Utilization of Naturally Occurring Seed Priming Agents in Enhancing Seed Germination and Seedling Emergence of Rice (Oryza Sativa L.)

Merivic Gapasin-Catada, Jehson T. Campos, Arjay R. Zamora

Seed priming is an effective tool to attain high germination and germination rate, uniformity of seedlings and crop stand. This study was conducted to determine the effectiveness of seed priming on the germination and early seedling establishment of rice. The experiment was laid out in a Randomized Complete Block Design (RCBD). Rice seeds were subjected to priming for 12 and 24 hours in tap water, coconut milk and coconut water. When the seed priming was accomplished, seeds were removed from the priming agents and placed on top of cloth; they were later air dried and sown. Seed priming in water for 12 hours enhanced the germination percentage to 71.80%; the rate of germination and plant height of the seedlings increased to 33.13 cm, and root length to 13.07 cm. After seed priming, the seedling height of rice considered ideal for transplanting was attained on the 13th day of sowing. The effects of priming in water and coconut water for 12 and 24 hours are comparable; however, seed priming in water for 12 hours resulted to the tallest seedling at 20.92 cm. Seed priming in water for 12 hours is sufficient in enhancing seed germination and the early seedling establishment of rice but coconut water if available can also be used.

Keywords: Seed priming, rice, coconut milk, coconut water, germination, germination rate.

INTRODUCTION

Rice (Oryza sativa L.) remains to be the primary staple food in the world. Specifically, Asia consumes the most rice (FAO, 2012). In the Philippines, the demand for rice has been steadily going up. In 2009-2010, Filipinos ate an average of 119 kg of rice annually; however, in 2008, the average consumption was 128 kg per person (Bureau of Agricultural Statistics [BAS] 2011). On the other hand, rice production in 2015 was lower by 4.31% compared to 2014 levels. The decrease in the production was attributed to the harvest and yield in the lowland areas brought about by insufficient water supply, dry spell or drought, and adverse effects of typhoons (Philippine Statistics Authority [PSA], 2015).

For field crops production, good seed viability is an important attribute. Low germination or non-uniformity of seedling emergence can result in financial losses, decreased crop production and reduced market value of the final product (Ghiyasi et al., 2008a). Most of the rice in the world is transplanted. Transplanting of rice in the field is limited due to unreliable water supply caused by variable rainfall or irrigation pattern, thus transplanting is sometimes delayed until enough water accumulates in the area. Importantly, seed
priming has shown its effectiveness in improving seed germination, seedling growth and crop stand against the negative impacts brought about by stress in the field.

Seed priming is a hydration treatment of seeds preceding sowing in the soil (Farooq, Basra, Wahid et al., 2009). The treatment can be accomplished by either overnight soaking of seeds (on-farm priming) or soaking in solutions of low water potential. The seeds are then dried down closer to the original moisture level. The procedure prepares the seeds for sowing later in the soil and warrants maximum and rapid germination (Farooq, Basra, Tabassum et al., 2006). Treatment with water, salt solution, certain hormones, organic and inorganic chemicals, and pesticides, etc. (Vasileva & Ilieva, 2007) are primarily practiced in seed priming. Also, pre-soaking, hardening, hormonal priming, hydropriming, halopriming, Osmo conditioning, and ascorbate priming are some common priming techniques to improve speed and synchrony of seed germination.

In a physiological state, seed priming allows the seeds to hydrate partially and allows advanced metabolic processes; however, it prevents the protrusion of the radicle. Seed priming resulted in increased germination rate, higher germination uniformity, better allometric (changes in growth of plant parts over time) attributes and the faster emergence of seedlings (Farooq, Basra, & Ahmad, 2007). Along with early germination and better stand establishment, priming leads to crops that grow faster, flowers earlier and produce higher yields (Du & Tuong, 2002).

Rice seed priming enhances its resistance to environmental stress (Goswami et al., 2013), weed suppressive ability and improves yield (Juraimi et al., 2012). Previous studies by Anwar, Iqbal, Raza, and Iqbal (2013) and Zarei and Sinaki (2012) have shown that rice seeds responded to seed priming in the early part of the germination stage and revealed that it could increase seed germination, vigor index, and germination speed. Also, primed seeds flowered earlier (vital in drought-prone areas), matured earlier and gave higher yields (Harris et al., 2001). Hydro-priming was found to fight the effects of drought (Kaya, Okçu, Atak, Cikılı, & Kolsarıcı, 2006) and other abiotic stresses (Jafar et al., 2012) on seedling emergence and crop establishment.

Seed priming is practically practiced with water and chemicals such as glycine betaine, proline, salicylic acid, ascorbate, hormones, etc. but these are costly compared to other natural products like coconut water and coconut milk. Coconut water is an essential growth supplement for plant tissue culture/micropropagation (Neumann, Kumar, & Imani, 2009). Cytokinins identified in coconut water are N6-isopentenyladenine, dihydrozeatin, trans-zeatin, kinetin, ortho-topolin, dihydrozeatin O-glucoside, trans-zeatin O-glucoside, trans-zeatin riboside, kinetin riboside, and trans-zeatin riboside-5’- monophosphate (Yong, Ge, Ng, & Tan, 2009). In seed priming, cytokinin functions in mobilizing the food reserves embedded in the seed coat resulting in the imbibition of water within the seed coat.

Exploring the potential of natural products is needed like coconut water and coconut milk as alternative priming agents. The primary objective of the study was to determine the effectiveness of seed priming on the germination and early seedling establishment of rice seeds after soaking in tap water, coconut water, and coconut milk for 12 and 24 hours. Specifically, the study aimed to determine and measure the germination percentage, rate of germination of seeds, and the characteristics of germinated rice seedlings.
MATERIALS AND METHODS

Experimental design and treatments

The experiment was conducted in a Randomized Complete Block Design (RCBD) with six treatments representing the various priming agents enumerated below as follows:

T0 = control (no priming)
T1 = 24 hours soaking in tap water
T2 = 12 hours soaking in tap water
T3 = 24 hours soaking in coconut water
T4 = 12 hours soaking coconut water
T5 = 24 hours soaking in coconut milk
T6 = 12 hours soaking in coconut milk

Each treatment replicated five times with fifty rice seeds per replicate.

Preparation of the priming agents

Extraction of coconut water

Fresh coconut water was derived from young green coconut of approximately 220 days old. Coconut water is the aqueous part of the coconut endosperm. The water was extracted by breaking the coconut with a bolo while the coconut water was descended into a clear container. Pure coconut water was used as seed priming agent.

Extraction of coconut milk

The coconut milk is a liquid product obtained from dividing the matured coconut into halves and grating the solid endosperm. The pure grated coconut was squeezed forcefully allowing the milk to pass through a strainer to strain the other parts of the coconut getting only the clean coconut milk.

Priming of the rice seeds

Priming was done by soaking the rice var RC 18 seeds in tap water, coconut milk and coconut water for 12 and 24 hours. Bottles were used as containers that can contain approximately 250 ml liquid and with wide mouth. Then, a ratio of 1 g/100 ml (%) for the weight of rice seeds and the amount of priming agents was used. Pure coconut water and coconut milk were used; water served as control including no seed priming. All primed seeds were soaked for 12 and 24 hours.

After the seed priming duration was accomplished, the seeds were removed from the priming agents, placed on top of a clean cloth, and air dried for approximately 5 hours. After which, the seeds were sown.

Sowing

Air-dried primed seeds were sown in seedling trays. All 50 primed seeds were sown singly in each hole on the seedling tray. The seedling trays were embedded on a seedbed in an open field. Three days after sowing, the germinating seedlings were flooded with water at 2-3 cm level and on the 19th day after sowing. The water level was increased to 5 cm on the 20th day onwards.

Data gathering

1. Germination Percentage. This proportion was taken by counting the total number of primed seeds that germinated ten days after seeds were sown. The germination percentage was computed using the formula:

   \[
   \text{Germination} \% = \left( \frac{\text{Number of germinated seeds}}{\text{Number of seeds sown}} \right) \times 100
   \]
2. Daily Germination Rate. This rate was taken by counting the number of primed seeds that germinated per day up to the 10th day after sowing. This was computed using the formula:

\[
\text{Germination rate} = \frac{\text{Number of normal seedlings}}{\text{Days to first count}} + \ldots + \frac{\text{Number of normal seedlings}}{\text{Days to final count}}
\]

3. Daily shoot length. This length was taken by measuring the shoots of the rice seedlings from the base to the highest tip of the shoots.

4. Final root length. This measure was taken by getting the length of the roots from the base up to the tip of the longest root of 10 seedlings 21 days after sowing.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using Statistical Tool for Agricultural Research (STAR) version 2.0.1 (IRRI, 2014). Where significant difference existed among treatments, the mean separation between the groups was subjected to Tukey’s HSD (honestly significant difference) test.

RESULTS

Germination Percentage

Primed treatments significantly affected the germination percentage (Table 1) of rice seeds. Seed priming in water for 12 hours showed the highest percentage germination (71.80%); however, this result is not significantly different from the effects of priming the seeds in water for 24 hours (63.80%) and in coconut water for 12 hours (63.40%).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Percentage (%)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0 – Control (no soaking)</td>
<td>40.20c</td>
<td>17.72</td>
</tr>
<tr>
<td>T1 - 24 hours soaking in water</td>
<td>63.80ab</td>
<td></td>
</tr>
<tr>
<td>T2 - 12 hours soaking in water</td>
<td>71.80a</td>
<td></td>
</tr>
<tr>
<td>T3 - 24 hours soaking in coconut water</td>
<td>54.00bc</td>
<td></td>
</tr>
<tr>
<td>T4 - 12 hours soaking in coconut water</td>
<td>63.40ab</td>
<td></td>
</tr>
<tr>
<td>T5 - 24 hours soaking in coconut milk</td>
<td>02.40d</td>
<td></td>
</tr>
<tr>
<td>T6 - 12 hours soaking coconut milk</td>
<td>00.80d</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. The average germination percentage (%) of rice (Oryza sativa L.) seeds as influenced by priming in water, coconut water and coconut milk for 12 and 24 hours.

Note: Mean separation by Tukey’s HSD (honestly significant difference) test, 5%..

Daily germination rate

The effect of priming rice seeds with different priming agents on their rate of germination was found significant (Table 2). Results show that rice seeds subjected to water priming for 24 and 12 hours initiated a greater number of seeds that germinated on the 3rd day; also, water priming showed the highest number of seeds that germinated up to the 10th day.

Daily plant height

The seedling height of rice was taken 7 days after transplanting. Seed priming in water, coconut milk and coconut water exhibited significant results in the daily plant height of the seedlings. On the 13th day, the seedlings had already attained the ideal seedling height for transplanting. Priming in water for 12 hours exhibited the highest plant height at 20.92 cm, but this is not significantly different in seedlings subjected to priming in water for 24 hours and in coconut water for 12 and 24 hours (19.77 cm and 19.67 cm).
**Table 2.** The average germination rate of *Oryza sativa* L. seeds as influenced by priming in water, coconut water and coconut milk for 12 and 24 hours.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>T0 – Control (no soaking)</td>
<td>0.80d</td>
</tr>
<tr>
<td>T1 - 24 hours soaking in water</td>
<td>7.50ab</td>
</tr>
<tr>
<td>T2 - 12 hours soaking in water</td>
<td>9.10a</td>
</tr>
<tr>
<td>T3 - 24 hours soaking in coconut water</td>
<td>3.50cd</td>
</tr>
<tr>
<td>T4 - 12 hours soaking in coconut water</td>
<td>4.80bc</td>
</tr>
<tr>
<td>T5 - 24 hours soaking in coconut milk</td>
<td>0.00d</td>
</tr>
<tr>
<td>T6 - 12 hours soaking in coconut milk</td>
<td>0.00d</td>
</tr>
<tr>
<td>CV (%)</td>
<td>50.29</td>
</tr>
</tbody>
</table>

Note: Mean separation by Tukey’s HSD (honestly significant difference) test, 5%.

**Table 3.** Daily plant height of *Oryza sativa* L. seeds as influenced by priming in water, coconut water and coconut milk for 12 and 24 hours.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>T0 – Control (no soaking)</td>
<td>2.65c</td>
</tr>
<tr>
<td>T1 - 24 Hours soaking in water</td>
<td>6.56a</td>
</tr>
<tr>
<td>T2 - 12 Hours soaking in water</td>
<td>6.42ab</td>
</tr>
<tr>
<td>T3 - 24 Hours soaking in coconut water</td>
<td>5.21b</td>
</tr>
<tr>
<td>T4 - 12 Hours soaking in coconut water</td>
<td>5.24b</td>
</tr>
<tr>
<td>T5 - 24 Hours soaking in coconut milk</td>
<td>0.1d</td>
</tr>
<tr>
<td>T6 - 12 Hours soaking in coconut milk</td>
<td>0.01d</td>
</tr>
<tr>
<td>CV (%)</td>
<td>16.54</td>
</tr>
</tbody>
</table>

Note: Mean separation by Tukey’s HSD (honestly significant difference) test, 5%.
Root Length (cm)

The effect of seed priming using water, coconut milk, and coconut water is significant. The seeds subjected to priming in water for 12 hours exhibited the longest root; however, its effect is not significantly different from the control seeds and those primed with water for 24 hours and with coconut water for 12 and 24 hours.

Table 4. Root length of rice (Oryza sativa L.) seedlings as influenced by priming in water, coconut water and coconut milk for 12 and 24 hours.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Root length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0 – Control (no soaking)</td>
<td>12.33a</td>
</tr>
<tr>
<td>T1 - 24 hours soaking in water</td>
<td>12.50a</td>
</tr>
<tr>
<td>T2 - 12 hours soaking in water</td>
<td>13.07a</td>
</tr>
<tr>
<td>T3 - 24 hours soaking in coconut water</td>
<td>11.69a</td>
</tr>
<tr>
<td>T4 - 12 hours soaking in coconut water</td>
<td>12.16a</td>
</tr>
<tr>
<td>T5 - 24 hours soaking in coconut milk</td>
<td>2.76b</td>
</tr>
<tr>
<td>T6 - 12 hours soaking in coconut milk</td>
<td>1.15b</td>
</tr>
<tr>
<td>CV (%)</td>
<td>13.42</td>
</tr>
</tbody>
</table>

Note: Mean separation by Tukey’s HSD (honestly significant difference) test, 5%.

DISCUSSION

Enhancing the viability of the seeds is important in any crop production. A high percentage and faster rate of germination and good crop stand are some measures of seed viability. The majority of rice varieties are transplanted and pre-germination is practiced before seedling transplanting. Typical seed germination follows a triphasic model (Bewley, 1997) and requires longer duration for germination to initiate. The Phase I which is the water imbibition the first 20 h results to a rapid increase in seed weight. This phase is followed by a stable plateau stage until 50 h (phase II); the coleoptiles elongation could be evident at this stage. Another rapid water uptake is observed in Phase III followed by the protrusion of the radicle (Yang et al., 2007). Phase II is regarded as the most significant step because germination process required active metabolic reactions during this period.

Seed priming lessens the time from germination to emergence. In its physiological state, seed priming allows the partial imbibitions of water and will facilitate build up of germination enhancing metabolites (Farooq, Basra, Tabassum et al., 2006), but preventing the protrusion of the radicle. Seed soaking allows the utilization of reserves from the storage parts of seed (e.g., cotyledons or endosperms) for partitioning to the embryo. This process is accomplished by the activation of enzymes primarily α- and β-amylase. These enzymes are useful for the conversion of complex sugars into simpler ones and are mobilized to the embryo (Farooq Basra, Wahid et al., 2009; Taiz & Zeiger, 2010). Following activation, the embryo is set for the rapid cell division and the emergence of embryonic tissues by rupture of the seed coat. Results of the study on percentage and the rate of germination conform to the finding of Harris, Joshi, Khan, Gothkar, and Sodhi (1999). They described that speed and uniformity of germination and emergence were enhanced by soaking the seeds in plain water prior to sowing, thereby leading to better crop stands, and stimulated seedlings to grow much more vigorously. Brocklehurst and Dearman (1983) also stressed that the germination of hydro-primed seeds occurs earlier; it is also higher and more synchronized and leads to a reduction in the lag time of imbibition. On coconut water as a priming agent, the result of the study conforms to the findings of Dahamarudin and Rivai (2013). The study found out that the use of coconut water has improved the viability as shown in the germination capacity of three (3) upland rice varieties from Indonesia like Situ Patenggang, Limboto, and Batutegi.
The result of the study also suggests that priming of seeds in water for 12 hours is effective in increasing the height of the seedlings. Priming has lessened the period by which the seedlings attained the suggested height for transplanting. The increment in plant height at a shorter duration (13 days after sowing) can be attributed to the effect of seed priming. At priming, seeds are at physiologically active state which may suggest that certain metabolic activities are switched on. As seeds imbibe water during seed priming, this condition allows the activation of enzymes that are necessary for germination to continue once seeds are sown. In particular, the growth of rice shoots of the primed seedlings can be ascribed to the soluble carbohydrate supply brought about by the increase in activated α-amylase. In rice seeds, Andoh and Kobata (2002) mentioned that water absorption with seed priming leads to the activation of the metabolic process related to α-amylase activity and drying the seeds after seed priming preserves its metabolic activities (Andoh & Kobata, 2002). The increment in the shoots of rice seedlings in this experiment could be due to the availability and supply of nutrients required for cell growth, which is achieved with seed priming. However, aside from tap water, priming can also be done using coconut water. The result showed that priming with coconut water at 12 hours enhanced the germination, germination rate, seedling height, and root length of rice seedlings. Coconut water which contains hormones is highly available especially during copra making.

CONCLUSION

Rice is responsive to seed priming. Seed priming in tap water for 12 hours enhanced the germination percentage, rate of germination and increased plant height and root length of the seedlings. The effect of seed priming for 12 hours in coconut water enhanced the germination percentage of rice. The ideal seedling height of rice for transplanting which is 18-20 cm was attained on the 13th day after sowing on seeds subjected to priming in tap water for 12 hours. Seed priming for 12 hours, tap water is sufficient in enhancing seed germination and the early seedling establishment of rice.

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