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Factors Promote Germination and Initial Growth of *Monochoria vaginalis*

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Abstract. The purpose of study was conducted to examine factors that influence on germination of *Monochoria vaginalis*, a major and problematic weed in paddy fields. It was found that seed coats of barnyardgrass and *Monochoria* itself strongly promoted germination of *Monochoria* seeds. Germinating rice seeds caused germination induction of *Monochoria*, but dehulled rice and rice hulls did not play a role. In general, rice root exudates from 2 to 22 day after germination did not exert significant influence on germination and initial growth of *Monochoria*. The increase of rice seeds showed no remarkable impact on germination, root length and shoot length of *Monochoria*. In conclusion, it is suggested that coats of weed seeds, residues of rice and other plants in paddy fields may play a critical role in promoting germination of *Monochoria* seeds. Therefore, the incorporation of these residues in paddy fields may provide negative impact to weed control as germination of *Monochoria* is much accelerated.

Introduction

Monochoria vaginalis is one of the most troublesome weeds in paddy fields [1] and was reported to reduce rice grain yield [2,3]. *Monochoria* seeds were reported to germinate well in light, flooded, and low oxygen conditions (<1%) [4], and CO2 was proposed to influence on germination of the seeds [5-7]. However, in the darkness, germination of submerged *Monochoria* seeds was accelerated by the presence of seeds, seed hulls and shoots of rice [8,9]. Therefore, to understand mechanism of germination in darkness of *M. vaginalis* will provide helpful information to manage this noxious weed.

Yokota et al. [10] reported that rice hull extracts accelerate the propagation of bacteria such as *Bacillus* sp., *Pedobacter* sp., *Pantoea* sp. *and Sphingomonas* sp., and they digested seed coats of *M. vaginalis*, therefore facilitated the weed germination. The role of possible germination stimulators from rice seeds in darkness was eliminated [10]. To understand the factors involved in germination of *Monochoria* seeds other than rice, such as barnyardgrass, is also a major problematic weeds in paddy fields is indispensable. The interaction between barnyardgrass and rice has been known to include both competition and allelopathy [11]. Many allelochemicals have been detected in barnyardgrass included phenolics, long-chain fatty acids, and lactones which inhibited growth of rice [11]. Other putative allelochemicals consisted of *p*-hydroxymandelic acid [12], *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, and hydroxyl benzene [13]. Plant growth inhibitors from barnyardgrass were found to inhibit growth of *M. vaginalis* [11]. However, to date, other interactions between the two weeds have not yet been reported.

In this study, it was examined that whether the submerged rice seeds were the principal factor to promote germination of *Monochoria*. Effects from barnyardgrass was evaluated as it is a major weed in paddy fields, and competed with rice and *Monochoria* in both allelopathy and competition [14]. Influences from dehulled rice and hull extracts and root exudates of rice, and seed coats of barnyardgrass and *Monochoria* itself on germination of *Monochoria* seeds were critically examined.

Materials and methods

Plants

Rice seeds (*Oryza sativa* var. *Koshihikari*) were received from Plant Breeding Laboratory, Faculty of Agriculture, University of the Ryukyus, Japan. Seeds of barnyardgrass (*Echinochloa crus-galli* [L.] Beauv.) and *Monochoria* (*Monochoria vaginalis* Presl var. *plataginea* Solms-Laub.) were collected from the experimental farm affiliated to University of the Ryukyus. The empty and undeveloped seeds of the weeds were discarded by floating them in tap water. The remaining seeds were air-dried and stored hermetically and -20^{o}C for breaking dormancy. The seeds of rice, barnyardgrass and *Monochoria* were sterilized with 1% sodium hypochlorite for 30 min and were rinsed many times with distilled water before use. In the germination tests, the germination percentage of the weed and rice seeds was randomly checked and shown to be >95%. Seeds coats of barnyardgrass and *Monochoria*, and rice hulls, and dehulled rice were carefully removed from rice seeds and stored at 5^{o}C until used. After germination, the seedlings of *Monochoria*, barnyardgrass and rice were placed in a growth chamber (25^{o}C, 4000 lux, with an 8h/day/16h night cycle, and humidity of 75 days) until measurements were conducted.

Effects of rice density on germination and initial growth of barnyardgrass and *Monochoria*

Rice at densities of 3, 5, 7, 10, 15, and 20 seeds were incubated with 10 seeds of each barnyardgrass at 27^{o}C in the dark in a 9 cm diameter Petri dish, added with 10 ml of distilled water, and 10 seeds of each barnyardgrass and *Monochoria*. After 4 days, the dishes were transferred to the growth chamber and measurements including germination, length of roots and shoots of the weeds and rice were conducted after 7 days.

Effects of exuding duration of rice seeds on germination and growth of *Monochoria*

An amount of 100 rice seeds was immersed in 100 ml distilled water in a glass beaker for 2, 4, 6, 8, 10, 12, 14, 16, 20, and 22 days in the growth chamber. The exudates were filtered by filter papers and an aliquot of 10 ml of the extracts was put into a 9 cm diameter Petri dish with 10 seeds of *Monochoria*. The dishes were incubated in darkness at 27^{o}C for 4 days and they were afterward transferred to the growth chamber. Germination, length of roots and shoots of *Monochoria* were calculated after 7 days.

Effects of aqueous extracts of dehulled rice and rice hulls on germination and growth of barnyardgrass and *Monochoria*

Five hundred g rice seeds were used. The hulls and the respective dehulled rice were extracted with distilled water (temperature of 30^{o}C) and shaking for a week at 60 rpm of an automatic shaker. The extracts were concentrated to dryness and dissolved in distilled water to give a 1000 ppm solution, and pH was adjusted to 7.0. The effects of the aqueous extracts on germination and growth of barnyardgrass and *Monochoria* were examined by a similar protocol described in the experiments 1 and 2 described above. Treatments with distilled water were as controls.

Effects of seed coats of barnyardgrass and *Monochoria* on germination and growth of *Monochoria*

An amount of 0.5 g of sterilized seed coats of each barnyardgrass and *Monochoria*, and rice hulls were immersed with distilled water and afterward placed in a 9 cm diameter Petri dish, filled with 10 ml of distilled water, and sown with 10 seeds of *Monochoria*. The dishes were incubated in the dark at 27°C for 4 days and they were then transferred to the growth chambers. Measurements including germination, length of roots and shoots were conducted after 7 days. Treatment with germinating rice seeds was used as control.

Statistical analysis

All trials of this experiment were conducted in a completely randomized block design with 3 replications. Data were analyzed using Minitab software version 16.0. Differences between means were declared significant at $p<0.05$ using Tukey's procedure for multiple comparisons.

Results

Effects of rice density on germination and initial growth of barnyardgrass and *Monochoria*

In the densities of rice seeds from 7 to 20 seeds, germination rates of barnyardgrass were always greater than *Monochoria*, however at 3-5 seeds, germination of *Monochoria* was negligibly higher than barnyardgrass (Table 1). The rates of germination reduction of both barnyardgrass and *Monochoria* were proportional to the seed densities. At the maximum density of 20 seeds, germination of barnyardgrass was 83.0% whereas that of *Monochoria* was reduced to 37.0%. However, statistically, the germination rates of both barnyardgrass and *Monochoria* were not significantly different, indicating that the increase of rice seeds did not influence remarkably germination of the two weeds.

Length of shoots and roots of the two weeds were not also markedly different among rice seed densities, with exception of the densities from 15-20 seeds that significantly reduce root length of barnyardgrass as compared to other treatments (Table 1). In addition, rice growth was not also significantly different among trials, with exception of the lowest density of 3 seeds which was remarkably reduced as compared with that of other densities.

Effects of exuding duration of rice seeds on germination and growth of *Monochoria*

Germination rates of *Monochoria* was the lowest when rice seeds were immersed for 2-4 days, increased from 6-14 days, but they were reduced from 16-22 days. However, the rates among treatments were negligible and insignificantly different (Table 2). The initial growth of shoots and roots of the weed received similar results, with exception of the exudate at 6 days, which significantly reduced the shoot and root elongation of the weed, however the germination was not influenced (Table 2). Results of this experiment revealed that duration of immersing rice seeds from 2-22 days did not markedly influence germination of *Monochoria*.

Table 1. Effects of rice seed densities on germination and growth of (A): barnyardgrass and (B): *Monochoria*

Number of rice seeds	Banyardgrass			Rice	
	Germination (%)	Root length (mm)	Shoot length (mm)	Root length (mm)	Shoot length (mm)
3	80.0±12.0a	50.4±3.3a	72.7±11.9a	40.6±14.0a	38.3±4.3b
5	80.0±10.0a	35.7±6.7ab	77.1±6.8a	50.1±15.1a	42.9±2.7ab
7	97.0±3.0a	39.7±1.7ab	92.1±7.9a	55.0±3.5a	47.5±0.7ab
10	97.0±3.0a	36.6±1.4ab	69.2±1.1a	50.7±2.8a	50.7±1.9a
15	87.0±9.0a	28.1±1.9b	66.1±4.0a	47.9±2.2a	51.3±1.8a
20	83.0±0.9a	33.0±3.0b	74.9±2.0a	52.3±5.4a	50.0±1.6a

A

Number of rice seeds	*Monochoria*			Rice	
	Germination (%)	Root length (mm)	Shoot length (mm)	Root length (mm)	Shoot length (mm)
3	83.0±9.0a	5.2±1.9a	5.3±0.8a	78.6±14.8a	49.3±4.1a
5	87.0±9.0a	3.4±1.7a	5.3±0.5a	90.0±12.7a	51.4±3.7a
7	87.0±9.0a	0.9±0.1a	6.4±0.1a	86.7±11.1a	55.3±2.1a
10	77.0±19.0a	1.1±0.4a	5.7±0.3a	67.5±11.9a	51.5±1.6a
15	60.0±10.0a	0.9±0.4a	5.3±0.1a	70.6±12.4a	50.1±2.7a
20	37.0±7.0a	0.4±0.1a	5.5±0.1a	66.3±14.3a	46.1±2.6a

B

Values are means ± SE (standard errors)
Columns with similar letters are not significantly different (p<0.05)

Effects of aqueous extracts of dehulled rice and rice hulls on germination and growth of barnyardgrass and *Monochoria*

It is found that aqueous extracts at concentration of 1000 ppm of dehulled rice and rice hulls, as well as distilled water in darkness did not induce any germination as well as initial growth of *Monochoria*. In contrast, germination of barnyardgrass was not influenced by any treatment as compared to that of the control (distilled water). However, shoot length of barnyardgrass at both dehulled rice and rice hulls were reduced, whereas their root elongation was significantly promoted (Table 3).

Table 2. Effects of root exudates of rice seeds on germination and growth of *Monochoria*

Exudates of rice seeds (d)	Germination (%)	Root length (mm)	Shoot length (mm)
2	77.0±19.0a	3.1±1.8ab	9.9±1.6a
4	77.0±19.0a	1.2±0.6ab	7.4±1.8ab
6	90.0±6.0a	0.1±0.1b	3.8±0.5b
8	97.0±3.0a	3.1±0.7ab	8.5±0.8ab
10	83.0±3.0a	3.6±0.5ab	7.0±0.3ab
12	97.0±3.0a	3.9±0.2ab	7.5±0.6ab
14	97.0±3.0a	4.1±0.4ab	7.3±0.4ab
16	83.0±1.2a	4.9±0.4a	7.5±0.7ab
20	87.0±9.0a	4.1±0.7ab	6.5±1.1ab
22	80.0±6.0a	3.9±1.2ab	6.9±1.2ab

Values are means ± SE (standard errors)
Columns with similar letters are not significantly different (p<0.05)

Table 3. Effects of extracts of dehulled rice and rice hulls on germination and growth of barnyardgrass and *Monochoria*

Extracted materials	Germination (%)	Root length (mm)	Shoot length (mm)
	Barnyardgrass		
Distilled water	100.0±0.0a	14.3±0.6b	52.5±1.6a
Dehulled rice	100.0±0.0a	22.5±2.4a	50.3±1.1a
Rice husks	100.0±0.0a	25.6±5.5a	42.0±2.1b
	Monochoria		
Distilled water	0.0±0.0b	0.0±0.0d	0.0±0.0d
Dehulled rice	0.0±0.0b	0.0±0.0d	0.0±0.0d
Rice hulls	0.0±0.0b	0.0±0.0d	0.0±0.0d

Values are means ± SE (standard errors)
Columns with similar letters are not significantly different (p<0.05)

Effects of seed coats of barnyardgrass and *Monochoria* on germination and growth of *Monochoria*

In this experiment, germinating rice seeds were used as the control, which shown 97% of germination, whereas treatments with seed coats of barnyardgrass and *Monochoria* itself provided 100% germination of *Monochoria* (Table 4). Seed coats of *Monochoria* recorded significantly higher elongation of roots and shoots of *Monochoria*, followed by germinating rice seeds and coats of barnyardgrass.

Table 4. Effects of seed coats of barnyardgrass and *Monochoria* and rice seeds on germination and growth of *Monochoria*

Materials	Germination (%)	Root length (mm)	Shoot length (mm)
Seed coats of barnyardgrass	100.0±0.0a	1.6±0.1b	4.9±0.2b
Seed coats of *Monochoria*	100.0±0.0a	2.3±0.3a	5.3±0.1a
Rice seeds	97.0±6.0a	1.9±0.1c	4.5±0.1c

Values are means ± SE (standard errors)
Columns with similar letters are not significantly different (p<0.05)

Discussion

The extracts of rice hulls have been reported to stimulate germination of *Monochoria* in both light [14,15] and darkness but with unsterilized rice hulls [10]. However, findings of this study, in contrast, showed that aqueous extracts of rice hulls did not provide induction of *Monochoria* germination. In this study, the concentration was 1000 ppm using only distilled water, but in the previous researches, the extracts from rice hulls were the combination of both distilled water and methanol [10] and ethyl acetate [15], which may contribute to the different results obtained from this study. However, Yokota et al. [10] reported that in darkness, sterilized rice hulls gave no induction of *Monochoria* germination, and explained that bacteria such as *Bacilus* sp., *Pedobacter* sp., and *Sphingomonas* sp. were responsible for the germination stimulation of the weeds, by digesting the seed coat of *Monochoria*. In contrast, the rice hulls as well as the dehulled rice used in this study were not sterilized, but no germination of *Monochoria* was found. Therefore, this research excluded the possible role of rice hulls and dehulled rice, and indicated that the germinating rice seeds play a critical role in promoting germination of *Monochoria*. Takeuchi et al. [16] proved that germination of *Monochoria* required light and flooded condition, but suggested that ethylene stimulated seed germination only in the light by increasing the embryo growth potential which is then high enough to overcome the mechanical restriction of the seed coat. The use of rice exudates from germinating rice at 2-20 seed density showed germination promotion, however, it was not examined whether ethylene or ethephon, an ethylene-releasing substance [17], was whether contained in the exudates.

This study was the first to find that seed coats of barnyardgrass and *Monochoria* itself also strongly induce germination of *Monochoria*. To date, the extracts of rice plants at early growing stages (10, 20, 40, and 120 days) were also reported to stimulate germination of *Monochoria*, but materials derived from plants other than rice involved in the germination promotion of the weed, have not been found. In our previous research, barnyardgrass obtain a number of plant growth inhibitors such as phenolics, lactones, long-chain fatty acids, and steroids which inhibited growth of *Monochoria* [11,18], however the germinated *Monochoria* seedlings were used, therefore the effects of germinating barnyardgrass seeds on germination of *Monochoria* were not examined. Rice allelopathy was also reported to obtain many allelochemicals to inhibit emergence of barnyardgrass and *Monochoria* [14]. In this study, elongations of roots and shoots of *Monochoria* and barnyardgrass were markedly reduced as compared to that of *Monochoria* seed coats, suggesting that possible allelochemicals from barnyardgrass and rice may be responsible for the inhibition.

Ethylene or its derivative ethephon released from germinating rice seeds and immersed seed coats of barnyardgrass and *Monochoria* may play a critical role in inducing germination of *Monochoria*, however experiments for germination were conducted in darkness, therefore it needs to elaborate whether it is true that ethylene is inactive to germination of *Monochoria*, as suggested by Takeuchi et al. [16]. Bacteria may also available in the seed coats of barnyardgrass and *Monochoria* and may play a role in the germination promotion of the weed as reported in Yokota et al. (2014) [10].

Conclusions

This study observed that, in addition to germinating rice seeds, coats of barnyardgrass and *Monochoria* seeds also promoted germination of *Monochoria* seed itself. The residue of other plant residues in paddy fields may also provide positive impacts on germination of the harmful seeds. Therefore, management of rice and other plant residues including weeds in paddy fields may be effective on weed control of Monochoria. Beside ethylene, the involvement of chemicals as germinating promoters in rice exudates and seed coats of barnyardgrass and *Monochoria* should also be conducted to further understand the actual germination promotion of the weed. The incorporation of crop residues including rice was suggested to reduce paddy weed emergence [19,14], but findings of this study alert that the use of residues of rice and paddy weeds to incorporate to paddy soils may also provide negative impacts to weed control by accelerating germination of *Monochoria*. The clarification of actual mechanism to induce germination promotion of *Monochoria* is helpful to control the emergence of this noxious weed in rice production.

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