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Hydropriming Treatment of Rice Seeds With Microbubble Water

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Abstract

In rice cultivation, seed emergence and seedling establishment tend to be unstable, and rice plants are likely to lodge during the ripening period in direct seeding, leading to an unsteady yield. Although the possibility of direct seeding in dry paddy fields is being re-examined from the viewpoint of reducing labor, unstable seed emergence and seedling establishment remain as challenges to be dealt with. Therefore, in order to improve unstable seed emergence and seedling establishment, we investigated the effects of hydropriming treatment of rice seeds with microbubble (MB)-water which have effect on promoting plant growth, on emergence and early growth of seedlings. In soil with 50% moisture content, the emergence rate, seedling height, longest root length, aboveground dry weight, underground dry weight, chlorophyll content, and α-amylase activity in seeds primed with MB-water were remarkably higher than those in seeds primed with dechlorinated-water and non-primed seeds. However, no significant differences were observed among the seeds primed the same way in soil with 25% moisture content. These results demonstrate that the hydropriming treatment of rice seeds with MB-water promotes their emergence in soil with 50% moisture content. In near future, we need to investigate seedling emergence of other cultivars hydropriming treatment with MB-water.

Keywords: emergence, hydropriming, microbubble, rice, priming

1. Introduction

In Japan, almost all rice farmers first sow rice seeds in seedling trays so that the seedlings can emerge and grow before they are transplanted in paddy fields. In rice cultivation, sowing rice seeds and planting the seedlings in the paddy fields account for about a quarter of the labor necessary for cultivating rice. Therefore, the introduction of direct seeding of rice in paddy fields is expected to reduce both labor and costs (Furuhata, 2009). However, direct seeding is not popular despite these benefits. Seed emergence and seedling establishment tend to be unstable, and rice plants are likely to lodge during the ripening period in direct seeding, leading to an unsteady yield. Although the possibility of direct seeding in dry paddy fields is being re-examined from the viewpoint of reducing labor, unstable seed emergence and seedling establishment remain as challenges to be dealt with (Ando & Kobata, 2002).

Seed priming is a technique to promote uniform germination by increasing germination vigor through forced water absorption and drying of seeds. Priming treatment is performed with water-based hydropriming; osmopriming based on high molecular compounds or inorganic salt solution; and matripriming, in which water is supplied via vermiculite and other materials (Horita & Saruyama, 2006; Farooq et al., 2011). Hydropriming treatment is easy, economical, and environmentally friendly because only water is used. Research on priming treatment with various seeds, including rice seeds, has been reported (Masuda et al., 2005; Horita & Saruyama, 2006). Ajirloo et al. (2013) evaluated the effects of different seed priming techniques, un-soaked seed (control), hydropriming (soaked with distill water), halopriming with KNO_3 and $CaCl_2$ (1% solution), on seed emergence and seedling growth of three maize varieties (SC-301, SC-604 and SC-711), and clarified that the response of varieties to different priming techniques approximately was similar and hydropriming gave significantly higher number of leaves compared to other treatments. Hamidreza et al. (2013) conducted to evaluate the effects of different time seed priming (osmo-hydropriming) on germination and seedling growth of *Secale montanume* seeds under drought conditions, and showed that the least priming time (6 hr) had more effects on germination percentage and the highest root/shoot length ratio was related to osmopriming with -0.5 MPa. Goswami et al.

(2013) have studied the effects of hydropriming, dehydration priming (induced by PEG), and osmopriming (induced by NaCl and KH_2PO_4) on subsequent germination of rice seed (*Oryza sativa* 'Kshitish'), and showed that germination percentage of hydropriming in water was 92% and the highest of other treatments. Dey et al. (2013) have also showed that the lowest mean germination time was observed from hydropriming of rice seeds ('BRRI dhan29') with 30 hours soaking at 35°C.

Thornton and Powell (1992) have reported that germination of Brassica oleracea seeds was improved when aerated water was used in hydropriming treatment. Recently, researchers are interested in using microbubbles (MBs) in the agricultural field since these ultrafine bubbles, which are less than 50 µm in diameter, have some unique characteristics (Li et al., 2009a, 2009b; Takahashi et al., 2003; Takahashi, 2005). Promoting the growth of lettuce with air-MBs in hydroponics (Park & Kurata, 2009) have been reported as applications of MBs in agriculture. Thus, MB treatment for hydropriming of seed may promote seed emergence and early growth of seedling.

This study was performed to investigate the effect of hydropriming treatment of rice seeds with MB-water on the emergence and early growth of seedlings by focusing on the high osmosis of MBs.

2. Method

2.1 Hydropriming Treatment

In this study, dechlorinated-water was used not only because such water more practical than deionized or distilled water in actual applications but also because the effect of chlorine in tap water must be eliminated. After tap water was aerated for 24 h at 25°C, the presence or absence of chlorine in the water was confirmed using a chlorine comparator (Photometer CL, OYWT-31, Oyalox Co., Ltd., Tokyo, Japan) (Ikeura et al., 2011). In the hydropriming treatment with dechlorinated-water, 50 rice seeds of the "Koshihikari" variety were immersed in 30 L of dechlorinated-water in a plastic container at 25°C for 12 h (dechlorinated-water seeds). 'Koshihikari' account for the greatest proportion of all rice cultivars in Japan (Statistics Agricultura, Foresty and Fishers). In the hydropriming treatment with MB-water, 50 rice seeds were soaked in a similar container of 30 L of dechlorinated-water for 12 h after MBs were generated using the MB generation equipment (Royal Electric Co., Ltd., Fukui, Japan) in the container for 15 min at a flow rate of 2.5 L/min (MB-water seeds). After being hydroprimed, the dechlorinated-water seeds and MB-water seeds were dried naturally to a pre-soaking moisture content of 12.6% in the experiment room. Non-primed seeds were used as the control.

2.2 Effect of Hydropriming Treatment on the Emergence of Rice Seeds

Commercial horticultural soil (organic culture soil for flowers and vegetables; Akagi Engei, Isesaki, Gunma, Japan) was first sprayed with dechlorinated-water in order to increase its moisture content. The soil moisture content was adjusted to 50%, which was optimum for the emergence of rice seeds, and 25%, so that emergence is inhibited (Ueyama, 1976). The soil was then put into 400-mL plastic pots where the abovementioned hydroprimed seeds were sown. The pots were covered with a transparent plastic film in order to maintain the moisture levels after the seeds were covered with the soil. Soil moisture content is the volume of water added to soil substances that contribute to the dry weight of the soil. The moisture levels were kept constant during the experiment. An emergence test was performed in a growth chamber (MLR-351H, Sanyo Co., Ltd., Osaka, Japan) under darkness at 30°C/25°C (12 h/12 h) for 14 days. Emergence was confirmed when a seedling grew 2 mm or higher from the soil surface. Time taken to reach an emergence ratio of 50% was indicated as E_{50} (Farooq et al., 2005). The tests were conducted in triplicate, with 3 plastic pots for each of the 2 types of hydropriming treatments and the control.

2.3 Effect of Hydropriming Treatment on Growth and Chlorophyll Content

The commercial horticultural soil was sprayed with dechlorinated-water to increase its moisture content to 25% or 50%. The soil was then put into 400-mL plastic pots, with 30 seeds sown in each pot after they were hydroprimed in a similar manner as the 50 seeds described above. The seeds were then cultured at 30°C/25°C (12-h bright period and 12-h dark period) with 150 µmol/m/s of photosynthetic photon flux density at the top of the pots and 80% relative humidity in a growth chamber for 20 days. Distilled water was sprayed from time to time in order to keep soil moisture content at 25% or 50% at 1 cm below the soil surface. The soil moisture content was constantly monitored using soil moisture censors (ECH2O EC-5, Decagon). One week after sowing, 20 seedlings that had grown relatively uniformly were left in each pot, while the others were eliminated. The test was performed in triplicate, with 3 pots for each of the 2 treatments and the control.

Ten seedlings were randomly chosen from each treatment, and their heights, longest root lengths, aboveground dry weight, and underground dry weight were measured. Chlorophyll content in the aboveground part of the remaining seedlings was quantified only in the soil with 50% moisture content. The fresh aboveground part was dissolved in 80% acetone, and the absorbance was analyzed using wavelengths of 645 nm and 663 nm by using a spectrophotometer (Shimadzu UV-1700, Shimadzu Co., Kyoto, Japan).

2.4 Effect of Hydropriming Treatment on α-Amylase Activity of Brown Rice

The chaff was removed from hydroprimed brown rice seeds by using tweezers, and the brown rice seeds was crushed and sifted with meshes of 0.5 mm to extract α-amylase by using an α-amylase measurement kit (Megazyme International Ireland Ltd., Ireland). The α-amylase activity was measured at 400 nm by using a spectrophotometer (Shimadzu UV-1700).

2.5 Statistical Analysis

The experiment was performed 3 times. Statistical differences were analyzed using the least significant difference method at $P < 0.05$.

3. Results and Discussion

Table 1 shows the effect of the hydropriming treatments on the emergence ratio of the rice seeds. The emergence ratios of the seeds in the soil with 25% moisture content were much lower than that of the seeds in the soil with 50% moisture content. No significant differences existed among the hydropriming treatments and the control in the soil with 25% moisture content. In the soil with 50% moisture content, the emergence ratios of the seeds hydroprimed with MB-water were significantly higher than those of the control and dechlorinated-water seeds at any culturing period. In particular, the ratio of the seeds hydroprimed with MB-water on the fifth day of culture was 2.1 times and 1.6 times higher than the ratios of the control and dechlorinated-water seeds, respectively. Moreover, the E_{50} values of the control, dechlorinated-water seeds, and MB-water seeds were 6.4, 6.3, and 5.6 days, respectively, and a significant difference was observed among the 3 treatments. In addition, the moisture content of rice seeds hydroprimed with the dechlorinated-water and MB-water immediately after water absorption was 23.7% and 27.7%, respectively (data not shown).

Matsushima and Sakagami (2013) reported that at 8% soil moisture content, rice seed ('Koshihikari') priming decreased emergence time by 26.8 h compared with that of the control. Our study showed that hydropriming decreased emergence time 18.2 h compared with that of the control at 50% soil moisture content. In addition, Dey et al. (2013) showed that the lowest germination time was observed from hydropriming of seeds ('BRRI dhan29') with 30 h soaking. These results were not similar to those of their results. It is thought to be factors that constitution in soil, priming temperature, and cultivars that we used differ from those of them. Furthurmore, Himuro (2007) pointed out that MBs probably reduce the surface tension of water by breaking hydrogen bonds among water molecules and promoting the permeability of water molecules. It is supposed that much lower emergence ratios in the soil with 25% moisture content than in the soil with 50% moisture content can be attributed to less water absorption by seeds in the soil with 25% moisture content. Thus, this clearly showed that even the hydropriming treatment of rice seeds with MB-water cannot promote emergence if soil moisture content is not suitable for germination.

These results demonstrated that the hydropriming treatment with MB-water have high effect on the emergence of rice seeds in the soil with 50% moisture content. It is thought that the ability of rice seeds to absorb moisture may quickly progress because of the remarkably high osmosis of MB-water.

Next, the effect of hydropriming treatment on the early growth of rice seedlings is shown in Table 2. Plant height, longest root length, aboveground dry weight, and underground dry weight of the control, dechlorinated-water, and MB-water seeds in the soil with 25% moisture content were lower than those of seedlings in the soil with 50% moisture content. In the soil with 25% moisture content, no significant differences were observed in growth among the 3 treatments. However, in the soil with 50% moisture content, plant height, aboveground dry weight, and underground dry weight of the MB-water seeds were higher than those of the dechlorinated-water seeds and control seeds.

Goswami et al. (2013) was reported that shoot and root length priming seeds with water, KH_2PO_4, NaCl and PEG were about 7-17 cm and 3-7 cm respectively, and higher growth rate than unprimed control seedlings. Dey et al. (2013) showed that shoot and root length when hydropriming treatment was highest germination of rooce seeds were 18.32 cm and 6.48 cm, respectively. Our result was greater than that of them. Therefore, the hydropriming treatment with MB-water may improve early growth in rice seedlings by promoting emergence.

Finally, in the soil with 50% moisture content, the effect of the hydropriming treatment on the chlorophyll content of the aboveground part of the seedlings and α-amylase activity of brown rice seeds are shown in Table 3. These were measured only in the soil with 50% moisture content because the effect of the MB-water on the emergence ratio and early growth in the soil with 25% moisture content could not be recognized. The chlorophyll content and α-amylase activity in the MB-water seeds were significantly higher than those in the dechlorinated-water seeds and control seeds.

The water content of seeds and chlorophyll amount in cotyledons in *Haloxylon persicum* could be reduced by salt stress, and this result suggests a correlation between chlorophyll content and seed moisture content (Zhang et al., 2010). Our results are similar to those obtained by Zhang et al. (2010). Therefore, the faster early growth of seedlings in MB-water can probably be explained by a boost in their photosynthesis ability caused by an increase in the chlorophyll content of their cotyledons.

Hydrolysis of starch in rice seeds primed with MB-water might be promoted by improved α-amylase activity. Kaur et al. (2002) reported that the α-amylase activity of chickpea shoots improved with hydropriming treatment. This finding is consistent with our results. Synthesis of α-amylase and other hydrolases in cereal seeds is induced by gibberellins, whose production is initiated by water absorption of seeds (Yoshioka & Seiwa, 2009). This indicates that α-amylase activity in rice seeds is remarkably improved by gibberellin synthesis, which is considerably stimulated by water absorption by rice seeds hydroprimed with highly osmotic MB-water. This result suggests that the growth promotion effect of the hydropriming treatment with MB-water on rice seeds might be attributable to the use of hexose produced by the hydrolysis of starch in the seeds for growing roots and shoots.

Table 1. Effect of hydropriming treatment on emergence ratio of rice seeds

Soil moisture content	Treatments	Emergence ratio (%) Culturing time (days)			E_{50}
		5.0	7.0	14.0	
25%	Control	0.0 ± 0.0 a[Z]	4.6 ± 0.6 a	32.0 ± 0.4 a	-
	Dechlorinated-water	0.0 ± 0.0 a	4.0 ± 0.4 a	31.3 ± 1.7 a	-
	MB-water	0.0 ± 0.0 a	4.2 ± 0.4 a	32.0 ± 1.8 a	-
50%	Control	14.0 ± 0.4 a	50.6 ± 0.6 a	78.6 ± 0.4 a	6.4 ± 0.1 a
	Dechlorinated-water	18.0 ± 1.6 a	51.3 ± 0.2 a	79.3 ± 0.6 a	6.3 ± 0.2 a
	MB-water	29.3 ± 1.2 b	64.0 ± 0.7 b	86.0 ± 0.3 b	5.6 ± 0.1 b

[Z] Different letters after figures indicate significant differences among treatments at $P<0.05$.

Table 2. Effect of hydropriming treatment on early growth of rice seedlings

Soil moisture content	Treatments	Height (cm)	Longest root length (cm)	Aboveground dry weight (g/plant)	Underground dry weight (g/plant)
25%	Control	12.5 ± 0.8 a[Z]	4.5 ± 0.4 a	7.1 ± 0.4 a	3.9 ± 0.2 a
	Dechlorinated-water	13.9 ± 1.5 a	5.0 ± 0.3 a	8.0 ± 0.4 a	3.6 ± 0.3 a
	MB-water	13.4 ± 1.1 a	5.2 ± 0.3 a	7.9 ± 0.4 a	3.8 ± 0.2 a
50%	Control	23.9 ± 1.0 a	6.4 ± 0.3 a	10.2 ± 0.5 a	4.2 ± 0.3 a
	Dechlorinated-water	24.3 ± 1.5 a	7.1 ± 0.4 ab	10.4 ± 0.6 a	4.3 ± 0.4 a
	MB-water	28.0 ± 1.0 b	7.6 ± 0.3 b	12.6 ± 0.6 b	5.1 ± 0.2 b

[Z] Different letters after figures indicate significant differences among treatments at $P<0.05$.

Table 3. Effect of hydropriming treatment on chlorophyll content and α-amylase activity of brown rice seeds in 50% soil moisture content

Treatments	Chlorophyll content (mg/100g)	α-amylase activity ($\times 10^{-2}$ Units/g)
Control	1.19 ± 0.08 a[Z]	5.3 ± 0.5 a
Dechlorinated-water	1.29 ± 0.06 a	5.4 ± 0.4 a
MB-water	1.60 ± 0.09 b	7.0 ± 0.3 b

[Z] Different letters after figures indicate significant differences among treatments at P<0.05.

4. Conclusion

The experiment was conducted to study the effects of hydropriming treatment of rice seeds 'Koshihikari' with microbubble (MB)-water which have effect on promoting plant growth, on emergence and early growth of seedlings. Hydropriming treatment with MB-water is extremely effective in improving the seedling emergence ratio and in promoting early growth. We believe that MB-water improved the emergence rate and promoted early growth by helping the seeds absorb water efficiently as a result of the considerably high osmosis of MB-water. In near future, we need to investigate seedling emergence of other cultivars hydropriming treatment with MB-water.

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