegashimaakamai and Tushimaakamai were determined and the relationship between the inhibitory activity of extracts and allelopathic activity in Japanese traditional rice varieties are discussed.

MATERIALS AND METHODS

Plant materials

Eight traditional Japanese rice (*Oryza sativa* L.) varieties, Awaakamai, Beniroman, Soujaakamai, Tanegashimaakamai, Touboshi, Tukushiakamochi, Tushimaakamai and Umenotani were chosen for determination of allelopathic activity. Cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.) and barnyardgrass (*Echinochloa crus-galli* L. Beauv.) were used for bioassay as test plants to evaluate rice allelopathic activity. Barnyardgrass was submerged in distilled water for one day to break seed dormancy just before the bioassay.

Inhibitory activity of rice extracts

One thousand seeds of each rice variety were germinated on a sheet of moist filter paper (No. 1; Toyo Ltd, Tokyo) in a growth chamber (25°C, 12-h photoperiod). After 10 days, rice seedlings (5-11 cm height) were harvested and homogenized with 70 % (v/v) aqueous methanol. After filtration through filter paper (No. 2; Toyo Ltd.), an aliquot of extract (final assay concentration was 0.5 g fresh weight equivalent extract ml^{-1}) was evaporated to dryness, dissolved in a 0.2 ml of methanol and added to a sheet of filter paper (No. 2) in a Petri dish (3-cm dia). Methanol was evaporated in a draft chamber. Then, the filter paper in the Petri dishes was moistened with 0.8 ml of 0.05% (v/v) aqueous solution of Tween 20. Ten seeds of test plants (cress, lettuce, alfalfa or barnyardgrass) were then placed into the Petri dishes as per treatments. The length of their shoots and roots was measured 48 h after incubation (25 °C, dark). In control treatments, methanol (0.2 ml) was added to the filter paper in Petri dish and evaporated as described above. Ten seeds of test plants (cress, lettuce, alfalfa or barnyardgrass) were then placed on filter paper moistened with aqueous solution of Tween 20 without methanol extract. Inhibition (%) was determined as following:

$$\text{Inhibition (\%)} = \frac{\text{control plant length - plant length incubated with rice extracts}}{\text{control plant length}} \times 100$$

The experiment was repeated six times using a randomize design with 10 plants for each determination. The data were analyzed by ANOVA test

Donor-receiver bioassay

One thousand seeds of each rice variety were germinated and grown for 4 days as described above. Uniform rice seedlings were then transferred in groups of six, to Petri dishes (5.5-cm dia) each containing a sheet of filter paper (No. 2) moistened with 3.5 ml of 1 mM phosphate buffer (pH 6.0) as described by Kato-Noguchi *et al*. (8) and grown for further 3 days. Ten seeds of test plants (cress, alfalfa, lettuce or barnyardgrass) were arranged on the filter paper in the Petri dishes and allowed to germinate and grow with the rice seedlings (25°C, 12-h photoperiod). After 3 days incubation, the lengths of the shoots and roots of test plants were measured. Control seedlings were incubated without rice seedlings. Inhibition (%) was then determined as described above. The experiment was repeated six times using a randomize design with 10 plants for each determination. The data were analyzed by ANOVA test.

RESULTS AND DISCUSSION

Rice seedling extracts

Rice varieties varied in their inhibitory potential and the test plants also responded differently to the extracts (Fig. 1). Tushimaakamai showed more than 50 % growth inhibition on cress and alfalfa roots and shoots and lettuce shoots. The growth inhibition over 50% was also recorded by Awaakamai (cress roots and shoots), Beniroman (alfalfa roots) and Tanegashimaakamai (lettuce roots). Tushimaakamai had the greatest activity with an average of 49.4 % of the growth inhibition on the roots and shoots of four test plants, followed by Awaakamai, Beniroman, Touboshi, Tanegashimaakamai, Tukushiakamochi, Umenotani and Soujaakamai. Cress shoots were the most sensitive to rice extracts with an average of 33.8% growth inhibition.

Root exudates

Rice varieties showed variable inhibitory activity (Fig. 2). Growth inhibition over 50% was caused by Awaakamai (cress shoots, lettuce roots and shoots, and alfalfa roots), Beniroman (cress and alfalfa roots), Tanegashimaakamai (cress and alfalfa roots), Tushimaakamai (cress roots, and alfalfa roots and shoots) and Umenotani (cress roots).

Awaakamai had the greatest inhibition with an average of 40.3 % inhibition of roots and shoots of four test plants, followed by Tushimaakamai, Beniroman, Tanegashimaakamai, Umenotani, Tukushiakamochi, Touboshi, and Soujaakamai. Test plants also showed different sensitivity to rice. However, Beniroman, Touboshi, Tukushiakamochi, Tushimaakamai varieties stimulated the shoot growth of barnyardgrass (Fig. 2).

Test plants can grow with the rice seedlings without interspecies competition for nutrients, because nutrients were not added in the bioassay (9). During the early development, seedlings mostly withdraw nutrients from their seed reserve (5). Besides there were no changes in pH in Petri dishes during the bioassay. Therefore, the inhibitory effect of rice seedlings on test plants may not be due to the competitive interference and pH changes in the medium, suggesting that all rice varieties studied in this experiment may possess allelopathic activity and this inhibition by rice may be caused by allelochemicals

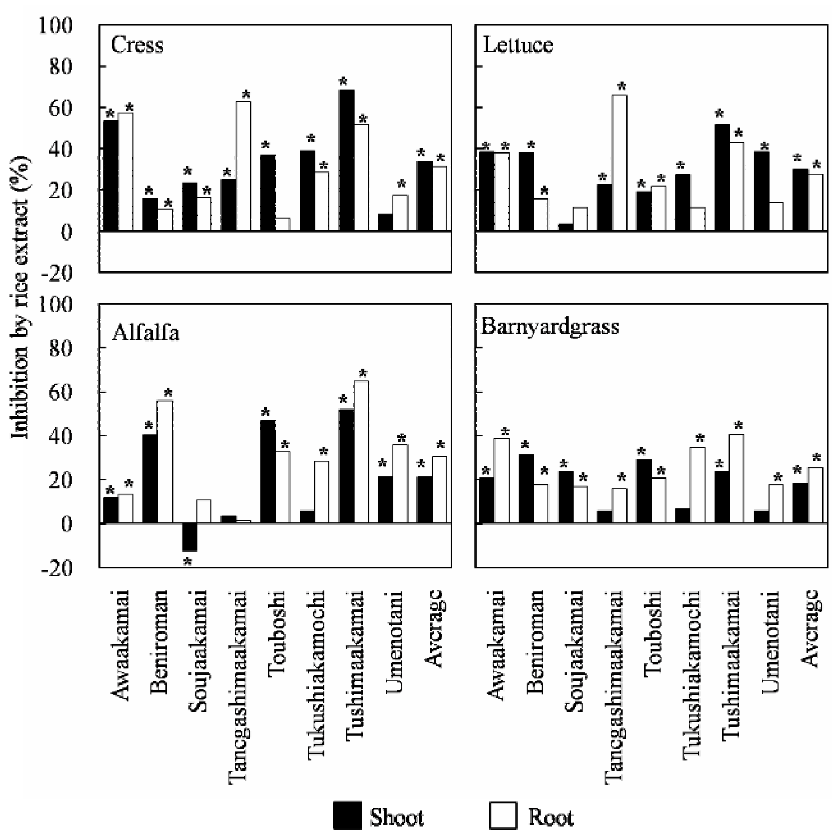

Figure 1. Inhibitory effects of rice seedlings extracts on root and shoot growth of test plants. *Indicates significant difference between control and treatment at $P < 0.001$ level.

but not by competitive interferences. In addition, there were differences in the allelopathic activity of rice varieties.

Rice seedling extracts versus root exudates

There was poor correlation between the inhibitory activity of rice extracts and rice root exudates (Fig. 3). Correlation coefficients (R) were 0.4561 (cress root), -0.1573 (cress shoot), 0.7946 (lettuce root), -0.1950 (lettuce shoot), 0.7806 (alfalfa root), 0.5199 (alfalfa shoot), 0.6528 (barnyardgrass root) and -0.5751 (barnyardgrass shoot). Significant correlations were only observed in alfalfa shoots and lettuce roots at 0.05 levels. Thus except for alfalfa shoots and lettuce roots, the responses of these test plants to rice extracts regarding the growth inhibition were different from those to rice root exudates, which suggest that the inhibitory substances in rice extracts may not be identical to those present in rice root exudates. Therefore, the growth inhibitory substances in the rice extracts and/or

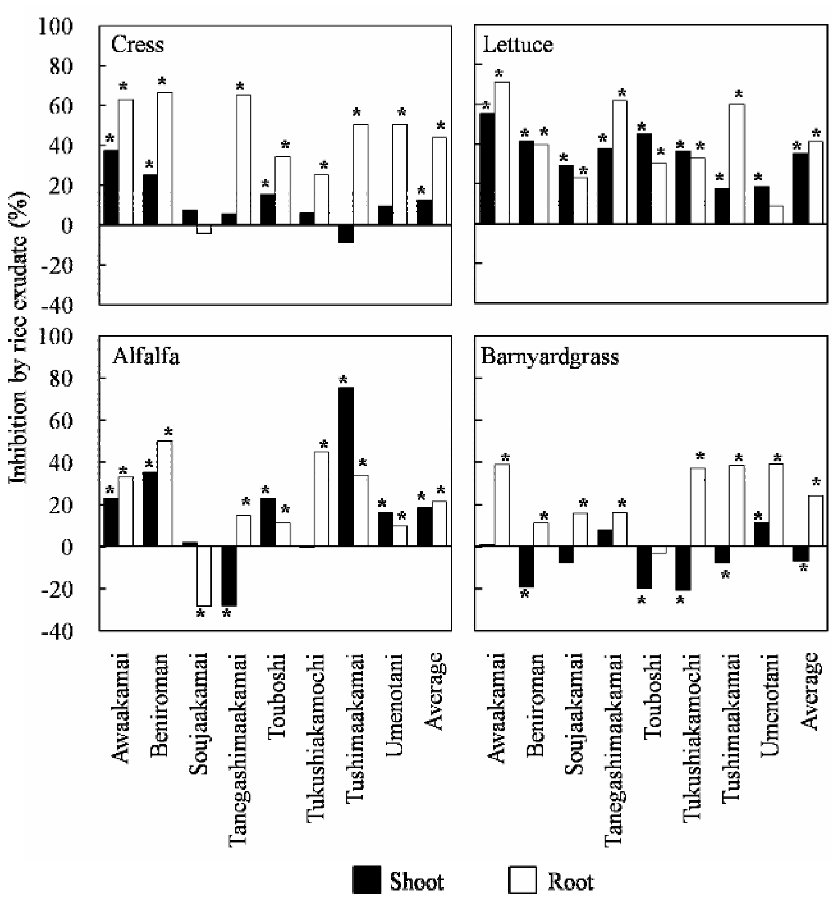

Figure 2. Effects of rice root exudates on root and shoot growth of test plants. *Indicates significant difference between control and treatment at P < 0.001 level.

rice plants may not necessarily release into rice rhizosphere as rice exudates. Inhibitory substances in plant root exudates are more important for plant allelopathy than inhibitory substances extracted from plants with some solvents, because the inhibitory substances in root exudates can work as allelochemicals soon after root exudation (9).

It was hypothesized that most allelochemicals are released during the early developmental stage of plants, when the plants undergo most stresses and compete with neighbouring plants for growth resources (light, nutrients and water) (3). In the present research, the extracts of Japanese traditional rice varieties inhibited the root and shoot growth of cress, alfalfa, lettuce and barnyardgrass, and Tushimaakamai had the greatest inhibitory activity (Fig. 1). All root exudates of Japanese traditional rice varieties also inhibited the root and shoot growth of test plants, and Awaakamai recorded the greatest

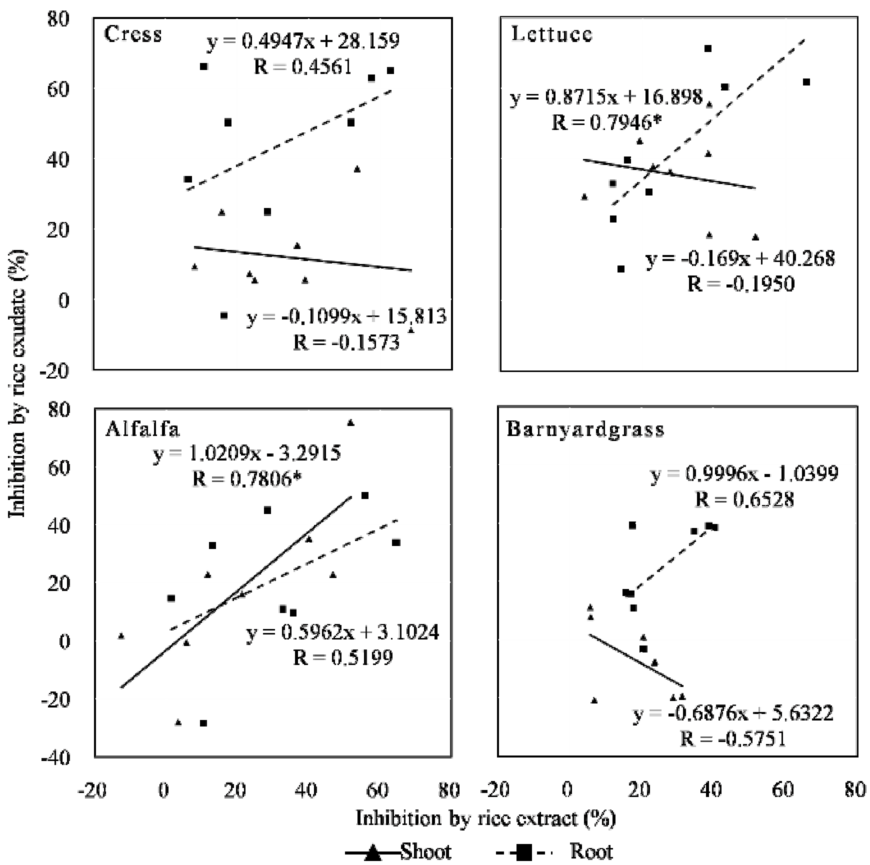

Figure 3. Relationship between inhibitory activity of rice extracts and root exudates. Calculated from the data shown in Tables 1 and 2. *, P < 0.05.

inhibitory activity (Fig. 2). These results suggest that the early developmental of rice seedlings of Japanese traditional varieties may produce allelochemicals (which are extractable) and release them into rice rhizosphere as rice exudates. However, these rice varieties showed different magnitudes of inhibitory activity between rice extracts and rice root exudates, and test plants also show different sensitivity (Fig. 1 and 2). Significant correlation between the inhibitory activity of rice extracts and rice root exudates were only found on alfalfa shoots and lettuce roots. Although many secondary compounds have been isolated from rice plants by solvents as putative rice allelochemicals (10,19), but the present research suggests that more attention should be paid in future allelopathy work to substances in root exudates than the substances in plant extracts as allelopathic agents.

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