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BRIEF COMMUNICATION

Assessment of allelopathic potential of root exudate of rice seedlings

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Abstract

To determine the allelopathic potential of root exudate from early developmental stage of rice (*Oryza sativa* L), 6-d-old seedlings of eight cultivars were grown with 3-d-old alfalfa (*Medicago sativa* L.), cress (*Lepidium sativum* L.) or lettuce (*Lactuca sativa* L.) seedlings in Petri dishes under controlled condition. All rice cultivars (cv. Norin 8, Kamenoo, Nipponbare, Kinuhikari, Koshihikari, Sasanishiki, Yukihikari and Hinohikari) inhibited growth of roots, shoots and fresh mass of alfalfa, cress and lettuce seedlings. Effectiveness of cv. Koshihikari was the greatest and more than 60 % inhibition was recorded in all bioassays, followed by that of cv. Norin 8 of which effectiveness was more than 40 %.

Additional key words: allelopathy, donor-receiver bioassay, growth inhibition, *Oryza sativa*.

Rice is one of the most important crops in the world and weeds are the most significant biological constraint to rice production. As the use of chemicals increases throughout the world, agricultural weed control alternatives to the present commercial herbicide dominated programs are now being given wide considerations (Duke 1986, Einhellig 1996, Olofsdotter 1998). 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, feruic, *p*-coumaric, and *o*-hydroxyphenylacetic acids, were found in aqueous extracts of rice residues or straws (Kuwatsuka and Shindo 1973, Chou and Lin 1976, Chou *et al.* 1991). It is not clear, however, whether these compounds are released from roots of living rice plants. Olofsdotter *et al.* (1995) claimed that extraction of plant tissue by water might force abnormal allelopathic effects to occur.

A large field experiments were conducted at the University of Arkansas, USA for screening 5000 rice accessions from 27 countries for allelopathic potential, and 200 rice accessions inhibited the growth of one or more weed species (Dilday *et al.* 1989, 1994, 1998). It is obscure, however, whether these inhibitions were caused by only allelopathic interference because plant-to-plant interference is a complex combination of competitive interference for resources and allelopathic chemical reactions (Fuerst and Putnam 1983, Putnam and Tang 1986, Leather and Einhellig 1988). Dekker and Meggitt (1983) found that most allelochemicals were released during germination and early growth of plants, and suggested that weed control research should concentrate on allelopathy during early developmental stages of crop plants. In this paper we described the assessment of the allelopathic potential of root exudates from rice plants during their early developmental stage by using Petri dish bioassay under laboratory condition. Separation of allelopathic effect from the complex combination of competitive interference and allelopathic reaction was also discussed.

Eight japonica type cultivars of rice (*Oryza sativa* L.), cv. Norin 8, Kamenoo, Nipponbare, Sasanishiki, Kinuhikari, Koshihikari, Hinohikari and Yukihikari, were chosen for bioassay as donor plants. These seeds were sterilized in an aqueous solution of 25 mM sodium hypochlorite for 15 min and rinsed in distilled water four

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times. Then, the seeds were allowed to germinate on two sheets of moist filter paper (No. 1; *Toyo Ltd,* Tokyo, Japan) at 25 °C and 12-h photoperiod. Light was provided from above with a white fluorescent lamp (irradiance of 2.9 W m^{-2} at plant level; *FL40SBR, National,* Tokyo, Japan). After 6 d, uniform seedlings were selected and used for bioassay.

It is difficult to use weed species as test plants under laboratory conditions, since their germination is at low rate and inconsistent (Inderjit and Olofsdotter 1998). Three plants, alfalfa (*Medicago sativa* L.), cress (*Lepidium sativum* L.) and lettuce (*Lactuca sativa* L.) were chosen for bioassay as receiver plants because of their known germination behaviours. Seeds of the plants were sterilized, rinsed and germinated as described above. After being kept in the daily cycles for 3 d, uniform seedlings were chosen for bioassay.

Rice seedlings were transferred, in groups of ten, to 9-cm Petri dishes which contained two sheets of filter paper (No. 2) moistened with 10 cm^3 of 3 mM phosphate buffer (pH 6.0). Then, each receiver plant (ten seedlings of alfalfa, cress or lettuce) was arranged on the same filter paper in the Petri dishes, and incubated under the same conditions as described above. The phosphate buffer was added in 6-h intervals. After 3 d, the shoot and root lengths and fresh mass of the receiver plants were measured. Control seedlings were grown alone without rice seedlings.

Just after bioassay, osmotic potential of the solution in each Petri dish was determined by a *Vapor Pressure Osmometer 5500* (*Wescor,* Logan, UT, USA). Standard solutions of mannitol were prepared at different concentrations (Hu and Jones 1997) and 3-d-old alfalfa, cress and lettuce seedlings were incubated in the solutions at 25 °C in the same daily cycle. After 3 d, the lengths of roots and shoots of the plants was measured as described above.

All experimental treatments were replicated seven times in complete randomized block designs. The percentages of seed germination and seedling length were scaled so that control was 100 % as described above, and means and SEs from seven replicate experiments with 10 plants each were calculated.

Growth of roots and shoots of several plants as well as germination were inhibited by extreme pH and osmotic potential in the test solutions for bioassay (Reynolds 1975, Wardle *et al.* 1992, Haugland and Brandsaeter 1996, Hu and Jones 1997). According to the test solution

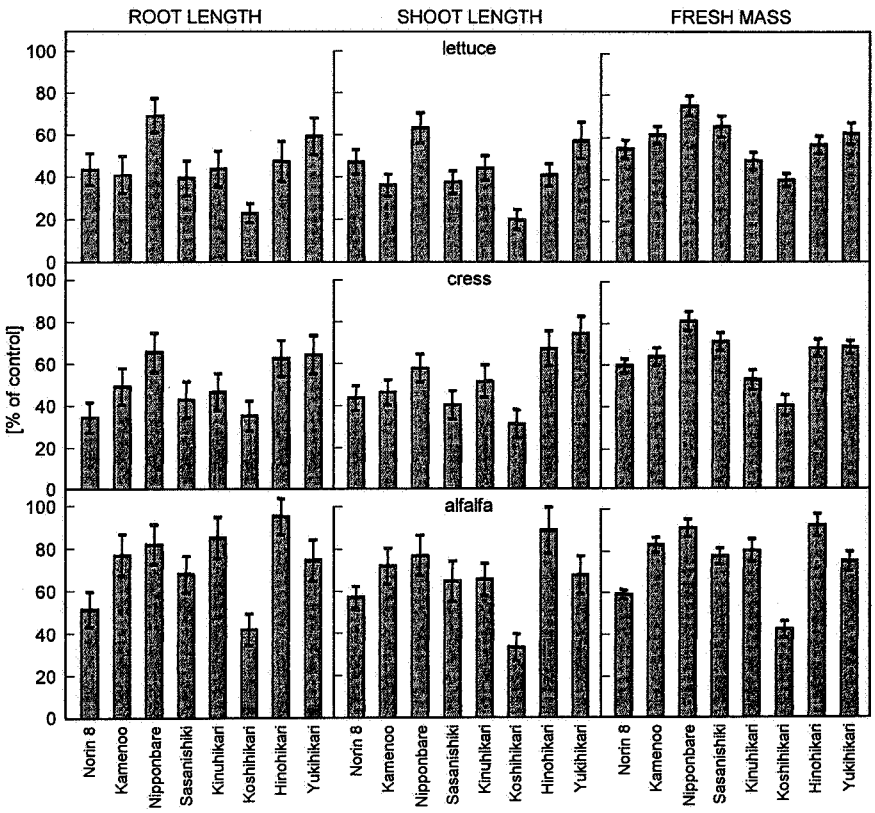

Fig. 1. Effects of rice seedlings on root and shoot growth, and fresh mass of alfalfa, cress and lettuce seedlings. Six-d-old rice seedlings (donor plants) were grown with 3-d-old cress, lettuce or alfalfa seedlings (receiver plants) in Petri dishes for 3 d. Then, the root and shoot length, and fresh mass of the receiver plants were measured. Percentage root and shoot length, and fresh mass of the seedlings, respectively, were calculated so that those of control seedlings were 100 %. Control seedlings were grown without rice seedlings. Means ± SE from 7 independent experiments with 10 plants for each determination are shown.

of Weidenhamer *et al.* (1987), 3 mM phosphate buffer (pH 6.0) was chosen as the test solution for the present experiment. The buffer did not affect the growth in rice, cress, lettuce and alfalfa seedlings (data not shown).

The osmótic potential of all test solutions used in this experiment for bioassay were less than 10 mmol kg^{-1}. All plants for the bioassay were also incubated in a range of solutions with known osmotic potential as described by Hu and Jones (1997). No effect of osmotic potential on growth of roots, shoots and fresh mass of these plants was detected up to 100, 150 and 100 mmol kg^{-1}, respectively. These data suggest that pH and osmotic potential of the test solutions did not significantly affect their growth of alfalfa, cress, lettuce and rice seedlings.

It was pointed out that research in allelopathy should design to eliminate the effects of the competitive interference (Leather and Einhellig 1986, Qasem and Hill 1989, Inderjit and Dakshini 1994). In our experiments, each cultivar of 6-d-old rice seedlings (8 cultivars, donor plants) was grown with 3-d-old alfalfa, cress, or lettuce seedlings (receiver plants) for 3 d in Petri dishes without interspecies competition for light and nutrients as well as water.

All rice cultivars inhibited the root and shoot growth of receiver plants (Fig. 1). However, the inhibition rate of both root and shoot growth differed with combinations of rice cultivars and receiver plant species. The root length of alfalfa, cress and lettuce seedlings was 44 - 95, 34 - 65 and 23 - 69 % those of the control plants, respectively, and the shoot length of alfalfa, cress and lettuce seedlings was 34 - 90, 32 - 75 and 21 - 64 % those of the control plants, respectively. All rice cultivars also inhibited growth of fresh mass of alfalfa, cress and lettuce seedlings (Fig. 1). Inhibition rate was also depending on the combination of rice cultivars and receiver plant species. The ranges for the fresh mass of alfalfa, cress and lettuce seedlings were 48 - 95, 41 - 81 and 39 - 75 % those of control plants. Thus, the growth of roots, shoots and fresh mass of receiver plants was inhibited by the presence of rice seedlings of all cultivars tested. Although there was no marked difference in the sensitively between roots, shoots and fresh mass in the same receiver plant species, the standard errors in the measurements in fresh mass is smaller than those in root and shoot growth.

Inhibition indexes (Table 1) were calculated from average inhibition rate of root length, shoot length and fresh mass (Fig. 1). These indexes indicate that effectiveness of cv. Koshihikari was the greatest and more than 60 % inhibition was recorded in all bioassay. More than 40 % inhibition was also record for cv. Norin 8. Thus, rice cultivars studied in the present research differed in their ability to alter the growth of alfalfa, cress and lettuce seedlings. Several genes because of the involvement of several chemicals in combination with the production and release of these chemicals (Dilday *et al.* 1994, 1998), may control allelopathic ability. In fact, the allelopathic activity was reported in various rice of both traditional and improved cultivars from different countries (Dilday *et al.* 1994, 1998).

Dekker and Meggitt (1983) have found that most allelochemicals were released during germination and early developmental stage of crop plants when the crops were most sensitive for competition. During the period, weeds also establish and create the basis for later major weed problems. Thus, early developmental stage of crop plants might determine the possible crop yield at the end of the season (Putnam and Tang 1986, Olofsdotter *et al.* 1995). Using laboratory Petri dish bioassay, 6-d-old rice seedlings were shown to affect the growth of roots, shoots and fresh mass of alfalfa, cress and lettuce seedlings (Fig. 1). These results suggest that the 6 to 9-d-old rice seedlings may produce and release alleochemical(s) into the environment and inhibit growth of neighbouring plants.

Inderjit and Olosfsdotter (1998) proposed that laboratory bioassay is one of the most important parts of research in rice allelopathy because bioassays under controlled environments permit distinctions to be made between allelopathic effects and interference by other means. Understanding of the chemical basis of the allelopathic system in rice plants as well as the field experiment of the allelochemicals are also essential (Duke 1986, Leather and Einhellig 1986). Thus, to clarify the chemical basis of such allelopathic system, purification and identification of the allelochemical(s) released from cv. Koshihikari which possess greatest allelopathic potential among eight cultivars is now underway.

Table 1. Inhibition index of rice seedlings on alfalfa, cress and lettuce seedlings. The inhibition indexes were calculated on average of inhibition rate of root length, shoot length and fresh mass. Inhibition rate of root length, shoot length and fresh mass, respectively, was scored so that those of control plants were 100 %.

Rice cultivars	Inhibition index [%] alfalfa	cress	lettuce
Norin 8	43.9	53.7	51.4
Kamenoo	22.3	46.6	53.2
Nipponbare	16.3	31.4	30.5
Sasanishiki	29.6	48.2	51.5
Kinuhikari	22.6	49.2	43.9
Koshihikari	60.6	63.2	71.9
Hinohikari	7.4	33.5	51.1
Yukihikari	27.1	30.4	39.9

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