Allelopathic Potential of White, Red and Black Rice Cultivars

Hisashi Kato-Noguchi¹, Kenji Nitta² and Tomio Itani²

¹Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Miki, Kagawa 761-0795, Japan; ²Department of Life Science, Faculty of Life and Environmental Sciences, Prefectural University of Hiroshima, Nanatsuka 562, Shobara 727-0023, Japan

Abstract: The inhibitory effect (allelopathic potential) of shoot, seed and root extracts of 8 white, 5 red and 5 black rice (Oryza sativa L.) cultivars was determined against the seedling growth of lettuce (Lactuca sativa L.) and white clover (Trifolium repens L.). All extracts inhibited hypocotyl and root growth of lettuce and white clover seedlings, and those inhibitory activities ranged from 1% – 96%. The average inhibitory activity of the extracts on the hypocotyl and root growth of lettuce and white clover was 42 – 88%. No apparent difference in the inhibitory activity was found either among the extracts of white, red and black rice, or among the extracts of shoots, seeds and roots. However, the red rice cultivar Tsushima-akamai marked the greatest inhibitory activity with an average of 88% growth inhibition on hypocotyls and roots of lettuce and white clover, followed by Souja-akamai and Koshihikari. These results suggest two red rice, Tsushima-akamai and Souja-akamai, may be potentially useful for weed management in agriculture.

Key words: Allelopathy, Colored rice, Growth inhibition, Lettuce, Tsushima-akamai, White clover.

Some plant species have been observed to provide excellent weed suppression after incorporation of their residues into soil (Narwal, 1999; Semidey, 1999; Caamal-Maldonado et al., 2001). Plants produce hundreds of secondary substances, and some of these substances play an important role in such weed suppression in the rhizosphere as allelopathic substances (Gross and Parthier, 1994; Duke et al., 2000). Chemicals with allelopathic activity are present in many plants and in many plant organs including leaves, flowers, fruits, and buds. Under the certain conditions these substances are released into the plant rhizosphere, either as exudates from living plants or by decomposition of plant residues in sufficient quantities to inhibit the growth of neighboring or successional plants (Rice, 1984; Putnam, 1988; Einhellig, 1996; Seigler, 1996).

Because of the agricultural importance of rice, rice allelopathy has been extensively studied and a great number of allelopathic compounds were identified in the rice extracts, root exudates and decomposing residues (review: Rimando and Duke, 2003; Khanh et al., 2007). Thus, rice may be one of the crop plants to exploit allelopathy for weed control in an agricultural setting.

Colored rice (red, black and green rice) has been recently paid much attention by rice consumers in Japan as a health food. These rice cultivars were characterized by pigments in the kernel and in other parts (Itani and Ogawa, 2004). These pigments mainly consist of tannin, anthocyanin and chlorophyll in red (reddish-brown), black (purple-black), and green rice, respectively (Nawa and Ootani, 1991; Itani, 1997, 2000). Of these colored rice groups, red, and black rice are used to produce several secondary products with their peculiar colors like colored noodles, cakes, alcoholic beverages, etc. Red rice is presumed to be an ancestor of the cultivated rice introduced into Japan from the Asian continent (Itani, 2000). Therefore it was considered interesting to assess the allelopathic potential of the colored rice cultivar for weed control purposes.

Three red rice and two black rice cultivars were reported to have strong allelopathic potential (Fujii et al., 2001; Araya et al., 2004; Matsuo et al., 2006). Kanesawa et al. (2009) also reported that Tsushima-akamai had the greatest inhibitory activity among 8 Japanese red rice cultivars. However, the number of cultivars examined was limited. There is also limited information available on the comparison of allelopathic potential among white, red and black rice groups. In the present research, allelopathic potential of shoots, roots and seeds of white, red and black rice was determined against hypocotyl and root growth of lettuce and white clover.
Materials and Methods

1. Plant materials

Eight white rice (common rice; *Oryza sativa* L.) cultivars; Koshihikari, Hinohikari, Hoshiyutaka, Nipponbare, PI312777, Rexmont, Nakateshinsenbon and Tanchomochi, 5 red rice cultivars; Souja-akamai, Tsushima-akamai, Tanegashima-akamai, Beniroman and Tsukushi-akamochi, and 5 black-rice cultivars; Asamurasaki, Okunomurasaki, Shihou, Ryoyokuromai and Syoshikuromai were selected. Red rice is characterized by the surface color of hulled rice, which is reddish-brown by tannin. Black rice is characterized by the surface color of hulled rice, which is purple-black by anthocyanin (Nawa and Ootani, 1991; Itani, 1997, 2000). Lettuce (*Lactuca sativa* L.) and white clover (*Trifolium repens* L.) were used as test plant species because of their different sensitivity to growth inhibitory substances (Itani et al., 1998; Matsuo et al., 2006).

2. Rice growth condition, extraction and bioassay

One hundred rice seeds, treated with the ipconazole-copper sterilizer, were sown on the filter paper (No. 1, Toyo Ltd. Tokyo) moistened with distilled water in the plastic container (6 × 6 × 10 cm) and grown in a growth chamber at 25°C under a 12-h photoperiod (25,000 lux). After incubation for 5 days, 10 mL distilled water was supplied and rice was grown another 5 days under the same condition. Rice seedlings were then harvested, divided into shoots, seeds and roots, and dried at 60°C for 24 hr. Dried rice shoots, seeds and roots were extracted with 20 mL of 70% (v/v) aqueous methanol for 48 hr. After filtration using filter paper (No. 1), the extract was evaporated to dryness in the draft chamber and dissolved in 1 mL methanol. An aliquot of the extract (final assay concentration was 100 mg dry weight rice plant equivalent extract mL⁻¹) was added to a sheet of filter paper (No. 1) in six-well multidish plastic plates (BD Biosciences, NJ, USA) and methanol was evaporated in the draft chamber. Then, the filter paper in each well (15 mm × height 18 mm) was moistened with 0.5 mL distilled water. Seven seeds of lettuce and white clover were then sown on the filter paper. The length of the shoots and roots of lettuce and white clover was measured after 72 hr of incubation in darkness at 20°C. Control seedlings were sown on the filter paper moistened with 0.5 mL distilled water. Inhibitory activity (%) of the extracts was then determined by the formula: [(length of control plant - length of treated plant) / length of control plant] × 100. Difference between the activities of extracts from treated and control plants was examined by Tukey’s test. The average of the inhibitory activities against hypocotyls and roots of lettuce and white clover was designated as “inhibitory activity of extract”, and the average of the “inhibitory activity of extract” from shoot, seed and root of rice as “inhibitory activity of cultivar”. In addition, the inhibitory activity of the extracts of shoots, seeds and roots of two red rice cultivars, Tsushima-akamai and Souja-akamai, was also determined at the concentrations 0, 3, 10, 30, 100 and 300 mg dry weight rice plant equivalent extract mL⁻¹ as described above. The bioassay was repeated three times using a randomized design.

Results and Discussion

Extracts of the shoots, seeds and roots of all rice cultivars inhibited the growth of hypocotyls and roots of lettuce and white clover in a range of −1% to 96%. Average inhibitory activity of the white rice shoot, seed and root extracts was 49 – 79%, 59 – 82% and 50 – 83%, respectively, those of 5 red rice cultivars were 40 – 86%, 71 – 92% and 52 – 78%, respectively, and those of 5 black rice cultivars were 13 – 68%, 57 – 86% and 35 – 86%, respectively (Table 1, Average extract). The two-way ANOVA analysis did not show any apparent difference in the inhibitory activities among the extracts of white, red and black rice cultivars. There was also no apparent difference in the activity among the extracts of shoots, seeds and roots, or between the sensitivities of both test plants to the extracts.

However, Tsushima-akamai marked the greatest inhibitory activity (Table 1) with an average of 88% growth inhibition on shoots and roots of lettuce and white clover, followed by Souja-akamai and Koshihikari (Table 1, Average cultivar). Ryoyokuromai had the lowest average inhibitory activity with 42% and the shoot extract of the cultivar did not significantly inhibit root growth of two test plants. Thus, Tsushima-akamai may be the most allelopathic followed by Souja-akamai. In addition, inhibitory activities of the extracts of shoots, seeds and roots of Tsushima-akamai and Souja-akamai were concentration-dependent (Fig. 1). These results are consistent with the previous reports (Fujii et al. 2001; Matsuo et al., 2006; Kanesawa et al., 2009).

In spite of the heavy use of commercial herbicides to control weeds, crop yield loss due to the remaining weeds is high (Putnam, 1988; Einhellig, 1996; Weston, 1996). The negative impact of commercial herbicide use on the environmental contamination makes it necessary to diversify weed management options. Controlling weeds through allelopathy is one strategy to reduce commercial herbicide dependency (Duke et al., 2000; Belz, 2007; Macias et al., 2007; Tesio and Ferrero, 2010). The present research suggests that, although the experiment was accomplished under laboratory condition and further investigation in field is essential, Tsushima-akamai and Souja-akamai may be potentially useful for weed management in an agricultural setting, such as a multi and/or soil admixture, which may reduce application of the commercial herbicide. Potent allelopathic substances, momilactone A and B, have been isolated from Koshihikari.
Table 1. Inhibitory activities of shoots, seeds and roots of white, red and black rice cultivars on hypocotyl and root growth of lettuce and white clover.

| Cultivar         | Lettuce Hypocotyl | Lettuce Root | White clover Hypocotyl | White clover Root | Average Extract | Average Cultivar |
|------------------|-------------------|-------------|------------------------|------------------|----------------|-----------------|
| Koshihikari      | Shoot 73***       | 47**        | 66**                   | 78***            | 66             | 75              |
|                  | Seed 83***        | 72***       | 77***                  | 85***            | 79             |                 |
|                  | Root 83***        | 72**        | 77***                  | 87***            | 80             |                 |
| Hinosihikari     | Shoot 82***       | 78***       | 76***                  | 81***            | 79             |                 |
|                  | Seed 78***        | 63**        | 72***                  | 70***            | 73             |                 |
|                  | Root 71***        | 25*         | 64**                   | 74***            | 59             | 70              |
| Hoshiyutaka      | Shoot 70**        | 25*         | 86***                  | 80***            | 65             |                 |
|                  | Seed 80***        | 75***       | 84***                  | 76***            | 79             |                 |
|                  | Root 62***        | 60**        | 68**                   | 76***            | 67             | 70              |
| Nipponbare       | Shoot 57**        | 50**        | 46**                   | 42***            | 49             |                 |
|                  | Seed 67**         | 60**        | 74**                   | 76***            | 69             |                 |
|                  | Root 60**         | 63**        | 54**                   | 58**             | 59             | 59              |
| P1512777         | Shoot 41**        | 25*         | 76**                   | 83***            | 56             |                 |
|                  | Seed 57**         | 41**        | 74**                   | 64**             | 59             |                 |
|                  | Root 80***        | 67**        | 92***                  | 92***            | 83             | 66              |
| Rexmont          | Shoot 72**        | 88***       | 76***                  | 31*              | 67             |                 |
|                  | Seed 71***        | 88***       | 77***                  | 73**             | 77             |                 |
|                  | Root 69***        | 81***       | 76**                   | 60**             | 72             | 72              |
| Nakatehishinbon  | Shoot 63**        | 49**        | 64**                   | 46**             | 56             |                 |
|                  | Seed 82***        | 81***       | 86***                  | 75**             | 81             |                 |
|                  | Root 46**         | 53**        | 64**                   | 50**             | 53             | 63              |
| Tanchomochi      | Shoot 78***       | 63**        | 78***                  | 76***            | 74             |                 |
|                  | Seed 87***        | 72**        | 88***                  | 79***            | 82             |                 |
|                  | Root 69***        | 53**        | 36**                   | 43**             | 50             | 69              |
| Souja-akamai     | Shoot 81***       | 60**        | 75***                  | 81***            | 77             |                 |
|                  | Seed 95***        | 91***       | 90***                  | 91***            | 92             |                 |
|                  | Root 71***        | 75***       | 64**                   | 74***            | 71             | 80              |
| Tsushima-akamai  | Shoot 89***       | 81***       | 88***                  | 87***            | 86             |                 |
|                  | Seed 89***        | 81***       | 96**                   | 95***            | 90             |                 |
|                  | Root 96***        | 81***       | 80***                  | 80***            | 87             | 88              |
| Tanegashima-akamai | Shoot 40*        | 27*         | 52**                   | 40**             | 40             |                 |
|                  | Seed 74***        | 63***       | 74*                    | 71***            | 71             |                 |
|                  | Root 57**         | 42**        | 68**                   | 43**             | 52             | 54              |
| Beniroman        | Shoot 67**        | 52**        | 68**                   | 74**             | 65             |                 |
|                  | Seed 84***        | 74***       | 84***                  | 76***            | 80             |                 |
|                  | Root 81***        | 67**        | 82***                  | 80***            | 78             | 74              |
| Tsukushi-akamochi | Shoot 67**        | 50**        | 78***                  | 72***            | 67             |                 |
|                  | Seed 86***        | 72***       | 72**                   | 71**             | 75             |                 |
|                  | Root 55**         | 47**        | 48**                   | 70**             | 55             | 66              |
| Asamurasaki      | Shoot 53**        | 22**        | 60**                   | 70***            | 51             |                 |
|                  | Seed 83***        | 72**        | 92**                   | 95***            | 86             |                 |
|                  | Root 96***        | 60**        | 92**                   | 95***            | 86             | 74              |
| Okumomurasaki    | Shoot 63**        | 47**        | 76***                  | 84***            | 68             |                 |
|                  | Seed 70**         | 63**        | 76**                   | 74**             | 71             |                 |
|                  | Root 67**         | 53**        | 80***                  | 84**             | 71             | 70              |
| Shihou           | Shoot 71**        | 63**        | 68**                   | 71***            | 68             |                 |
|                  | Seed 80***        | 78***       | 88***                  | 84***            | 85             |                 |
|                  | Root 67**         | 44**        | 76**                   | 79**             | 67             | 73              |
| Ryoyokuromai     | Shoot 22*         | 8           | 9*                     | –                | 13             |                 |
|                  | Seed 89***        | 83***       | 76**                   | 68**             | 79             |                 |
|                  | Root 55**         | 35*         | 288                    | 23*              | 35             | 42              |
| Syoshikuromai    | Shoot 59**        | 41**        | 44**                   | 60**             | 51             |                 |
|                  | Seed 62**         | 47**        | 54**                   | 66**             | 57             |                 |
|                  | Root 70**         | 63**        | 61**                   | 70**             | 66             | 58              |

Concentration of tested samples corresponded to the extract obtained from 100 mg dry weight rice per mL. Inhibitory activity (%) was determined by the formula: \[
\left(\frac{\text{length of control plant} - \text{length of treated plant}}{\text{length of control plant}}\right) \times 100.
\]

Means from 3 independent experiments are shown. Asterisk indicates significant difference between control and treatment: * \(P < 0.05\), ** \(P < 0.01\), *** \(P < 0.001\). Average extract indicates the average inhibitory activity of an extract of rice shoots, seeds or roots on hypocotyl and root growth of lettuce and white clover. Average cultivar indicates the average inhibitory activity of all extracts (shoot, seed and root extracts) of one rice cultivar on hypocotyl and root growth of lettuce and white clover.
and later from other rice cultivars (Kato-Noguchi et al., 2002; Kong et al., 2004; Kato-Noguchi et al., 2010). The number of secondary substances in rice plant extracts and decomposing residues were also identified as potential allelochemicals (Rimando and Duke, 2003; Khanh et al., 2007), but since there is no information about the allelopathic substances in Tsushima-akamai, it is therefore important to identify allelopathic substances in Tsushima-akamai.

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