Rice Science, 2010, 17(2): 135−140

DOI: 10.1016/S1672-6308(08)60116-X

Induced Effects of Exogenous Phenolic Acids on Allelopathy of a Wild Rice Accession (*Oryza longistaminata*, S37)

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Abstract: Four exogenous phenolic acids, including salicylic acid, fumalic acid, *p*-coumaric acid and *p*-hydroxybenzonic acid, were used to investigate the regulatory effects on allelopathy of a wild rice accession of S37 (*Oryza longistaminata*), which is a known allelopathic rice. The four exogenous phenolic acids induced the enhancement of the allelopathic potential of wild rice S37 in target weeds though the weed-suppressive activities were low, and the inducible effects were dependent on the specific phenolic acid, concentration and treatment time. After foliar application of exogenous phenolic acids, the inhibition rates for plant height, root length and fresh weight of barnyard grass (*Echinochloa crus-galli*) were significantly higher than those of the control. Especially at the concentration of 100 mg/L, the inhibition rates for plant height and fresh weight of barnyard grass by fumalic acid were 38.12% and 26.31% higher than those of the control, showing that fumalic acid was more effective compared with other phenolic acids in inhibiting monocotyledon weed growth. Furthermore, the weed-suppressive activity of aqueous extract from the leaves of wild rice S37 treated with exogenous phenolic acids was increased, and it peaked at 48 h after the treatment with the aqueous extract, and then gradually declined.
Key words: salicylic acid; fumalic acid; *p*-coumaric acid; *p*-hydroxybenzoic acid; phenolic acid; allelopathy; inducible effect; *Oryza longistaminata*

Rice is a major food crop worldwide, and its yield and quality are negatively impacted by weeds in paddy field, especially by barnyard grass. Weeds generally cause a yield loss of more than 10% rice production under current cultivation practices, though yield loss might reach 45%–96% if no measures were applied for weed control [1]. Thus, it needs to apply herbicide to control weeds in rice fields. The negative effects of herbicides on environment and agricultural ecosystem are gradually highlighted due to long-term and large-scale herbicide application. In sustainable cropping system, the utilization of rice allelopathy, an important biological engineering technology and environmentally friendly method to control paddy field weeds, has been explored by weed researchers since the mid 1980s [2-4].

In recent years, most research on allelopathy in rice has focused on identification of allelochemicals, including identification, confirmation, bioactivity test and structural analysis of allelopathic product. However, the environmental factors which induced the dynamic changes of allelopathic activity in rice were neglected, especially the biological factors regulating allelopathic activity [5-7]. Preliminary genetic analysis suggested that allelopathy in rice was quantitatively inherited and controlled by several gene loci, which indicates that allelopathy in rice is not only genetically controlled, but affected by environmental factors as well [8]. Previous studies showed that allelopathy in rice was instable and the synthesized and released quantity of rice allelochemicals varied with growth stages and environmental conditions. It would be feasible to explore effective ways to enhance rice allelopathy and maintain its stability using regulating environmental factors [9].

It had been documented by Tang et al [10] that allelopathic phenolic and terpenoid compounds in plants increased under environmental stress, and the allelopathic effect on neighbouring plants was enhanced as well. Tang et al [10] observed that water stress enhanced phytotoxic secondary metabolites in both the plant tissue and the rhizosphere of purple nutsedge (*Cyperus roturdus*). McKey et al [11] found that phenolic acid contents in mature leaves of abundant trees in rain-forest vegetation were different in two sites with different soil types, and increased in the site with low nutrient soil. A strong correlation between phenolic acid

Received: 4 January 2010; **Accepted:** 16 April 2010
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This is an English version of the paper published in Chinese in *Chinese Journal of Rice Science*, Vol. 24, No. 1, 2010, Pages 62–66.

content and allelopathic activity has been reported [5, 12-14]. Applying exogenous salicylic acid to improve the weed-suppression of allelopathic rice PI312777 had been confirmed by Qiu et al [15], which indicates that allelopathy in rice could be regulated by exogenous phenolics. In this experiment, four kinds of exogenous phenolics (salicylic acid, fumalic acid, *p*-coumaric acid and *p*-hydroxybenzonic acid) were applied to S37, a known allelopathic wild rice (*Oryza longisatminata*) accession [16-17], to investigate the effects of four exogenous phenolics on the allelopathic activity of rice. We hope that the results of this experiment will help to determine the feasibility of exogenous phenolics in regulating the inherent allelopathic activity of rice.

MATERIALS AND METHODS

Test materials

S37, an accession of wild rice (*O. longisatminata*, AA-genome species) with allelopathy, was provided by the Rice Research Institute, Yunnan Academy of Agricultural Sciences, Kunming, China. The seeds of barnyard grass (*Echinochloa crus-galli*) were collected from a paddy field in the north suburb of Kunming in 2008, and stored at room temperature. The soil was collected from the paddy field at the Agricultural Environment & Resource Research Institute, Yunnan Academy of Agricultural Sciences (YAAS), Kunming, China, and its properties were analyzed by the Analysis and Testing Center, Agricultural Environment & Resource Research Institute, YAAS, Kunming, China according to the Chinese Standards NY/T 1121.2-2006, NY/T 1121.6-2006, GB 7173-1987, GB 9837-1988 and GB 9836-1988 [18]. The properties of soil were: pH 6.2, organic content (18.14±0.17) g/kg, total N (1.63±0.12) g/kg, total P (0.37±0.08) g/kg and total K (8.41±0.17) g/kg. Salicylic acid, fumalic acid, *p*-coumaric acid and *p*-hydroxybenzonic acid were purchased from the SIGMA corporation, which were at analytical reagent grade (>99.0% purity).

Experimental methods

Impact of four phenolic acids to Echinochloa crus-galli growth

Dry paddy soil (250 g, sieved through 50 mesh screen) was placed into glass containers (diameter 12 cm and height 15 cm). Fifteen germinated barnyard grass seeds were placed on soil surface and covered with 2 mm depth of soil after the soil was moistened. Four phenolic acids solutions (concentrations of 250, 500, 750 and 1000 mg/L) were sprayed individually at 50 mL/m^2 for each treatment using a commercial Potter tower sprayer at a 1.5 MPa work pressure and 40 μm droplet diameter. The control was simultaneously sprayed with the same amount of distilled water. All the treatments were raised in an artificial growth chamber with a light intensity of >5000 lx, an illumination time of 12 h/d, a relative humidity of 50% during the day and 70% at night, and a temperature constant at 28°C. Plant height and fresh weight of barnyard grass shoots were measured at 20 days after treatment. Treatments were replicated four times for each phenolic acid treatment.

Regulatory effects of four exogenous phenolic acids on the allelopathy of wild rice S37

The 4-leaf-old seedlings of wild rice S37, which raised in a greenhouse, were planted in containers (diameter 12 cm and height 15 cm), and grown in an artificial growth chamber with the growth conditions similar to those described above. At 10 days after rice planting, pre-germinated barnyard grass seeds were planted around the rice plants and covered by 2 mm depth of soil. Four phenolic acids solutions were applied to shoots of wild rice S37 using a Potter tower sprayer. Only shoots of tested rice were treated and the soil was covered by a film wrap to prevent soil contamination by the solution. The concentrations of exogenous phenolic acids were formulated at 25, 50, 75 and 100 mg/L. After spraying, the film was removed and the treated plants were placed back into the artificial growth chamber under the growth conditions as described above. Twenty days after treatment, plant height and fresh weight of barnyard grass shoots were measured. Treatments were replicated four times for each phenolic acid treatment.

Impact of aqueous extracts from wild rice S37 treated with exogenous phenolic acids

Seedlings of wild rice S37 were grown in an artificial growth chamber for 10 days. The four exogenous phenolic acids solutions at a concentration of 50 mg/L were sprayed to aboveground plant parts of rice using a Potter tower sprayer in a similar manner as described above. Each phenolic acids treatment was