Seed germination potential of different local varieties of *Oryza sativa* (rice) as affected by different seed priming methods

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Abstract

Seed priming is a technique used to improve the overall germination behavior of rice through the imbibition of solutions. This study aimed to investigate the effects of different priming methods on two local varieties of *Oryza sativa* (rice). This study employed three different priming methods using mannitol, glycerol, and sorbitol on red and black varieties of *Oryza sativa* and compared the germination behavior between primed and unprimed seeds. The researchers compared three different parameters such as germination percentage, germination index, and shoot/root ratio by performing a Kruskal-Wallis test using SPSS. Seeds primed with mannitol in black rice resulted in a significant difference in the germination index and shoot/root ratio compared to unprimed seeds. Seeds primed with glycerol in red rice resulted in a significant difference in the germination index compared to unprimed seeds.

Keywords: *seed priming, imbibition, germination percentage, germination index, shoot/root ratio*

Introduction. Seed priming is a technique used to improve the overall germination behavior of rice. This is done by starting all processes necessary for germination but inhibit radicle protrusion preventing the seed to germinate. Osmotic solutions are used in order to partially hydrate seeds up until a point necessary for germination process to start but not enough for radicle protrusion [1].

Priming has shown to increase resistance in crops against both biotic and abiotic stresses [2]. Multiple studies conducted on seed priming have yielded mostly positive results with most of them focusing on studying the effects of multiple types of stresses, be it biotic or abiotic, along with different crop varieties and priming agents [3,4,5].

There are several types of seed priming including hydropriming, halopriming, osmopriming, and hormonal priming, and all have shown great promise in improving seed germination [6]. Osmopriming utilizes osmotic sugars to induce germination in seeds. This specific method has shown great potential, with polyethylene glycol being one of the most common solutions used [7,8]. A previous study has shown a significant difference between osmopriming and hydropriming when it comes to seed germination [9]. However, there are still knowledge gaps found in this field. Some priming agents are yet to be tested on rice or compared against other agents. Also, the effect of seed priming on multiple local rice varieties has not been tested.

Commonly used rice varieties include Rc 216, Rc 160, Rc 300, and Rc 222. However, due to the lack of research regarding high-value rice varieties, the researchers chose to investigate the black and red rice varieties. In this study, the effects of different osmopriming methods on different rice varieties were investigated and compared.

For this study, the parameters germination percentage, germination index, and shoot/root ratio for each rice cultivar treated with the different osmopriming methods (mannitol, glycerol and sorbitol), the number of seeds that germinate every day, and the length of the seedling, shoot and root on the seventh day were measured. Using these parameters, the germination percentage, germination index and shoot/root ratio were computed. These were used to compare the effect of different osmopriming methods on seed germination within each cultivar.

This research aimed to investigate the effects of different osmopriming methods (mannitol, glycerol and sorbitol) on the seed germination behavior of two local varieties (red and black) of *Oryza sativa* (rice). Specifically, it aimed to:

(i) determine the parameters for each rice cultivar treated with the different osmopriming methods such as the number of seeds that germinate every day and the length of seedling, shoot, and root on the seventh day;

(ii) compute for the parameters for each rice cultivar treated with the different osmopriming methods such as germination percentage, germination index, and shoot/root ratio; and

(iii) compare the effect of different osmopriming methods on seed germination parameters within each cultivar in terms of germination percentage, germination index, and shoot/root ratio.

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Methods. This study tested the effects of three different priming methods using mannitol, glycerol and sorbitol with specific concentrations on two different local varieties of rice (red and black). Several germination parameters were used to test the seed priming effects on seed germination. The methods were done in correspondence with the International Rice Research Institute (IRRI), Los Baños, Laguna.

This study was conducted in one of the laboratories of Philippine Science High School - Western Visayas Campus' (PSHS-WVC) SLRC Building over a 16-day period, including a 10-day germination test.

Three different priming methods and one unprimed control for each variety were used in the study. The concentrations indicated below were based on previous studies on seed priming [11,12]. Table 1 shows the priming agent, concentration, and treatment numbers.

Table 1. Priming agents, concentrations, and treatment.

Treatment Number	Priming Agent	Concentration*
1	mannitol	2%
2	glycerol	5%
3	sorbitol	0.25 M

* References: [11] Seed priming alleviated salt stress effects on rice seedlings by improving Na+/K+ and maintaining membrane integrity; and [12] Effects of different priming methods and duration on seedling characters maize (Zea mays L.)

Seeds, Priming Agents, and Equipment. Black and red rice seeds were purchased from a local farm. The priming agents mannitol, glycerol, and sorbitol were all obtained from Patagonian Enterprises. The beakers, stirring rods, Petri dishes, flasks and graduated cylinders were all from PSHS-WVC's Chemistry Science Research Assistant (SRA).

Moisture Content Test. Seeds were tested for their moisture content at Western Visayas Agricultural Research Center (WESVIARC). A grain moisture tester (Riceter f511) was used to measure the moisture content. The test chamber was filled with seeds, then the handle was turned to crush the grain, and the moisture content was instantly provided. Optimum moisture content for rice is at 14.0% or lower after drying.

Preparation of Priming Agents. Twenty-five grams of mannitol was measured using an analytical balance (Shimadzu aux-220), then dissolved in 500 mL of water in a 2 L beaker and stirred using a stirring rod in order to achieve the desired 2% concentration. A 100 mL graduated cylinder was used to measure 25 mL of glycerol and 475 mL of water to create a 5% glycerol solution, and both solutions were mixed using a stirring rod in a 2 L beaker. The same process was repeated to achieve a 0.25 M sorbitol solution using 15 mL of sorbitol and 485 mL of water.

Treatment of Seeds. During the priming process, the seed weight to solution volume ratio of 1:5 was used. Using 100 g of seeds from each variety, the seeds were soaked in 5% solution of glycerol for 24 h. The same was done for mannitol and sorbitol. Soaked seeds were then recovered from the solution, spread on a metal tray with paper towels and allowed to dry for 24 h. The seeds were then brought to WESVIARC to have their moisture contents measured, following the same methods as the first test.

Germination Test. A total of eight (8) setups were used for the two varieties (red and black). Three different priming agents and a control of untreated seeds were used in each variety. Twenty-five seeds were sown in one 90 mm Petri dish which contained one layer of Whatman No. 2 filter paper moistened with 10 mL of water which was measured using a syringe. Each setup had three replicates which were made up of six Petri plates each. A total of 150 seeds were used in each replicate and 144 Petri plates were used for the study.

Every day, at 7:00 AM and at 3:00 PM, seeds were watered as needed to maintain a 100% moisture level using a syringe. Natural light was allowed to enter through the windows while an artificial source of light was turned on from 7:00 AM to 6:00 PM. External factors were also observed and recorded such as the temperature, presence of insects, animals or any other factor that might affect the study. At 3:00 PM, the number of germinated seeds were recorded. A seed was deemed to have germinated once it reached a radicle length of 2 mm which was measured using a vernier caliper.

Measuring of Seedling Shoot and Root Length. On the seventh day of the experiment, the seedling shoot and root length were measured. The two longest germinated seeds in each Petri plate were chosen, and their length was measured using a vernier caliper.

Calculation of Parameters. The germination percentage, germination index and shoot/root ratio were calculated using the formulas stated by Li [13] and Wilson [14];

$$Germination\ Percentage = (GS/ST) \times 100$$

where GS is the total number of germinated seeds and ST is the total number of seeds tested.

$$Germination\ Index = \sum (Gt/Dt)$$

where Gt is the number of germinated seeds on day t and Dt is the time corresponding to Gt in days.

$$Shoot/Root\ Ratio = SL : RL$$

where SL is the length of the shoot and RL is the length of the root.

Data Analysis. Using IBM SPSS Statistics, a Kruskal-Wallis test was conducted with a significance level of 0.05 to examine the differences in the germination behavior of *Oryza sativa* according to the priming technique used. It was followed by a Dunn Test for pairwise comparisons in a post hoc manner.

Safety Procedure. Before the conduct of the study, the chemical safety data sheet of chemicals to be used were reviewed. During the conduct of the study, the proper use of personal protective equipment was observed inside the laboratory and proper precautions were followed. The chemicals obtained were placed inside appropriate containers and were handled properly according to their safety protocol. After the conduct of the experiment, the seedlings were thrown away properly into the garbage bins, and chemical wastes were properly disposed.

Results and Discussion. During the moisture content test, red rice yielded a moisture content of 12.6% while black rice yielded a moisture content of 14.0% which are both within the optimal 14.0% moisture content. The moisture content should be within the optimal percentage in order to proceed to priming.

The table below shows the results of the germination test for red rice. The germination percentage of the primed seeds relative to the unprimed seeds showed an overall increase. The means of the germination index of the primed seeds were comparable to one another and were higher than those of the unprimed seeds. The means of the shoot/root ratio were also comparable.

Table 2. The germination percentage and the germination index at the end of the 10 d experiment and the shoot/root ratio on the seventh day of the experiment sorted into four groups according to the priming technique (mean ± standard error) for RED RICE.

Treatment	Germination %	Germination index*	Shoot/Root Ratio
Mannitol	98.00 ± 0.00	49.30 ± 0.25	0.85 ± 0.05
Glycerol	98.00 ± 0.77	49.44 ± 0.23 a	0.85 ± 0.06
Sorbitol	98.00 ± 1.02	50.33 ± 0.83	0.82 ± 0.10
Unprimed	97.56 ± 0.80	44.44 ± 1.34 a	0.81 ± 0.05

* Means followed by the same letter are significantly different at p ≤ 0.05 (Dunn Test).

A significant difference (h = 8.273, p = 0.041) was found between the germination index of red *Oryza sativa* seeds that were primed with glycerol and the unprimed seeds based on pairwise comparison of treatments. This would mean that priming with glycerol does indeed lead to a better germination index compared to non-priming.

The test showed that there was no significant difference between germination percentage and shoot/root ratio between primed and unprimed seeds.

Table 3 shows the results of the germination test for black rice. Similar to the red variety, the germination percentage of the primed seeds relative to the unprimed seeds showed an overall increase. The means of the germination index of the primed seeds were comparable to one another and were higher than those of the unprimed seeds. The shoot/root ratio among all treatments vary with mannitol having the lowest shoot/root ratio and unprimed seeds having the highest.

Table 3. The germination percentage and the germination index at the end of the 10 d experiment and the shoot/root ratio on the seventh day of the experiment sorted into four groups according to the priming technique (mean ± standard error) for BLACK RICE.

Treatment	Germination %	Germination index*	Shoot/Root Ratio*
Mannitol	97.78 % ± 0.45	47.92 ± 0.74 a	0.93 ± 0.04 a
Glycerol	96.45% ± 0.80	44.29 ± 0.37	1.28 ± 0.14
Sorbitol	97.58 % ± 0.22	46.02 ± 0.49	1.46 ± 0.12
Unprimed	93.11 % ± 1.74	36.58 ± 0.19 a	1.75 ± 0.17 a

* Means followed by the same letter are significantly different at p ≤ 0.05 (Dunn Test).

A significant difference (h = 9.974, p = 0.019) was found between the germination index of black *Oryza sativa* seeds that were primed using mannitol and unprimed seeds based on pairwise comparison of treatments. This would mean that priming with mannitol does indeed lead to a better germination index compared to non-priming for black *Oryza sativa* seeds.

A significant difference (h = 8.435, p = 0.038) was found between the shoot/root of seeds primed with mannitol and unprimed seeds.

The test showed that there was no significant difference between germination percentage between primed and unprimed seeds.

Seed priming has been used to reduce the time between seed sowing and seedling emergence, and to synchronize that emergence [15]. Primed seeds have shown to have earlier, more uniform and sometimes greater germination and seedling establishment and growth [16]. Currently, many rice varieties have been tested with priming such as Inpago 8, Situ Bagendit, IR64 and more. Seed priming using mannitol, glycerol, and sorbitol has not yet been tested on local varieties such as red and black varieties. Investigation of the effects of seed priming on red and black rice will allow a better understanding of seed priming and its effects.

A significant difference in the germination index (GI) of both red and black rice varieties was found. In red rice, the GI of seeds treated with glycerol had a significant difference among the GI of unprimed seeds. In black rice, the GI of seeds treated with mannitol had a significant difference compared to the GI of unprimed seeds. These results are consistent with the results of a previous study [3], where primed aerobic cultivars were compared with unprimed aerobic cultivars, showed an improved GI. This could be attributed to how the priming process works, wherein seeds that have undergone seed priming have already completed the processes necessary for germination without the occurrence of the germination itself [1]. This gives the primed seeds an advantage for an earlier germination compared to the unprimed seeds, thus increasing the speed of germination and the germination index.

Germination percentage has shown to have no significant difference among the treatments and the control, although there was an observed increase in the germination percentages of primed seeds when compared to the germination percentage of the unprimed seeds over a period of ten days. The lack of significant difference goes against the existing literature which has shown a significant difference in the germination percentage of seeds with the use of priming in different crops such as maize, wheat, rice, and canola [17,18]. Granted that the seeds were obtained from one source, the seeds could have had similar seed quality, which refers to the physical, physiological and genetic attributes that determine the performance of the seed [19], and could be influenced by both genetic background and environmental conditions of the mother plant during seed development [20]. Priming, despite being able to speed up the germination process and therefore increasing resistance against biotic and abiotic resources [21], cannot salvage seeds from undergoing the deterioration process [22].

Regarding the shoot/root ratio between primed and unprimed seedlings, there was no significant difference in red rice varieties. However, there was a significant difference in black rice with mannitol-primed seeds having the minimum shoot/root ratio. The results for the black rice variety are supported by a previous study [23], which shows that seed priming caused a significant difference in the shoot/root ratio. However, the results of this study led to a lower shoot/root ratio of seeds primed with mannitol compared with unprimed seeds. This indicates a longer root than shoot after seven days. The shoot/root ratio is defined as the ratio of the amount of plant tissues that have supportive functions to the amount of those that have growth functions [24]. Previous studies have shown that plants show different reactions based on different limitations in the environment. For example, root growth is favored when there is a lack of nitrogen, phosphorus, or sulfur [25].

Biotic factors that could affect germination such as insects and pests were present. While these factors were not fully controlled, the researchers employed several techniques to minimize their effects such as using an insecticide chalk to line the perimeter around the set-up, closing the windows to prevent insects from infesting the seedlings, distributing water equally among all Petri dishes, and using an artificial source of light from 7:00 AM to 6:00 PM while allowing natural light to enter through the windows.

Limitations. This study was limited to the germination stage alone, and while germination is one of the initial factors influencing yield, there are still other factors during the other stages of crop production that should be considered such as infestations and forces of nature. Abiotic factors such as light, moisture content, and temperature are largely dependent on nature and could not be controlled. Therefore, temperature was measured and recorded every day at 3:00 PM using a room thermometer. Also, several natural factors could not be controlled such as the presence of pests and sunlight exposure.

Conclusion. This study had shown that seed priming does indeed have a significant effect on some of the germination behaviors of *Oryza sativa* — specifically its germination index and in the case of black rice, its shoot/root ratio — but does not affect the germination percentage. Mannitol is the best priming agent for black rice, while glycerol is the best priming agent for red rice.

Recommendations. For future experiments similar to this study, a longer period for the data gathering is recommended. The crop yield may also be another parameter to measure and compare in future studies. Other priming agents and other varieties could also be employed in future studies on the subject.

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