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VARIETAL DIFFERENCES OF DOSE RESPONSE RICE ALLELOPATHY ON ANNUAL RYEGRASS

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Abstract

Weeds are continuing a constant problem in Australian cropping system. Annual ryegrass (*Lolium rigidum*) is a ubiquitous weed of southern Australia, infesting the majority of crops. The prospects of herbicide resistance necessitate considering alternative options such as the allelopathic potential of crops for weed suppression. To assess the dose response allelopathic prospects of rice, a laboratory-based root exudates bioassay was conducted using ECAM (Equal-Compartment-Agar Method). The allelopathic effects of two different genotypes (Quest and M 205) and five different densities (0, 5, 10, 15 and 20 seeds/beaker) of rice against annual ryegrass were investigated. During harvest the inhibited root, and shoot length was measured, the tissue structure of ryegrass root was also observed under electronic microscope. The experiment was designed as a randomized complete block with three replications and conducted under control conditions. Results showed that rice root exudates reduced the seedling growth of ryegrass with increasing density and genotype. Quest showed strong allelopathic potentiality than M 205 against ryegrass. The root growth of ryegrass was most infected than shoot by rice root exudates. The cellular study also suggests high density of rice increase high amount of allelochemicals in agar medium that caused damage of ryegrass root tissue system. These investigations also suggest that there are opportunities to explore dose response (hormesis) allelopathic effect of rice genotypes to control ryegrass.

Key words: Rice, Allelopathy, Dose and Annual ryegrass

Introduction

Rice has been extensively studied with respect to its allelopathy as part of a strategy for sustainable weed management, such as breeding allelopathic rice strains (Olofsdotter 2001; 2002, Takeuchi *et al.,* 2001). A large number of rice varieties were found to inhibit the growth of several plant species when grown together under field and/or laboratory conditions (Dilday *et al.,* 1998, Kim *et al.,* 1999, Olofsdotter *et al.,* 1999, Azmi *et al.,* 2000, Seal, 2003). These findings suggest that rice may produce and release allelochemicals(s) into the neighboring environment, thus encouraging the exploration of allelochemicals in rice those suppress the growth of *Heteranthera limosa* (Dilday *et al.,* 1989), *Ammannia coccinea* (Dilday *et al.,* 1994, 1998), *Echinochlola crus-galli* and *Cyperus difformis*, (Hassan *et al.,* 1998), arrowhead (*Sagittaria montevidensis*) (Seal *et al.,* 2003). Annual ryegrass (*Lolium rigidum*) is Australia's most economically damaging crop weed (Yu, and Powles; 2004). A heavy infestation of 200 plants m^{-2} can cause a 50% yield loss in wheat costing \$100- \$250 ha^{-1} (Wu *et al.,* 1998). Surveys across southern Australia found annual ryegrass in 86% of rice crops (Lemerle *et al.,* 1999) and 69% of cereal crops (Lemerle *et al.,* 1996). It is a primary weed of cropping systems in the Murrumbidgee catchment where rice has been cultivating. It is predicted that resistance to several herbicides of ryegrass may become a problem into rice field introduction by seed movement with irrigation water and or other sources. Such references have been occurred in California of USA (Barb, 1990). Although weed control herbicide options are available for rice, the prospects of herbicide resistance necessitate considering allelopathic potential of rice for ryegrass weed suppression.

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In rice allelopathy, research also indicated that a large number of compounds like phenolic acids, fatty acids, indoles and terpenes had been identified in rice root exudates and decomposing rice residues as putative allelochemicals which possed the growth inhibitory activity against neighboring plants (Kata-Nagauchu, 2008). Research also documented that the concentration of these allelochemicals in a culture medium would also depend on the density (Inderjit, 1996; Inderjit *et al.,* 1996). A high density of donor plants and a stressful environment may be important for the allelopathic effect. Moreover, ecological interactions are often density dependent and density dependent allelopathic studies were tested previously and it was reported that with high density plant got more advantages to compete with neighboring species (Weidenhamer *et al.,* 1989; Levin, 1988). The density dependent rice allelopathy was also examined and it was noted that high density of rice cultivars could noticeably reduce the seedling growth of arrowhead (*Sagittaria montevidensis*) (Seal *et al.,* 2003; Xu *et al.,* 2004). Thus, rice with high density as a donor crops of putative allechemicals may have agronomic potential in non-chemical weed management fields but no studies have shown that ryegrass is allelopathized by rice. Therefore, a laboratory based rice density dependent allelopathic study was conducted to determine the allelopathic impacts of rice on ryegrass.

Materials and Methods

Annual ryegrass and rice cultivars

Two rice cultivars were tested for their allelopathic activity on annual ryegrass. The ryegrass seeds were collected from Australia. Origin and pedigree information for rice cultivars used in the current experiments is provided in Table 1. The rice germplasm collection place located at New South Wales DPI, Yanco, Australia.

Table 1. Basic information of rice genotypes

Cultivar	Origin	Grain size	Attribute
Quest	Australia	Standard medium	Short season, water saving
M 205	USA	Medium (longer than Quest)	Short season, water loving

Bioassay

The Equal Compartment Agar Method (ECAM) developed by Wu *et al.,* (2000) was used for the screening of rice accessions. Five different densities (0, 5, 10, 15, and 20 seedlings/beaker) of two rice varieties were used in the experiment. The pre-germinated seedlings were sown in 500 ml beakers filled with 30 ml of 0.3% nutrient-free water agar media. 500 ml beakers filled with 30 ml of 0.3% nutrient-free water-agar and as per treatment germinated rice seedlings of rice cultivars were sown in agar medium. The beakers were sealed with parafilm and kept in a controlled growth chamber (light/dark 12/12 h at 20^{o}C/18^{o}C respectively). After one week of growth, pre-germinated ryegrass seedlings were added to the other half of the beaker. A piece of pre-autoclaved white paperboard was inserted across the centre and down the middle of the beaker with the lower edge of the paper board kept 1 cm above agar surface. The beaker was divided into two equal compartments to minimize competition for space and light between rice and ryegrass seedlings. The arrangement was such that the roots of rice can freely enter the ryegrass compartment so that any allelochemicals produced and released by the rice seedlings can diffuse throughout the entire agar medium to influence ryegrass root growth. After ryegrass sowing, the beakers were again wrapped with parafilm and placed back in the growth chamber for seven days. The weed seedlings were removed after one week of co-existence with the rice seedlings and their root lengths measured to the nearest 0.5 mm. Weeds grown in absence of rice were the control treatments. Comparisons between cultivars were made on the basis of percent control data.

Highest and lowest ranking cultivars were determined by calculating the mean root inhibition of each cultivar. The experiment was repeated twice at the same condition to verify the results.

Observation of ryegrass root cells

A segment of root tissue of inhibited ryegrass (caused by allelopathic cultivar) was placed on a slide and root tissues were examined under a transmitted light compound microscope (X 100 objective). The photomicrographs were taken using a compound microscope (Nikon Eclipse 50i).

Statistical analysis

Root length data were analyzed using Genstat 7 (version 13). Replicated means were converted to percentage of control. Least significant differences LSD at $P = 0.05$ for mean root length of weeds were calculated to enable varietal comparisons.

Results and Discussion

Root length of ryegrass

Root growth of ryegrass was significantly influenced by both rice varieties compared to the control ($P < 0.05$). The allelopathic effect increased with increased rice density. The main effects of seedlings densities were significant for all densities up to 20 rice seedlings $beaker^{-1}$ (Figure 1) and thus, it indicated that high density of rice seedlings resulted with accumulation of high amount of allelochemicals in agar medium causing reduced root growth of ryegrass. Our finding is also a supportive to Seal *et al.* (2004) who reported that root growth of arrowhead weed was significantly affected by high rice seedling density. Apart from the density effect of rice seedlings, variety also showed significant result on the said parameter. The most allelopathic cultivar was Quest, which was more effective at suppressing ryegrass root growth than M 205. The root growth reduction of ryegrass was more than 50% at density 5 rice seedling/beaker which when was increased reached maximum at density 15 seedlings/beaker. Although the variety M-206 showed less allelopathic potential but it significantly reduced the root length of ryegrass and the allelopathic pattern was similar with Quest.

Difference in degree of ryegrass root inhibition was more at density 15 and 20 by Quest showing strong allelopathic potentiality towards ryegrass. Whereas M 205 showed a linear form his allelopathic phytotoxicity with increased its density, this findings indicates rice allelopathy phenomenon was probably genetically controlled and each genotype has its own typical density to maximum inhibition of a particular weed species. Seal *et al*., (2004) documented that a particular density of rice produce a typical dose of rice root exudates which was more toxic to weeds. Similar ryegrass root inhibition also claimed by Asaduzzaman *et al.* (2012) in canola density.

Shoot length of ryegrass

Shoot length of ryegrass species was influenced by rice seedlings allelopathy ($P< 0.05$). Increasing rice densities after 10 seedlings/beaker significantly reduced the shoot length of ryegrass (Figure 2). Differences in degree of ryegrass shoot inhibition between the allelopathic and less allelopathic rice varieties were not significant at densities at 5 and 10. However, at such densities, both varieties had significant difference to suppress the shoot length of ryegrass. It suggests that the concentration of allelochemicals of allelopathic variety is less beyond this density and a minimum density is required to produce an allelopathic effect. Olofsdotter *et al.* (2002) noted that any allelochemical compounds couls be toxic if applied at a high dose and acted as a stimulator at low density.

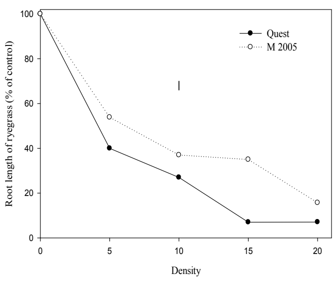	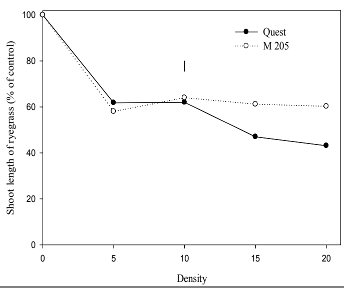	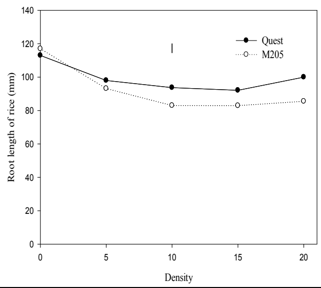
Fig. 1. Effect of rice density dependent allelopathy on root growth of ryegrass (LSD = 3.94, P< 0.05)	**Fig. 2. Effect of rice density dependent allelopathy on shoot growth of ryegrass (LSD = 4.5, P< 0.05)**	**Fig. 3. Effect of rice density dependent auto allelopathy on root growth of rice (LSD =5.4, P< 0.05)**

Root length of rice

To address the autotoxicity of rice, the root growth of both rice cultivars were also observed. Root length of rice was not affected due to co-growth of rice and ryegrass. However, at density 5 seedlings root length of rice was affected (Figure 3), which might be due to that the metabolism of rice root exudates was affected by ryegrass causing root length of rice to be decreased. But increased rice density produced more allelochemicals in medium which was not diluted by ryegrass therefore root length of rice was static at this density. Similarly root length of allelopathic rice genotype was significantly higher than non-allelopathic cultivar. One explanation could be that strong allelopathic rice cultivar (Quest) diluted the ryegrass allelochemicals in agar medium and reduced the seedling growth of ryegrass significantly compared to non-allelopathic cultivars.

Effect on root tissue system of ryegrass

The promotion of cell division as well as dimension of ryegrass root were not affected by rice root exudates, however, an usual root tissue damage was observed in root tissue systems at density 15 and 20 seedlings/beaker (Figure 4). It was also observed that the type and dimension of damage was different at high density. There was a clear hole in root tissue but the size of the damage was small at low density whereas at low density there was an initial and large size of tissue damage as was observed under microscope. It might be due to that high density of rice secreted more and strong allelochemicals that directly damaged and made hole on particular root cell. Similar observation was also reported by Iqbal *et al*. (2012) at laboratory condition where they noted that allelochemicals could influence the cell dimension and wound on root tissues.

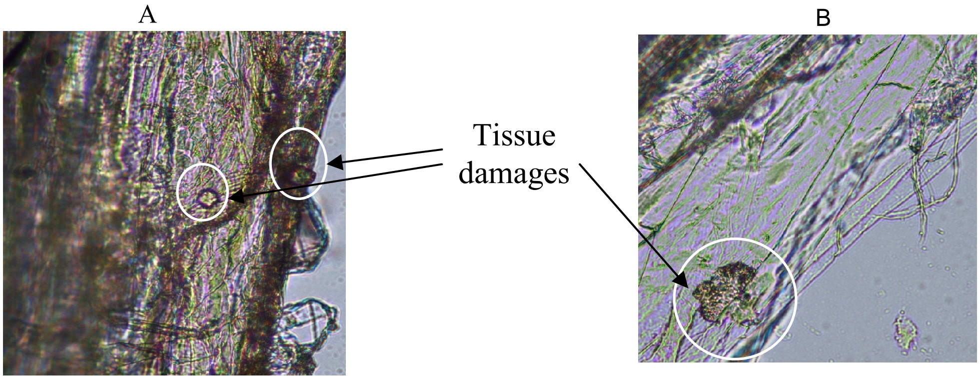

Fig. 4 Effect of rice density dependent allelochemicals on ryegrass root tissue (A = grown with 15 rice seeds/beaker and B = grown with 20 rice seeds/beaker)

These observations support the findings of Seal *et al.* (2004) and An *et al.* (2005). The results show that the inhibitory activity or allelopathy phenomenon of rice is increased with density. More the density, more the allelochemicals were found in agar medium leading to more ryegrass root growth inhibition. Genotypic influence also played a major role in the suppression of ryegrass root and shoot growth. Between two genotypes, Quest had more allelopathic nature than M 205, and increasing seedlings density influenced the root and shoot growth of ryegrass presumably due to increased concentration of root exudates in the agar medium. This suggests that increase in rice competitiveness can be achieved at high density leading to greater suppression of target weed species including ryegrass. So, the root exudates of rice could be used as potential natural herbicides resources but these must be properly identified.

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