Germination of *Oryza sativa* L. NSIC Rc222 under sodium chloride (NaCl) stress hydroprimed at various time durations

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Abstract

Soil salinity decreases plant productivity and induces slow growth especially under very high levels ranging from 9 dS/m to 15 dS/m. To overcome the negative effects of soil salinity, hydropriming or soaking of seeds in distilled water is used as a seed priming technique to activate various metabolic processes in the seed which can promote growth under stress. The study evaluated the germination of *Oryza sativa* L. NSIC Rc222 for seven days under 15 dS/m salt stress hydroprimed at 12 h, 24 h, and 48 h, with unprimed seeds as the control. Germinated seeds were counted every day, and seedling height was measured to compute for the final germination percentage (FGP), speed of germination (SG) and seedling vigor index (SVI). Results show that hydropriming the seeds for 48 h resulted in the highest SG and SVI values while no significant differences were recorded among the FGP of all treatments. Increasing hydropriming duration improves the germination parameters of *Oryza sativa* L. NSIC Rc222 seeds under sodium chloride (NaCl) stress. In conclusion, hydropriming is effective in mitigating the negative effects of saline stress on SVI and SG but it has no effect on germination viability.

Introduction. - One major environmental constraint faced in agriculture is soil salinity, wherein it induces slow growth among plants and subsequently decreases crop yield. Soil salinity is the amount of dissolved salts in the soil which occur as ions, particularly sodium chloride ions. Under increased soil salinity, the osmotic potential in the soil decreases due to the direct effects of ion toxicity which leads to the inhibition of the plant's ability to take up water from the soil [1]. This results in the reduction of several germination parameters such as final germination percentage (FGP) and speed of germination (SG), yielding less vigorous seedlings [2].

The germination and early seedling growth of *Oryza sativa* L. can be negatively affected by increasing salt concentrations [3]. About 48 million hectares of land in humid regions of South and Southeast Asia are fit for *Oryza sativa* L. production but are constrained by soil salinity due to the surface intrusion of saline water from the flooding of rivers and streams during tidal fluctuations and typhoon surges, high actual surface evaporation on dry periods, usage of salinized groundwater for irrigation, and accumulated fertilizer residues [4].

Among the seed priming techniques, hydropriming is the most economical and accessible option in germinating seeds under a variety of stress conditions [5]. Hydropriming is done by soaking seeds in distilled water for a specified number of hours before sowing. It was reported by Sher et al. [6] that hydropriming may improve the stand establishment, seedling vigor, and productivity of field crops under optimal and suboptimal conditions.

Khafagy et al. [7] evaluated the effects of hydropriming on the germination of different *Oryza sativa* L. rice varieties under normal and saline conditions, while Prasad et al. [8] studied rice seedling vigor. Farooq et al. [9] looked at rice hydropriming optimization, but its effect particularly on the NSIC Rc222 variety has not yet been studied. In these three studies mentioned, rice germination was significantly improved which suggests that hydropriming might also be a useful seed priming technique for the Rc222 variety.

The NSIC Rc222 variety of rice is classified by the International Rice Research Institute (IRRI) in the study of Mondal and Borromeo [10] as salt-sensitive rice, susceptible to soils of very high salinity level (9.0-15 dS/m). The evaluation of the germination of *Oryza sativa* L. NSIC Rc222 under salt stress hydroprimed at various time durations is significant to identify more convenient seed treatment methods which can improve its germination performance.

This study evaluated the germination of *Oryza sativa* L. NSIC RC222 under sodium chloride (NaCl) stress hydroprimed for 12 h, 24 h and 48 h. The specific aims are:

(i) evaluate the germination of *Oryza sativa* L. NSIC Rc222 subjected to saline stress hydroprimed for various time durations (0 h, 12 h, 24 h, and 48 h) using the following parameters:

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a. Final germination percentage (FGP),
b. Speed of germination (SG), and
c. Seedling vigor index (SVI);

(ii) evaluate if there is a significant difference among the treatments using Analysis of Variance (ANOVA) at $\alpha = 0.05$ and Least Significant Difference (LSD) using Microsoft Excel Software for Windows 10.

Methods. - *Oryza sativa* L. NSIC Rc222 seeds were hydroprimed by soaking in distilled water for 12, 24, and 48 hours following a randomized complete block design while the control group was left unprimed. Seeds were air-dried for two (2) hours and were subjected to 15 dS/m saline solution to simulate salt stress and to 0 dS/m as control in their corresponding petri dishes. These were left to germinate for seven (7) days inside a temperature-controlled incubator [8]. Germinated seeds were counted every 24 hours and all the germination parameters were measured at the end of seven (7) days. Data analysis was then carried out.

Acquisition of Seeds. Certified *Oryza sativa L.* NSIC RC222 seeds were acquired from the Department of Agriculture - Western Visayas Integrated Agricultural Research Center. The seeds were stored in an airtight container at room temperature until ready for use.

Construction of the Incubator. An incubator with dimensions 0.6m x 0.45 m x 0.45 m was constructed (Figure 1) and covered with glass on top. A heat lamp was attached to a temperature controller set to 29 ± 0.2 degrees Celsius and four (4) LED tubes are fixed on top of the incubator at a 12-hour light and 12-hour dark photoperiodic cycle with the light intensity maintained at 4000 lux during the light cycle [11].

Figure 1. Incubator setup.
Left: top view, with petri dishes containing RC222 seeds.
Right: front view.

Preparation of Seeds. The seeds were surface-sterilized following the procedures used by Khafagy et al. [7]. Seeds were soaked in 1.0% (v/v) sodium hypochlorite solution for three minutes. The residual chlorine was washed out using distilled water. Seeds were divided into four sub-samples and assigned to treatment groups with a total of six replicates each using randomized complete block design. One petri dish containing 25 seeds was used per replicate and each was labeled according to the treatment group and replicate number.

Seed Hydropriming. The seeds were hydroprimed by soaking in a beaker filled with distilled water for 12, 24, and 48 hours, with a ratio of six seeds per ten mL of water. The seeds were subsequently air-dried for two hours.

Preparation of Growing Media and Sowing of Seeds. Two layers of Whatman No, 1 filter paper were placed in each of the 24 petri dishes (9 cm diameter) to serve as the growing medium for the seeds. The 25 seeds were sown in a 1:8:16 circular fashion and stored in the growing media to germinate for seven days.

Simulation of Saline Stress. The 15 dS/m saline solution was prepared by dissolving 9.6 grams of NaCl in one liter of distilled water. A PASCO conductivity probe with ± 10% accuracy was used to measure the salinity in a stepwise manner. Three replicates of each treatment were treated with 10 mL of the 15 dS/m saline solution while the remaining three replicates were given 10 mL of distilled water for control.

Counting of Germinated Seeds and Measurement of Seedling Height. The number of germinated seeds were counted every day for seven days while the seedling height was measured at the end of the germination period. Ten (10) seedlings were randomly selected from each replicate for the measurement of the seedling height using a vernier caliper [7]. The mean of the selected seedling heights per replicate was then calculated.

Data Analysis. The germination parameters, namely the final germination percentage (FGP), speed of germination (SG), and seedling vigor index (SVI) of the germinated seedlings for each treatment group were compared using One-Way Analysis of Variance (ANOVA). If the One-Way ANOVA result is significant, then Fisher's Least Significant Difference (LSD) was used as the post-hoc test for multiple comparisons at $\alpha = 0.05$. All data analyses were done using Microsoft Excel.

$$FGP = \frac{Number\ of\ germinated\ seeds}{Number\ of\ total\ seeds} \times 100\%$$

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

$$SG = \frac{Number\ of\ germinated\ seeds}{Days\ of\ first\ count} + \ldots\ldots + \frac{Number\ of\ germinated\ seeds}{Days\ of\ final\ count}$$

Safety Procedure. Sodium chloride is an eye irritant, thus wearing external protective clothing such as gloves, eye, and face protection were practiced. Eye irritations were mitigated by washing the eyes thoroughly with water. There were also risks associated with the use of sodium hypochlorite such as skin irritation and serious eye damage. Throughout the experiment, the chemicals were handled with care and external protective clothing was always worn. Direct contact with the chemical was mitigated by thorough washing.

Results and Discussion. - The study aimed to evaluate the germination of *Oryza sativa* L., NSIC Rc222 under NaCl stress hydroprimed for various time durations.

Final Germination Percentage. The highest FGP mean among Rc222 seeds subjected to 15 dS/m saline stress was found in 48 h hydropriming duration with 94.67% mean FGP, followed by 12 h and 24 h while the lowest was found in the unprimed

seeds with 82.67% mean FGP (Figure 2). It must be noted that the mean FGP of the unprimed seeds was lower than 85%, which indicates a significant breakdown in its processes as the seeds were adversely affected by salt stress. Meanwhile, in the 0 dS/m treatment, the 24 h hydropriming treatment yielded the highest FGP mean with 96.00%, followed by 48 h and the lowest in the 12 h treatment and unprimed seeds with both an FGP mean of 93.33%.

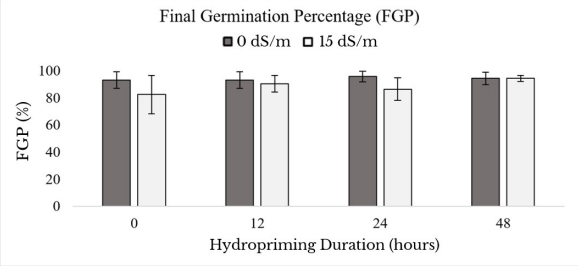

Figure 2. Final Germination Percentage (FGP) between control and saline-stressed conditions (mean values, n=3 per condition).

There is no significant difference among the mean FGP values of all treatment groups in both 0 dS/m and 15 dS/m saline level with a p-value of 0.05 (Table 1). It was observed that the seeds hydroprimed at longer durations and subjected to saline stress exhibited maximum germination in the fourth or fifth day, while most of the unprimed seeds germinated by the sixth and seventh day. At the end of seven days, however, most seeds from all treatment groups have already germinated. Hydropriming, in general, and the various soaking durations did not significantly affect the FGP of Rc222 seeds grown in 0 dS/m (no saline) and 15 dS/m saline treatments.

Table 1. Multiple mean comparisons for the FGP values among all treatment groups (n=3 per condition).

Saline Concentration	Final Germination Percentage (%)				
	0 h	12 h	24 h	48 h	p-value
0 dS/m	93.33	93.33	96.00	94.67	ns
15 dS/m	82.67	90.67	86.67	94.67	ns

ns = not significantly different at $p \leq 0.05$

Hydropriming seemed to have affected the germination of *Oryza sativa L.* only at the earlier stages, especially among seeds hydroprimed at longer durations. This could be due to the different biological mechanisms triggered by hydropriming such as the release of enzymes that produce soluble food nutrients which enabled the seeds to germinate upon sowing [8]. After some time, however, seeds hydroprimed at shorter durations germinated as well due to the optimum light and temperature conditions present inside the incubator [12]. These conditions eventually allowed the seeds from all treatment groups to grow to a minimum of 2 mm radicle length by the end of seven days. Due to this, the FGP values among all treatment groups in the study were almost similar in both normal and saline conditions, and thus, no significant difference was recorded.

Speed of Germination. The highest SG mean recorded for the 15 dS/m saline treatments was with 48 h hydropriming with SG mean of 34.8, followed by 24 h, 12 h, and the lowest from unprimed seeds with 7.69 (Figure 3). Similar results were observed among the seeds subjected to 0 dS/m, wherein the highest SG mean was recorded in 48 h hydropriming with mean of 43.37, followed by 24 h, 12 h, and the lowest from the unprimed seeds with SG mean of 21.6.

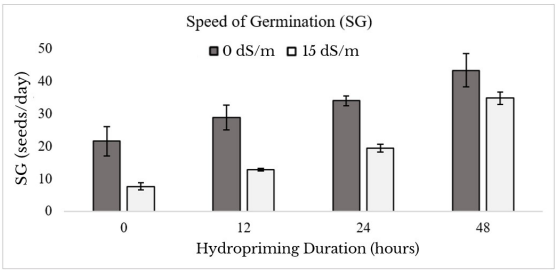

Figure 3. Speed of Germination (SG) between control and saline-stressed conditions (mean values, n=3 per condition)

Hydropriming significantly increased the speed of germination of Rc222 seeds, wherein under both 0 dS/m and 15 dS/m saline treatments, 48 h hydropriming obtained the highest SG values with 34.80 seeds/day and 43.37 seeds/day, respectively (Table 2). It was observed that the seeds hydroprimed at these durations germinated the earliest upon sowing while the unprimed seeds and 12 h hydropriming have only started to germinate on the third day. Under 0 dS/m, the SG values are comparable between the unprimed seeds and the 12 h hydropriming, as well as between 12 h and 24 h hydropriming treatments. Subjecting saline stress significantly decreased the mean SG values among Rc222 seeds but hydropriming at particularly longer durations mitigated these negative effects.

Table 2. Multiple mean comparisons for the SG values among all treatment groups (n=3 per condition).

Saline Concentration	Speed of Germination (seeds/day)				
	0 h	12 h	24 h	48 h	p-value
0 dS/m	21.56[b]	28.93[a b]	33.98[a]	43.37	<0.001*
15 dS/m	7.69	12.91	19.42	34.80	<0.001*

* Significantly different at $p \leq 0.05$
[z]In a row per saline concentration, means with the same letters are not significantly different at 5% level of significance.

Hydropriming *Oryza sativa L.* NSIC Rc222 seeds mitigated the effects of salt stress because the speed of germination (SG) increased as hydropriming duration was increased. The faster speed of germination (SG) in longer soaking durations was caused by biological and physiological processes such as the acceleration of the emergence phase and

multiplication of radical cells which limit the exposure of the seeds to the stressful conditions in the environment [13]. Findings were similar in studies conducted on *Triticum aestivum L*. (wheat) by Basra et al. [14] and *Momordica charantia* (gourd) seeds by Adhikari et al. [15] wherein increasing the duration of hydropriming up to 48 hours significantly improved the duration of germination. Hydropriming seeds enabled the completion of metabolic activities prior to planting which reduced the time for the seed to germinate [16].

Seedling Vigor Index. Among the seeds subjected to 15 dS/m saline stress, SVI increased with longer durations of hydropriming, wherein the 48 h hydropriming yielded the highest SVI with 7320.20, followed by the 24 h, 12 h, and the lowest in the unprimed seeds with 2453.00 (Figure 4). The seeds grown in 0 dS/m also yielded an increasing SVI with longer hydropriming durations except for the 48 h hydropriming which obtained a lower SVI of 8912.16 than the 24 h hydropriming which yielded an SVI mean of 10348.47.

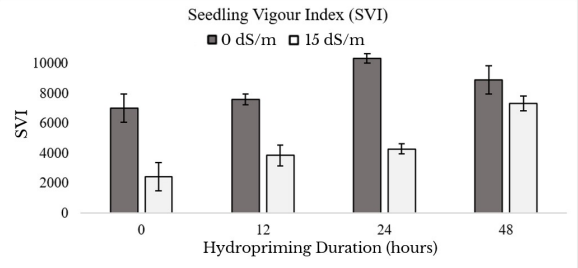

Figure 4. Seedling Vigor Index (SVI) in control and saline conditions (mean values, n=3 per condition).

Seeds hydroprimed for longer durations and subjected to saline stress have visibly grown higher seedling heights in a shorter period, which explains the increasing SVI values except for the 24 h hydropriming which obtained a significantly higher SVI compared to the 48 h hydropriming under 0 dS/m (Table 3). The seeds subjected to 15 dS/m salt stress exhibited the highest SVI with 48 h hydropriming, where the mean value was found to be significantly higher compared to the 24 h, 12 h hydropriming treatments and the unprimed seeds.

Table 3. Multiple mean comparisons for the SVI values among all treatment groups (n=3 per condition).

Saline Concen-tration	Seedling Vigor Index				
	0 h	12 h	24 h	48 h	p-value
0 dS/m	7019[b]	7617[ab]	10348	8912[a]	0.002*
15 dS/m	2453	3860[a]	4293[a]	7320	<0.001*

* Significantly different at $p \leq 0.05$
[z]In a row per saline concentration, means with the same letters are not significantly different at 5% level of significance.

Under 0 dS/m treatment, 24 h hydropriming resulted in a higher SVI than 48 h hydropriming treatment. This correlates with a previous study by Kumar et al. [17] that determined 24 h as the maximum length of time for which *Oryza sativa L.* seeds should be soaked, as exceeding it could cause seed deterioration. Although imbibition is vital for the re-constitution of biomembranes, activation of enzymes, mobilization of storage compounds, and protein synthesis in the seed, it can also cause imbibition damage, particularly when water is taken up rapidly [7]. Meanwhile, the SVI values obtained for the 15 dS/m saline treatment in the current study were similar to Khafagy et al. [7] where the highest SVI was with *Oryza sativa L.* seeds hydroprimed for 48 h compared to 12 h, 24 h, and 36 h. This suggests that hydropriming for longer durations increases the SVI especially when the conditions such as saline stress deem seed repair to be necessary.

Limitations. In the preparation of the 15 dS/m saline solution, table salt was used which may have possibly contained other additives aside from the 97% NaCl that could induce negative effects on the germination parameters of the seeds. Moreover, there might have been some discrepancies in the computation of the SG values due to the inconsistencies in the schedule of counting of germinated seeds as caused by the conflicting schedules of the researchers. Lastly, in the measurement of the seedling height using vernier caliper, the roots and leaves were straightened out by hand and laid flat on a piece of paper. Due to the risk of breaking the seedling, the roots may not have been straightened out entirely and uniformly, which rendered the measurement of the seedling height and the computation of the SVI to be less precise.

Conclusion. - Hydropriming was concluded to not have any significant effect on the final germination percentage but can effectively accelerate the growth and significantly improve the seedling vigor of Rc222 seeds subject to 15 dS/m salt stress. Moreover, 48 h hydropriming is found to be the most effective soaking duration in alleviating the effects of saline stress among *Oryza sativa L.* NSIC Rc222 seeds.

Recommendations. - It is recommended that *Oryza sativa* L. NSIC Rc222 (rice) seeds should be hydroprimed for 48 hours to mitigate the negative effects on its germination. Furthermore, it is recommended that a uniform schedule for data collection will be imposed, especially for the counting of germinated seeds. Use of digital software such as ImageJ may also be considered for a more precise measurement of the seedling height. Lastly, it is recommended that technical grade sodium chloride salt shall be used in making the saline solution to minimize the presence of other compounds that may also affect the germination of the *Oryza sativa* L. NSIC Rc222 seeds.

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References

[1] Munns R, James R, Lauchi A. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. J. Exp. Bot. *57*(5): 1025–1043. DOI: 10.1093/jxb/erj100

[2] Ucarli C. 2020. Effects of salinity on seed germination and early seedling stage. IntechOpen. DOI: 10.5772/intechopen.93647

[3] Hakim MA, Juraimi AS, Begum M, Hanafi MM, Ismail MR, Selamat A. 2010. Effect of salt stress on germination and early seedling growth of rice (*Oryza sativa* L.). Afr. j. biotechnol. 9(13) : 1911-1918. DOI: 10.15835/nsb.6.2.9163

[4] Department of Agriculture. 2001. Management of salt-affected soils for rice production. Rice Technol. Bulletin. 2001(40). URL: bit.ly/3NDyZyY

[5] Toselli ME, Casenave EC (2005) Hydropriming and cottonseed germination under unfavorable conditions: Modifications in hydrotime model parameters. Seed Sci. Technol. 33(1): 87-96. DOI: 10.15258/sst.2005.33.1.09

[6] Sher, A., Sarwar, T., Nawaz, A., Ijaz, M., Sattar, A., Ahmad, S., 2019. Methods of Seed Priming. In M. Hasanuzzaman V. Fotopoulos (Eds), Priming and Pretreatment of Seeds and Seedlings, pp. 1-11. DOI: 10.1007/978-981-13-8625-1_1

[7] Khafagy, M.A., Darowish, M.M., Salama, S.M. and Abo-El-Kheer, E.A.M. 2014. Effect of water priming duration on rice (*Oryza sativa* L.) germination and seedling growth under ISO-osmotic solution of NaCl and PEG. J. Plant Production, Mansoura Univ., 5(12):2141 -2157. DOI: 10.21608/jpp.2014.64853

[8] Prasad S, Prasad B, Singh RK (2012) Effect of hydro-priming duration on germination and seedling vigour of rice (*Oryza sativa* L.) cv. Prasad. J. Crop Weed, 8(1):65-71. URL: bit.ly/3QbMqrI

[9] Farooq M, Basra SMA, Afzal I, Khaliq A (2006) Optimization of hydropriming techniques for rice seed invigoration. SeedSci. Technol., 34: 507-512. DOI: 10.15258/sst.2006.34.2.25

[10] Mondal S, Borromeo TH (2016) Screening of salinity tolerance of rice at early seedling stage. J. Biosci. Agric. Res., 10(1): 843-847. DOI: 10.18801/jbar.100116.102

[11] Anuradha S, Rao SSR. 2003. Application of brassinosteroids to rice seeds (*Oryza sativa* L.) reduced the impact of salt stress on growth, prevented photosynthetic pigment loss, and increased nitrate reductase activity. Plant Growth Regul. 40(1): 29–32. DOI: 10.1023/A:1023080720374

[12] Damalas CA, Koutroubas SD, Fotiadis S. 2019. Hydro-Priming Effects on Seed Germination and Field Performance of Faba Bean in Spring Sowing. Agric., 9(201). DOI: 10.3390/agriculture9090201

[13] Ibrahim N, Bhadmus Z, Singh A. 2013. Hydro-Priming and Re-Drying Effects on Germination, Emergence, and Growth of Upland Rice (*Oryza sativa* L.). Nig J Basic Appl Sci., 2013: 21(2). DOI: 10.4314/njbas.v21i2.11

[14] Basra SMA, Farooq M, Tabassum R and Ahmed N. 2005. Physiological and biochemical aspects of seed vigor enhancement treatments in fine rice *(Oryza sativa* L.). SeedSci. Technol., 33: 623-628. DOI: 10.15258/sst.2005.33.3.09

[15] Adhikari B, Dhital PR, Ranabhat S, Poudel H. 2021. Effect of seed hydro-priming durations on germination and seedling growth of bitter gourd (*Momordica charantia*). PLoS ONE 16(8): e0255258. DOI: 10.1371/journal.pone.0255258

[16] Sadeghi H, Khazaei F, Yari L, Sheidaei S. 2011. Effect of seed osmopriming on seed germination behavior and vigor of soybean (Glycine max L.). ARPN J Agricult Biol Sci 6(1):39-43. URL: bit.ly/3NEPuuR

[17] Kumar A, Gangwar JS, Prasad SC and Harris D. 2002. On-farm seed priming increases yield of direct–sown finger millet in India. International Sorghum Millets Newsletter 43: 90–2. DOI: 10.1017/CBO9781107415324.00