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Research Article
Competitive and Allelopathic Effects of Wild Rice Accessions (*Oryza longistaminata*) at Different Growth Stages

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Abstract
The competitive and allelopathic effects of wild rice (*Oryza longistaminata*) accessions on barnyard grass at different growth stages determined by days after sowing (0, 30, 60 and 90 days) were studied in greenhouse pot experiments. Wild rice accession RL159 exhibited the greatest height and tillering. The weed suppression rates of wild rice accessions OL and F_1 on barnyard grass were significantly higher than for other rice accessions, with the lowest being *O. sativa* cultivar RD23. The highest suppression rates of OL and F_1 were 80.23 and 73.96% at barnyard grass growth stages of 90 days and 60 days. At a 90 growth stage, wild rice accessions RL159 and RL169 caused 61.33 and 54.51% inhibition in barnyard grass growth, respectively. Under the same conditions, the competitive inhibition rates of OL, F_1, RL159, RL169 and RL219 against barnyard grass were markedly lower than their weed suppressive effects, but were relatively similar for RD23. The allelopathic inhibition of OL and F_1 on barnyard grass was significantly higher than other rice accessions. The highest allelopathic rates of OL and F_1 were 60.61 and 56.87% at the 0 day growth stage. It is concluded that wild rice accessions OL and F_1 exhibited the highest allelopathic activity along with moderate competitive ability against barnyard grass; wild rice accession RL159 had the highest competitive ability and moderate allelopathic activity on barnyard grass. Thus, the three wild rice accessions OL, F_1 and RL159 could be used as ideal breeding materials for cultivated rice improvement.

Key words: Weed-suppression, competition, allelopathy, *Oryza longistaminata*, barnyard grass, growth stage

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rice (*Oryza sativa*) is one of the most important crops in the world both in terms of acreage planted and human consumption (Evans, 1998). It is a staple food for more than 60% of the world's population and plays a crucial role in the economic and social stability of the world (You, 1986). During rice production, weeds are one of the most important yield-limiting biological constraints worldwide. Weeds usually cause a yield loss of more than 10% of rice production under current cultivation practices, though yield loss might reach 45-96% if no measures were applied for weed control (Kim, 2001). In China, an estimated ten millions tons of rice is lost annually due to weed competition (Evans, 1998).

In order to increase crop productivity and reduce labour requirements, chemical herbicides have been widely applied for weed control in rice fields. However, due to long-term and large scale herbicide applications, many negative effects of herbicides on environments, agricultural ecosystems and human and animal health have been recognized, including soil erosion, biodiversity loss, herbicide contamination of offsite or nontarget areas and herbicide resistant weeds (Kong *et al*., 2006; Duke, 2007; Bastiaans *et al*., 2008). In sustainable farming system, the utilization of rice allelopathy to control paddy field weeds, has been explored by weed researchers since the mid-1980s (Dilday *et al*., 1994; Xuan *et al*., 2005; Khanh *et al*., 2007). Allelopathy is defined as the direct or indirect harmful or beneficial effects of one plant on another, including microorganisms, through the production of chemical compounds that escape from plants into the environment (Rice, 1984). Currently, much attention given to allelopathy in rice has focused on allelopathy screenings, identification of allelochemicals and biochemical mechanisms of allelopathy, including identification, confirmation, bioactivity test and structural analysis of allelopathic products (Guo *et al*., 2005; Kong *et al*., 2007, 2008; Guo and Lu, 2015). Weed suppression by rice is not only determined by allelopathic activity, but also influenced by competitive interactions (Gaudet and Keddy, 1988; Bastiaans *et al*., 1997; Olofsdotter *et al*., 1999). Therefore, screening rice cultivars which suppress weeds through both strong competitive and allelopathic abilities could be a promising strategy for developing sustainable rice production.

Oryza longistaminata A. Chev. and Roehr. is a rhizomatous perennial wild rice species native to Africa. The genome of *O. longistaminata* is type AA, similar to *O. sativa* and is regarded as an excellent gene pool for Asian cultivated rice improvement because of its strong resistance to biotic and abiotic stress. Valuable traits of *O. longistaminata* that could be used for improvement of rice cultivars, include long anther, large biomass, high nitrogen use efficiency (Yang *et al*., 2010; Xu *et al*., 2014), resistance to insect pests and disease (Yang *et al*., 2010), drought tolerance and dual regeneration ability through both seeds and rhizomes (Zhang *et al*., 2004; Xu *et al*., 2014). Besides its strong competitive ability, previous studies in our laboratory demonstrated that *O. longistaminata* exhibited high allelopathic activity on barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv.) (Zhang *et al*., 2008). Further research showed that weed suppressive effects of *O. longistaminata* and its F_1 and F_2 accessions on barnyard grass were related to allelopathy, seedling stage, plant height, tiller number and other environmental factors in the field (Xu *et al*., 2014).

Building on our former studies (Zhang *et al*., 2004, 2008; Xu *et al*., 2010, 2014), the present research examined the competitive and allelopathic effects of wild rice accessions (*O. longistaminata*) on barnyard grass at different growing periods. Results of our study should aid in developing a better understanding of the competitive and allelopathic relationship between *O. longistaminata* and barnyard grass and provide practical recommendations for controlling barnyard grass and other paddy weeds.

MATERIALS AND METHODS

Study species: The RD23, an indica cultivar of *O. sativa* from Thailand and wild rice *O. longisatminata* accession (S37), F_1 accession (*O. longisatminata*×RD23) and F_2 accessions (RL219, RL169 and RL159) were obtained from the Rice Research Institute, Yunnan Academy of Agricultural Sciences (YAAS), Kunming, China. The seeds of barnyard grass were collected from a paddy field in the north suburb of Kunming in 2014 and stored at room temperature. The soil was collected from the paddy field at the Agricultural Environment and Resource Research Institute, YAAS, Kunming, China and its properties were analyzed by the Analysis and Testing Center, Agricultural Environment and Resource Research Institute, YAAS 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 (Min *et al*., 1991). The properties of soil were: pH 6.2, organic content (18.14±0.17) g kg^{-1}, total N (1.63±0.12) g kg^{-1}, total P (0.37±0.08) g kg^{-1} and total K (8.41±0.17) g kg^{-1} (Xu *et al*., 2010).

Experiment design and data collection: The weed-suppression and competition experiments were conducted from May to August, 2015 in a greenhouse at the Agricultural

Environment and Resource Research Institute, YAAS. For the weed-suppression test, 2-leaf-stage seedlings of rice accessions were prepared and transplanted into a plastic box (55×15×15 cm) with paddy soil on 1 May, 2015. Each box contained 2 rows of rice plants spaced 9 cm apart and 10 rice seedlings were planted per row, i.e., plants were spaced 4.5 cm apart. After transplanting, on 1 May (0 days), 1 June (30 days), 1 July (60 days) and 1 August (90 days), barnyard grass seeds were sown in 2 rows between the rice rows and 30 seeds were sown per row, i.e., barnyard grass rows were 3 cm apart only, as the rice rows were 9 cm apart. Barnyard grass seeds were placed on soil surface and covered with 2 mm depth of soil after the soil was moistened. A control treatment without rice was included. The plant height and tillering number of six rice accessions were measured at same time. All the plastic boxes were kept in the greenhouse (25-28°C with a 12/12h (day/light) photoperiod). All plots were arranged in randomized block design with 4 replications. Fifteen days after sowing, height and fresh weight of each barnyard grass aboveground were measured. During experiments, the plots were often weeded and no synthetic fertilizers were used.

Simultaneously, the competitive effects between rice accessions and barnyard grass were tested in the same greenhouse. In this test, all treatments were the same as per the weed-suppression test but rice seedlings were transplanted into paddy soil though the holes in plastic films placed on the surface of paddy plastic boxes. Paddy soil was added on the plastic films after transplanting and barnyard grass seeds were sown over the plastic film. All rice accessions were transplanted on 1 May and barnyard grass seeds were sown on 1 May (0 days), 1 June (30 days), 1 July (60 days) and 1 August (90 days), i.e., the same methodology used in the weed-suppression test. A control treatment without rice was included. In this situation, the roots of barnyard grass and rice were separated by plastic films, so that rice root exudates did not affect barnyard grass growth. Water was supplied separately by drip irrigation to the two soil layers. Treatments were replicated four times for each growth period. Fifteen days after sowing, height and fresh weight of barnyard grass aboveground were measured. During experiments, the plots were weeded frequently and no synthetic fertilizers were used.

Data analyses: For weed-suppression experiments, the weed suppression was determined from fresh weight of barnyard grass comparing control versus treatment according to the formula: (1-treatment/control)×100%. For competition experiments, the competitive inhibition was determined from fresh weight of barnyard grass comparing control versus treatment according to the formula: (1-treatment/control)×100%. Finally, the allelopathic inhibition was determined according to the formula: the weed suppression-the competitive inhibition. Positive and negative values of inhibition rate indicate suppression and promotion of allelopathy in rice, respectively and absolute values of the inhibition rate indicate the degree of suppression or promotion.

Data was analyzed by analysis of variance (one-way ANOVA). If significant differences were detected by ANOVA, Duncan's multiple range tests were used to detect differences among treatments at a 5% level of significance.

RESULTS

Plant growth of rice accessions: At the 0 day growth stage, plant height of five wild rice accessions (OL, F_1, RL219, RL169 and RL159) was significantly higher than cultivated rice RD23 ($p<0.05$) and there was no tillering for all rice accessions (Table 1). The plant height of six rice accessions was increased significantly with succeeding growth stages from 0-90 days and the most rapid growth progression was between 30 and 60 days. From 30-90 days, the plant height of RL159 was the highest, followed by RL159, F_1 and OL and the lowest heights

Table 1: Plant height and tillering number of the test six rice materials under different growth stages

Variables	Days	Rice accessions					
		OL	F_1	RL219	RL169	RL159	RD23
Plant height (cm)	0	23.66 ± 0.41^a	23.59 ± 0.46^a	23.64 ± 0.35^a	23.55 ± 0.43^a	23.50 ± 0.27^a	16.10 ± 0.09^b
	30	32.62 ± 0.76^c	33.02 ± 0.23^c	26.83 ± 0.68^d	33.18 ± 0.43^b	35.93 ± 0.72^a	25.45 ± 0.61^e
	60	43.94 ± 0.97^c	46.39 ± 0.86^b	38.43 ± 0.50^d	46.93 ± 0.25^b	52.91 ± 1.52^a	36.18 ± 0.67^e
	90	50.93 ± 0.20^d	54.17 ± 1.03^c	48.41 ± 0.51^e	56.91 ± 0.20^b	62.92 ± 1.50^a	44.43 ± 0.89^f
Tillering number	0	-	-	-	-	-	-
	30	1.81 ± 0.05^b	1.86 ± 0.06^b	1.31 ± 0.05^d	1.72 ± 0.04^c	1.95 ± 0.03^a	1.39 ± 0.08^d
	60	2.16 ± 0.08^{bc}	2.30 ± 0.15^{ab}	1.48 ± 0.06^d	2.05 ± 0.08^c	2.34 ± 0.18^a	1.42 ± 0.06^d
	90	2.25 ± 0.09^c	2.44 ± 0.17^b	1.55 ± 0.08^d	2.17 ± 0.08^c	2.73 ± 0.12^a	1.47 ± 0.08^d

Data are expressed as mean±standard deviation, the different letters within same row signify significant differences at $p<0.05$

were seen in RL219 and RD23. The tillering number for all rice accessions was significantly increased from 0-30 days and then continued to increase gradually after 30 days. The tillering number of RL159 was the highest, the next highest were RL159, F_1 and OL and the lowest were RL219 and RD23 within the 30-90 days stretch. Thus the data showed that RL159 had more vigorous growth and tillering ability than the other five rice accessions and RL219 and RD23 had the lowest levels of growth and tillering ability.

Plant biomass of barnyard grass: The biomass of barnyard grass in the control experiments was significantly higher than in both weed-suppression and competition experiments (Table 2). For weed-suppression experiments, the biomass of barnyard grass for rice accessions RL219 and RD23 was the highest, followed by RL159 and RL169 and the lowest was F_1 and OL from 0-90 days. In the competition experiments, biomass of barnyard grass only varied slightly among the six rice accession from 30-90 days. For both weed-suppression and competition experiments, the biomass of barnyard grass among six rice accessions at the 0 day growth stage was the greatest, followed by that at 30 and 90 days, with the lowest at 60 days, indicating that barnyard grass grew more rapidly at the early and late experimental stages.

Competition and allelopathy of rice accessions on barnyard grass: The weed suppression rates of OL and F_1 on barnyard grass were significantly higher than other rice accessions, followed by RL159, RL169 and RL219 and the lowest was RD23 (Fig. 1). The highest suppression rates were for OL and F_1. In the case of OL is was 80.23% at 90 days while the highest rate for F_1 was 73.96% at 60 days, the lowest suppression rates seen for OL and F_1 were still 62.85 and 52.24%, respectively, at a 30 day growth stage. From 0-90 days, rates of inhibition of barnyard grass by RL159, RL169, RL219 and RD23 increased with growth stage. At the 90 day growth stage, rice accessions RL159 and RL169 caused 61.33 and 54.51% inhibition in barnyard grass growth, respectively, which was higher than that of rice accession F_1 (52.24%) at the 30 day growth stage (Fig. 1).

The competitive ability of six rice accessions increased with growth stage (Fig. 2). Under the same conditions, the competitive inhibition rates of OL, F_1, RL159, RL169 and RL219 against barnyard grass were clearly lower than their weed

Table 2: Biomass of barnyard grass for six rice accessions under different growth stages

		Biomass of barnyard grass (g)						
Variables	Days	OL	F1	RL219	RL169	RL159	RD23	Control
a	0	0.042 ± 0.001^{g}	0.049 ± 0.001^{f}	0.109 ± 0.001^{c}	0.096 ± 0.001^{d}	0.094 ± 0.001^{e}	0.130 ± 0.001^{b}	0.142 ± 0.001^{a}
	30	0.036 ± 0.001^{g}	0.046 ± 0.001^{f}	0.070 ± 0.001^{c}	0.058 ± 0.002^{d}	0.055 ± 0.001^{e}	0.085 ± 0.002^{b}	0.096 ± 0.003^{a}
	60	0.017 ± 0.000^{f}	0.017 ± 0.001^{f}	0.046 ± 0.001^{c}	0.036 ± 0.000^{d}	0.033 ± 0.001^{e}	0.056 ± 0.001^{b}	0.066 ± 0.001^{a}
	90	0.019 ± 0.000^{g}	0.027 ± 0.001^{f}	0.063 ± 0.002^{c}	0.043 ± 0.001^{d}	0.036 ± 0.001^{e}	0.078 ± 0.001^{b}	0.094 ± 0.001^{a}
b	0	0.068 ± 0.001^{d}	0.069 ± 0.001^{c}	0.069 ± 0.001^{c}	0.068 ± 0.001^{d}	0.066 ± 0.000^{e}	0.070 ± 0.001^{b}	0.075 ± 0.001^{a}
	30	0.044 ± 0.001^{d}	0.045 ± 0.001^{bcd}	0.047 ± 0.001^{bc}	0.047 ± 0.002^{bc}	0.045 ± 0.001^{cd}	0.048 ± 0.001^{b}	0.053 ± 0.002^{a}
	60	0.037 ± 0.000^{d}	0.039 ± 0.001^{c}	0.039 ± 0.001^{c}	0.039 ± 0.000^{c}	0.037 ± 0.001^{d}	0.042 ± 0.001^{b}	0.047 ± 0.001^{a}
	90	0.039 ± 0.000^{f}	0.043 ± 0.001^{d}	0.045 ± 0.001^{c}	0.041 ± 0.001^{e}	0.040 ± 0.001^{ef}	0.047 ± 0.001^{b}	0.057 ± 0.001^{a}

Data are expressed as mean±standard deviation, the different letters within same row mean significant differences at $p<0.05$, a and b signify the weed-suppression test and the competition test, respectively

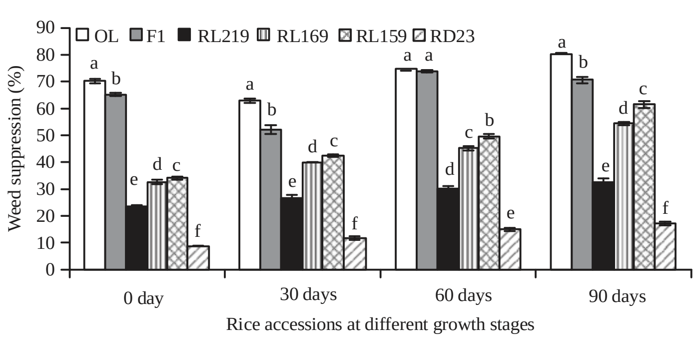

Fig. 1: Weed suppression of rice accessions on barnyard grass at different growth stages. Weed suppression was determined from fresh weight of barnyard grass comparing control versus treatment according to the formula: (1-treatment/control)×100%

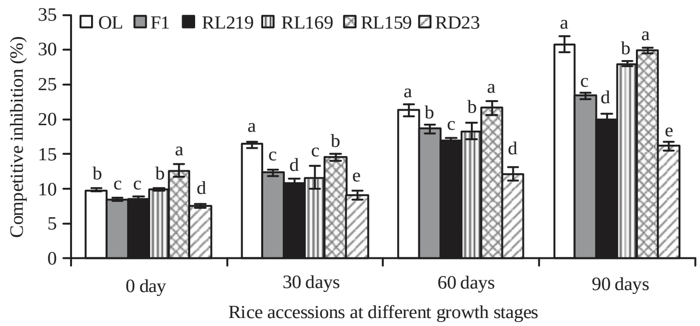

Fig. 2: Competitive inhibition of rice accessions on barnyard grass at different growth stages. Competitive inhibition was determined from fresh weight of barnyard grass comparing control versus treatment according to the formula: (1-treatment/control)×100%

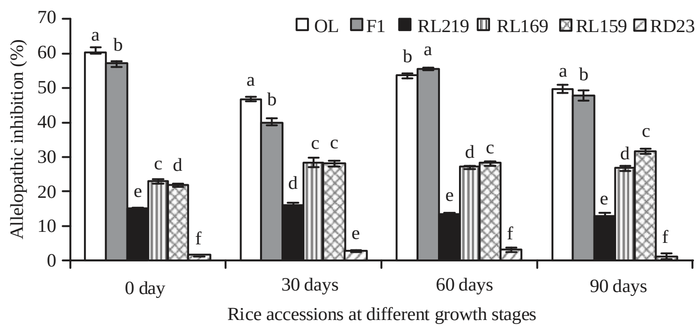

Fig. 3: Allelopathic inhibition of rice accessions on barnyard grass at different growth stages. Allelopathic inhibition was determined according to the formula: the weed suppression the-competitive inhibition

suppressive effects, but there was little difference seen between suppression and competition treatments for RD23. The competitive inhibition of OL and RL159 was significantly higher than other rice accessions but there was no difference between the two groups. Rice accessions RL219 and RD23 had lower competitive inhibition with comparing to other rice accessions from 0-90 days. The results collectively indicate that RL159 and OL have stronger competitive ability and that the competitive ability of RD23 is very low.

The allelopathic inhibition rates of OL and F_1 on barnyard grass were significantly higher than other rice accessions, followed by RL159, RL169 and RL219 and finally the lowest was RD23 (Fig. 3). The highest allelopathic rates were for OL and F_1 at 60.61 and 56.87% for the 0 day growth stage and the lowest allelopathic rates these accessions (OL and F_1) were 46.52 and 39.87% at growth stage of 60 days, respectively. The allelopathic inhibition rates of RL159 and RL169 were all over 20% from 0-90 days.

DISCUSSION

Weed suppression of rice is a complex chemical and ecological phenomenon, which is often influenced by rice/weed competition, allelopathy, their interaction and/or environmental conditions (Olofsdotter *et al*., 1999; Gealya *et al*., 2003; Hu *et al*., 2003; Kong *et al*., 2006). Xu *et al*. (2014) found that weed suppression effects of OL and its F_1 and F_2 accessions on barnyard grass are dependent on allelopathy, seedling stage, plant height, tiller number and other environmental factors of the rice field. The present study found that wild rice accessions OL and F_1 had the highest weed suppressed barnyard grass the most and that RL159 and RL169 also had a fairly strong inhibitory impact on barnyard grass. Thus these four rice accessions have higher competitive ability and stronger allelopathic activity than the others.

Research on the competition of rice with weeds have suggested that competitive ability seems to be correlated with rice agronomic features (Bastiaans *et al*., 1997; Xu *et al*., 2013). Competitive ability of rice is conferred by a combination of morphological traits that allow the rice to access more limited resources than neighboring weeds. Competition is based on the ability of a crop cultivar to access scarce light, nutrients and water resources in a limited space, thus suppressing the growth and reproduction of nearby weed species. Generally, rice plants with greater height and larger crowns cause more suppression of the weeds (Bastiaans *et al*., 1997; Xu *et al*., 2002). The study found that the competitive ability of six rice accessions increased with growth stage, with the competitive inhibition rates of OL and RL159 on barnyard grass significantly higher than other rice accessions, indicating that RL159 and OL have stronger competitive ability. Among six rice accessions, RL159 and OL had the greatest plant height and tillering number, thus they are able to compete more effectively for sunlight and soil nutrients in comparison to the other rice accessions tested.

In contrast to competitive effects, rice allelopathy has generally been only weakly correlated with agronomic features and often observed during the germination and seedling growth but not at later stages (Dilday *et al*., 1994; Olofsdotter *et al*., 2002; Hu *et al*., 2003; Zhou *et al*., 2005; Kong *et al*., 2007, 2008). Moreover, allelopathy in rice tends to be unstable and the quantity of rice allelochemicals synthesized and released generally varies with growth stages and environmental conditions. Zhang *et al*. (2008) reported that wild rice accessions OL and F_1 exhibited their strongest allelopathic activity at the 2 leaf-stage. In the current study we found that the allelopathic activity of wild rice accessions OL and F_1 on barnyard grass was stronger at 0 and 60 day growth stages than other growth stages.

A timeline of the interaction between wild rice and these cultivars emerges from the experiments we conducted. While just after transplanting, growth of wild rice accessions OL and F_1 is relatively slow as these cultivars become established in the new environment, primarily depending on allelopathy to suppress barnyard grass. From 30-60 days, wild rice accessions OL and F_1 grow very rapidly provided there is enough soil nutrient availability and thus they suppress barnyard grass via their higher competitive ability. After 60 days, because of scarce soil nutrients and reduction of living space, wild rice accessions OL and F_1 once more depend on allelopathy to suppress barnyard grass.

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