Effects of hydropriming on the germination of *Oryza sativa* L. NSIC Rc 216 (rice) under sodium chloride (NaCl) stress

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Abstract

Elevated salt concentration can be toxic to plant development. Hydropriming can overcome this by increasing the seeds' stress tolerance. This study determined the effects of hydropriming on the germination of *Oryza sativa* L. var. NSIC Rc 216, a widely used rice variety in the Philippines, subjected to sodium chloride stress. Seeds were hydroprimed for 12, 24, or 48 hours with unprimed rice seeds as control. Seeds were then allowed to germinate for seven days and germination parameters were recorded. Significant differences were recorded with the germination energy percentage (GEP) and speed of germination means (SG). The 48-hour treatment had significantly higher GEP and SG means when compared to the control set-up; however, no significant differences were recorded with the final germination percentage (FGP) and seedling vigor index (SVI). In conclusion, hydropriming had effects on the germination rate of rice under salt stress but not with its overall germination performance.

Introduction. - Rice (*Oryza sativa* L.) is an important staple food crop in the world and in the Philippines, feeding half of the human population [1].

However one of the major problems of the agriculture industry is soil salinity. It affects the plant at almost all of its growth stages and impacts the germination and growth of plants [2]. Highly saline environments can decrease the osmotic potential of soil and make it toxic to seedlings [3]. The growth of rice, in particular, can be negatively affected by increased salt concentration that leads to the reduction of several germination parameters such as its final germination percentage (FGP), germination energy percentage (GEP), and speed of germination (SG) [4].

Hydropriming has been recommended to address the effects of soil salinity [5,6,7]. It is a simple method that only requires distilled water as the priming medium for the seeds before sowing [8]. This process enables the seeds to imbibe water which facilitates the emergence of the seeds' radicle [9,10]. With this, it has the potential to upregulate the tolerance of plants from abiotic stresses by enhancing seed germination, seedling growth, and development [7,10].

Although there have been few studies on the effect of hydropriming on the germination of *O. sativa* L, under salt stress [11, 12, 13], there is limited research on its effect on the variety NSIC Rc 216 subjected to elevated salinity levels.

NSIC Rc 216 rice variety has a wide adaptation under different stresses presented by varying climates across the country thus making it one of the most popular rice varieties in the Philippines [14]. Although it is considered versatile, it was classified as salt-sensitive by Imai and Sevilla [15] which may lead to poor germination and plant growth that can cause yield losses during harvest if subjected to salt stress during germination [4].

This study tested the efficacy of hydropriming in counteracting salt stress in *O. sativa* L., variety NSIC Rc 216, helping determine how generalizable is hydropriming's pro-germination effects to other rice varieties.

More specifically, it aimed to:

(i) determine and calculate the number of seedlings per day, the height of seedlings, and the seed germination parameters: final germination percentage (FGP), germination energy percentage (GEP), speed of germination (SG), and seedling vigor index (SVI);

(ii) determine the effects of different hydropriming durations (12 hours, 24 hours, and 48 hours) on the calculated germination parameters of *Oryza sativa* L. variety NSIC Rc 216 under saline stress; and

(iii) compare and determine if there is a significant difference among the treatments using one-way analysis of variance (ANOVA) and

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Least Significant Difference (LSD) post-hoc analysis using Rstudio and R programming language.

Methods. - Rice seeds were hydroprimed at varying durations (12, 24, or 48 hours) following a completely randomized design (CRD), with unprimed rice seeds as control, as seen in Table 1. They were subsequently air-dried for three (3) hours, then they were allowed to germinate in the prepared germination media and chamber. After seven (7) days, all the germination parameters were measured and recorded. Statistical analysis was then performed.

Table 1. The different hydropriming duration, replicates, and corresponding labels of the different set-ups used.

Hydropriming duration (hours)	No. of seeds per replicate	Replicates
12	50	3
24	50	3
48	50	3
0 (Control)	50	3

Seed authentication, storage, and selection. Rice variety NSIC Rc 216 was acquired from the local farmers at Oluangan, Leon, Iloilo, and authenticated with the help of the Department of Agriculture at Leon, Iloilo.

They were then stored in an airtight container at room temperature until use. The seeds were tested for moisture content to ensure seed viability. Seeds that had moisture content of 14% or less were considered viable, while the rest were discarded.

Seed hydropriming. Fifty (50) seeds per replicate per treatment were selected. They were hydroprimed for 0 (control), 12, 24, and 48 hours using distilled water (Absolute Pure Distilled Drinking Water) with a ratio of 5 mL of water for every 12 rice seeds. The seeds were then air-dried for 3 hours and stored in growing media for germination.

Growing media. A total of 12 Petri dishes, with three (3) layers of filter paper each, were used as growing media. They were kept sealed during the experiment to prevent moisture loss.

Saline stress simulation. To induce salt stress, a 0.15 M saline solution was prepared using a technical grade sodium chloride (NaCl) and distilled water. Ten (10) mL of the prepared solution was then administered evenly to each replicate of each treatment after the hydroprimed and control seeds were sowed on the growing media.

According to Chunthaburee et al. [16], a 0.15 M salt concentration generally induces hyperosmotic stress to rice seeds through ion imbalance.

Growth period and conditions. The Petri dishes were then stored in a germination chamber with LED tubes at a 12-hour light and 12-hour dark photoperiodic cycle with the light intensity maintained at 4000 lux during the light cycle [17]. The seeds were then allowed to germinate for seven (7) days.

Data collection and calculation. Germinated seeds were counted every 24 hours at 6:00 AM, following the procedure by the International Research Institute (IRRI) where both plumule and radicle must be present [18]. After the germination period, 15 sprouted seedlings with the longest lengths (root+shoot) per replicate per set-up were selected and their lengths were recorded. The final germination percentage (FGP), speed of germination (SG), germination energy percentage (GEP), and seedling vigor index (SVI) were then calculated using the following equations [4, 19]:

$$FGP = \frac{No. of\ germinated\ seeds\ on\ the\ 7th\ day}{Number} \times 100$$

$$SG = \frac{No. of\ ger. seeds}{Days\ of\ first\ count} + \cdots + \frac{No. of\ ger. seeds}{Days\ of\ last\ count}$$

$$GEP = \frac{No. of\ seeds\ germinated\ on\ the\ fourth\ day}{Total\ number\ of\ seeds} x\ 100$$

$$SVI = FGP \times seedling\ length\ (root + shoot)$$

Statistical analysis. One-way analysis of variance (ANOVA) was conducted for each calculated parameter of all treatments with a confidence interval of 95% (α=0.05). Least Significant Difference (LSD) post-hoc analysis was then performed using Rstudio (version 1.4.1106, Open Source License).

Safety procedure. The safety data sheet (SDS) for NaCl was secured and the hazards of handling were considered beforehand. NaCl was disposed of in chemical waste containers while the discarded seeds were segregated properly. Proper protective equipment was worn at all times while performing all experimental procedures. All the procedures were done at home to prevent COVID-19 infection.

Results and Discussion. - The study aimed to determine the effects of hydropriming on the germination of *O. sativa* L. var. NSIC Rc 216 under NaCl stress.

After seven days, a germination lag was observed with the control setup for two (2) days and both 12 and 24-hour setup for one (1) day. No germination lag has been observed with the seeds hydroprimed for 48 hours.

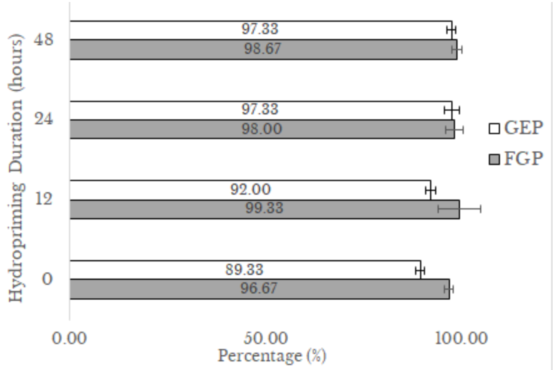

Figure 1. The calculated Final Germination Percentage (FGP) and Germination Energy Percentage (GEP) for all the experimental set-ups.

Final Germination Percentage and Germination Energy Percentage. The highest FGP mean was recorded with the 12 hours of hydropriming of rice, as seen in Figure 1; however, this was not significant when compared to other treatments. The highest recorded GEP mean, on the other hand, was with 24 and 48 hours of hydropriming, both having the same value of 97.33% and were significantly different when compared to the rest of the set-ups with a p-value of 0.03.

The values of both parameters (FGP and GEP) may indicate that hydropriming affects the germination of rice seeds at the earlier stages. This was suggested by the significantly different values for GEP which was a parameter calculated using the data on the 4th day.

With that, seeds germinated faster when hydroprimed at longer durations; however, after some time, seeds hydroprimed at shorter durations germinated as well. This may have caused the FGP values, which was a parameter calculated on the 7th day, to be non-significant.

This is in accordance with the results of Prasad [20] in which GEP also increased with longer durations of hydropriming, with the highest GEP mean recorded with 28 hours of hydropriming. This effect was attributed to the different biological mechanisms triggered by hydropriming, such as the release of enzymes that produce soluble food nutrients for the seeds. This may have enabled the seeds to germinate upon sowing [20].

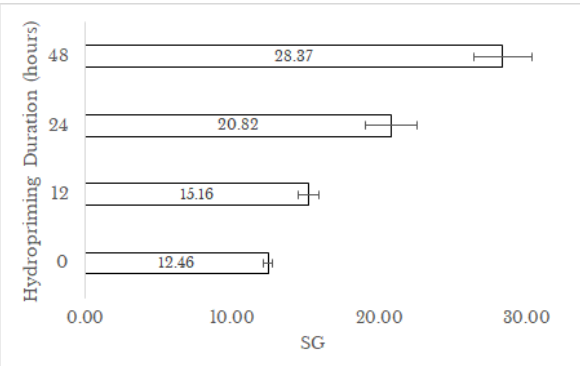

Figure 2. The calculated Speed of Germination (SG) for all the experimental set-ups.

Speed of Germination. The highest SG mean was recorded with the 48-hour hydroprimed seeds, as seen in Figure 2. The SG also increased with longer durations of treatment. This may be caused by the jumpstart in germination through a series of biological and physiological processes such as the acceleration of the emergence phase and multiplication of radicle cells [11, 21, 22]. In the study of Amooaghie [23], it was stated that the early germination stage of plants was "from sowing to seedling emergence" in which they are most vulnerable to external conditions such as salt stress. Hydropriming speeds up the germination process through stimulatory effects through cell division mediation and thus limits the exposure of the seeds to the stressful conditions presented by the environment [11, 24]. This was also in line with the findings of Kaya et al. [9] in which hydropriming of *Helianthus annuus* L. seeds resulted in the acceleration of germination even in low osmotic potential (i.e. salt stress).

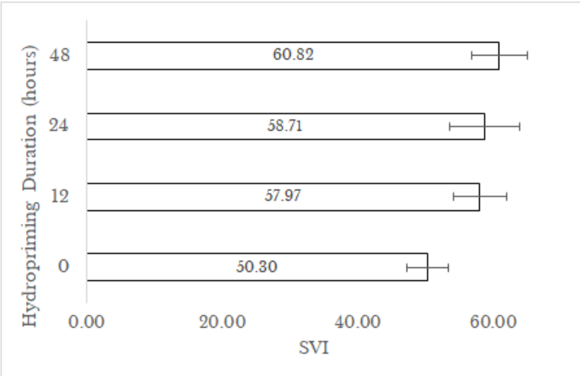

Figure 3. The calculated Seedling Vigor Index (SVI) for all the experimental set-ups.

Seedling Vigor Index. The highest SVI was recorded with 48-hour hydroprimed seeds, as seen in Figure 3. The seedling vigor index increased with the increasing duration of hydropriming; however, it was determined that these values are not significant with a p-value of 0.06. Similar to the FGP, the SVI was not affected probably because the parameter was recorded over a longer period and hydropriming may have only affected the earlier germination stages of rice seeds [24].

The study of Elyasirad et al. [25] had contrasting findings to these results. The study observed that hydropriming *Ferula assa-foetida* has a significant effect on the germination parameters of the seeds, including the SVI [25].

This may be explained by the positive effects of saline content observed by previous studies. An example of this is with Lutts et al. [13] which found out that increased NaCl concentration of up to 50 mM, caused proline accumulation of rice seedlings. This proline accumulation may be responsible for improving the germination of rice seeds by counteracting the effects of salinity by ion detoxification. This protects the plant at the cellular level from osmotic imbalance presented by the saline content of the environment [26]. This may have happened to the unprimed seeds that caused the germination performance in this setup to be comparable to the performance of 48-hour hydroprimed seeds, as evaluated by the SVI.

Limitations. Due to time constraints, this study only observed the effects of hydropriming on limited parameters and only one salinity level has been used. The entire experiment has been done at home which may have affected the overall results of this study specifically with the unavoidable external factors such as humidity, light from other sources within the study site, and resident presence.

Conclusion. - Hydropriming was concluded to only have effects on the early days of rice seed germination, primarily affecting the germination rate but not with the overall germination performance while the rice seeds were being subjected to saline stress. Hydropriming may also be used to accelerate germination of *O. sativa* L.in saline conditions.

Recommendations. - It is highly encouraged to use other Philippine rice varieties to further assess the effects of hydropriming on their germination while being subjected under saline stress. A larger scale of this experiment with a longer duration of observation is also recommended. The replication of the experiment in laboratory and field conditions may also be considered to minimize or completely eliminate the effects of external conditions that can affect the study.

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