Effective Bioassay methods for evaluating rice allelopathic potential

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Objectives

Bioassays are a necessary tool in allelopathy research, enabling qualification of biological responses in living organisms. Bioassays also frequently detect biological activity of chemicals in concentrations beyond of the limit of detection. Thus, bioassays are needed both in initial screening for allelopathic potential and in the extraction and purification of allelopathic substances. For screening rice allelopathic potential, several methods such as stair step method, hydroponic culture test, relay seeding technique, agar medium selection, cluster analysis using HPLC, and water extract method have been developed and tested for bioassays. Each screening method has its own advantages, but what we need is a reliable and universal method which gives a similar result in both the lab and the field. For this purpose, water extract method was employed as an alternative method for evaluating rice allelopathic potential.

Materials and Methods

Water extract from leaf : Foliar portion of 35-day-old rice plants of each cultivar (Dongjinbyeo, IAC 165, Koketsumochi, Sathi, Musashikogane, PI 312777, and Tang gan) grown in plastic box following the standard culture method was harvested and macerated in a mortar. One hundred ml of distilled water was mixed with 10.0 g of macerated sample of fresh rice leaf. Water extraction was done for 24-h at $26\pm 2^{\circ}\text{C}$. Then water extraction was filtered through Whatman no. 1. Dilution was made to make 1 and 5% solution for bioassay. To determine the effect of water extract on the growth of barnyardgrass, ten ml of 1, 5 and 10% solution of water extract was applied on a petri dish containing 20 barnyardgrass seeds with 3 replications and this experiment was conducted at $26\pm 2^{\circ}\text{C}$ with 12-h in light and 12-h in dark conditions (growth chamber). At day 10, effect on the growth of barnyardgrass root was determined.

Water extract from seed : Rice seeds from the same rice cultivars in above were grinded in a grinder (Ika-Werke, Germany). One hundred ml of distilled water was added to 10 grams of grinded rice sample. Water extraction was done for 48-h at $26\pm 2^{\circ}\text{C}$. Water extraction was filtered through Whatman no. 2 paper. Dilution was made to make 1 and 5% solution. Ten ml of 1, 5 and 10% solution of water extract was treated on a petri dish containing 20 barnyardgrass seeds with 3 replications. At day 10, germination and growth of barnyardgrass were determined.

Results

Water extract from leaf : Among 7 cultivars, Kouketsumochi, IAC 165 and Sathi showed over 50% inhibitory effect on barnyardgrass shoot growth at 10%, but at 1 and 5%, water extracts from all rice cultivars showed no clear inhibitory effects on barnyardgrass shoot growth (Fig. 1). However, 5% solution from Kouketsumochi, PI 312777, Sathi and Tan gan resulted in strong inhibitory effects (ranging from 20 to 40%) on barnyardgrass root growth, indicating that barnyardgrass root is more sensitive to water extract made from fresh rice leaf (Fig. 2).

Water extract from seed : Figures 3 and 4 indicate the effect of water extract made directly from seeds of 7 cultivars. There is a very clear difference among rice cultivars tested. All levels of water extract from Kouketsumochi, potential allelopathic cultivar, showed very high inhibitory effects on barnyardgrass root growth, but 10% of water extract from Dongjinbyeo showed no inhibitory effects on both barnyardgrass shoot and root growth. Almost no growth of barnyardgrass root was observed in 10% water extract from Kouketsumochi and Musashikogane. However, barnyardgrass root grew normal at all levels of water extract from Donjinbyeo. Response of barnyardgrass root growth can be used as a screening method for rice allelopathic potential by use of water extraction from seed (Figs. 3 and 4).

Thus water extraction from leaf and seed might be an alternative for evaluating rice allelopathic potential. Particularly, water extraction from seed might be a very simple bioassay method to screen rice allelopathic potential, strongly suggesting that rice seeds of each cultivar may possess different levels of allelopathic potential.

Fig. 1. Effect of water extract from rice leaf on barnyardgrass shoot growth.

Fig. 2. Effect of water extract from rice leaf on barnyardgrass root growth.

Fig. 3. Effect of water extract from seed on barnyardgrass shoot growth.

Fig. 4. Effect of water extract from seed on barnyardgrass root growth.