DOI: https://doi.org/10.4038/atrsj.v4i1.57

ISSN: 2792 – 1360 *Journal of Agro-Technology and Rural Sciences* *Volume 4, Issue 1; July 2024*

SODIUM CHLORIDE PRIMING IMPROVES SEED GERMINATION AND INITIAL GROWTH OF CHILI (*Capsicum annuum* L.)

Hasara Y.A.K., Sugathadasa M.V.U.M, Indurugalla I.A.Y.R., D. Manimekala D.G.H.D.S., Harshana Galahitigama* and Dissanayake D.D.M.O

Department of Export Agriculture, Faculty of Agricultural Sciences, Sabaragamuwa University of Sri Lanka, Belihuloya

**Corresponding Author: harshana@agri.sab.ac.lk (https://orcid.org/0000-0003-0158-2519)*

ABSTRACT

Among various priming methods, "Halo priming" emerges as a simple and cost-effective agro-technique employed by farmers to mitigate several consequences. Hence, a laboratory experiment was conducted to assess the impact of sodium chloride (NaCl) priming on chili seed germination and initial seedling growth performances. Four concentration levels of NaCl solutions (1%, 5%, 10%, and 15% w/v basis) were utilized, with different immersion durations (3, 6, 9, and 12 hours) used as treatments. Petri dishes were arranged according to a Completely Randomized Design (CRD) with tri-replicates. Germination parameters, including seed germination, mean germination time, coefficient of germination velocity, germination index, and seedling vigor index, were measured. Additionally, seedling parameters such as shoot length, root length, leaf area, plant fresh and dry weights were recorded. Data were analyzed using two-way ANOVA, and mean separation was conducted using Dunnett's test. Correlation analysis was performed to identify relationships among the tested parameters. Among the tested concentrations, the highest germination percentage (76.67±8.8%), germination index (31.08±4.0), coefficient of germination velocity (1.12±0.05), vigor index (2.68±0.14), and the lowest mean germination time (6.91±0.4 days) were reported in the 10% NaCl treatment. The 10% NaCl solution significantly enhanced seed germination-related parameters compared to hydro-priming. Furthermore, 10% NaCl treatment promoted initial seedling growth compared to hydro priming. Correlation analysis revealed a positive correlation between shoot length and germination index. However, seed priming duration had no observable effect on chili seed germination.

Keywords: Chili, Germination, Halo priming, Seed priming, Sodium chloride

INTRODUCTION

Seed priming is a pre-sowing treatment involving the controlled hydration of seeds, sufficient to allow pre-germinative metabolic events to occur but insufficient to allow radicle protrusion through the seed coat (Indurugalla et al., 2023). It also interferes with complex physiological and biochemical processes, which offers an effective means to improve seed quality, seed germination and vigor. However, many factors affect the response of seed germination to priming, such as crop variety, priming agent and its concentration, priming duration and temperature (Rhaman et al., 2020). Furthermore, improving specific physiological, biochemical, cellular and molecular changes, including rapid, uniform and increased germination, and improved seedling vigor and growth under a broad range of environmental conditions resulting in better field-level stand establishment (Galahitigama and Wathugala, 2016). Though there are various seed-priming methods available, halo-priming is defined as submerging seeds in inorganic salt solutions, as a cheap and simple seed-priming method among other different priming techniques (Galahitigama et al., 2021). Halo priming can affect osmoregulation in seeds by the active uptake of inorganic ions, and such ions play a vital role in balancing membrane potential and turgor, activating enzymes, and regulating osmotic pressure in cells (Ibrahim, 2016).

Chili is an important Solanaceae crop cultivated for spice production from its fresh, ripe and unripe fruits. Chilies are also used in various forms, whereas it plays a crucial role in adding heat, flavor, and spice to many traditional dishes (Yadav et al., 2011). Proper seed germination at field is important for chili farmers because it ensures higher crop production, crop uniformity, predictability, crop health, and economic efficiency (Yadav et al., 2011). Thus, the initial stage is more critical in the plant's life cycle setting the foundation for a successful and productive farming operation. However, it has been reported that seed germination of chili is non-uniform and slow under unprimed conditions and in different abiotic stresses (Yadav et al., 2011). Simultaneously, in halo-priming studies, researchers used very low-concentrated NaCl solutions (<1% NaCl w/v basis), and the impact of higher concentrations is still remains as a mystery to the world of science. Considering these aspects, a laboratory and a pot trial was conducted to investigate the sodium chloride halo priming impact on seed germination and initial growth of chili.

MATERIALS AND METHODS

Experiment 1

The experiment was carried out in the Agronomy laboratory, Faculty of Agricultural Sciences, Sabaragamuwa University of Sri Lanka located at A 6°42' North latitude and 80°47' East longitude. “MICH-3” chili variety (most of local farmers widely grown in the area) was used for the experiment. Chili seeds were purified with 1% NaClO (sodium hypochlorite) for few seconds and washed with tap for 5 minutes before applying priming solutions. Under the experiment 1, four different NaCl concentrations (5%, 10%, 15%, and 20% w/v basis) were used to promote seed germination with four immersion durations (3, 6, 9, and 12 hours) respectively. Therefore, sixteen treatments were applied with three replicates and the experiment was laid out in Completely Randomized Design (CRD). Primed and non-primed seeds were placed in 9 cm glass petri dishes on a layer of filter paper (Whatman 42). Ten seeds, were placed in each petri dish. Petri dishes were placed in a germinator at 25°±1^{0}C with 12 hours of light and 12 hours of dark condition. Final germination as percentage of total seeds, mean germination time, coefficient of germination velocity, germination index, and seedling vigor index were measured as germination parameters. Seeds were considered to have germinated normally when radical and two leaves were visible. In addition, root and shoot lengths were measured by using ruler. Plant fresh weight was measured by using an analytical balance.

The final germination percentage, mean germination time, Coefficient of Velocity of Germination, and germination index were calculated by using the formula (Kadeer, 2005).

Final germination percentage (FGP)

FGP= (final number of seeds germinated / total seed) × 100

Mean germination time (MGT)

Mean germination time **= Σ giti/gi,**
where:
gi = number of seeds newly germinated on ith day of observation
ti = number of days on which observation is taken

Coefficient of Velocity of Germination (CVG)

CVG=N1 + N2 + · + Nx/100 × N1T1 + · + NxTx
N=number of seeds germinated each day
T=number of days from seeding corresponding to N

Germination Index (GI)

GI=(10×n1) + (9×n2) + · · · + (1×n10)
n1, n2 . . . n10 = number of germinated seeds on the first, second and subsequent days until the 10th day

Vigor index (VI)

The vigor index was calculated by using following formula (Sadeghi et al., 2011)
VI=seedling length (cm) × germination percentage

Experiment 2

The pot trial was conducted in a greenhouse at theFaculty of Agricultural Sciences, Sabaragamuwa University of Sri Lanka. The monthly average maximum and minimum temperatures were 29.3°C and 18.8°C respectively, while relative humidity was 80%. Pots of diameter 6 cm and height 8 cm were filled by adding an equal quantity of potting media (sand: sieved topsoil: compost at 1:1:1 ratio). Then the mixture was sterilized with fungicide (Captan 50% w/w). Seedling parameters such as shoot length, root length, plant fresh and dry weights were recorded four weeks after seed establishment. Seedling fresh weight was recorded by using analytical balance, and dry weight was taken by keeping 10 seedlings from each replication for drying in oven at 45°C for 48 hours (Bajehbaj, 2010). The leaf area was calculated by using following equation (Lakitan et al., 2022).
Leaf area = [Leaf width (cm) × Leaf length (cm)] × 0.658

Statistical analysis

The statistical analysis was performed using the “SAS® On Demand for Academics” platform. Significant differences between treatments were evaluated using the two-way ANOVA procedure. Means were compared between treatments using Tukey's multiple comparison test at the 0.05 confidence level. R software was used to conduct correlation analysis among germination parameters.

RESULTS AND DISCUSSION

Overall, seed priming treatments were not significantly affected by immersion duration (p> 0.05), indicating that priming time can be adjusted in this experiment. However, NaCl concentration significantly affected germination and other measured parameters. According to previous studies, significant behavior from the seed immersion time duration has been reported in multiple studies using more considerable seed immersion times (e.g; 24, 48, and 72 hours) and lower NaCl concentrations (<1% NaCl w/v) (Quintero et al., 2018). Thus, the influence of the immersion period on the parameters under consideration may have been modified by employing such periods.

Table 1: Germination parameters of chili plants after exposed to water and different concentrations of NaCl concentrations

*FGP-final germination parameter; VI-vigor index; CVG-coefficient of germination velocity; GI-germination index; MGT-mean germination time. The data represent mean ± standard error. Different letters in the table indicate significant differences between treatments at P < 0.05.

Priming treatment	Measured parameters							
	FGP	MGT	GI	CVG	VI	Root length (cm)	Shoot length (cm)	Fresh weight (mg)
NaCl 1%	53.33±8.8^{ab}	8.45±1.1^{b}	14.75±6.4^{b}	1.08±0.1^{ab}	2.28±0.2^{b}	1.89±0.2^{b}	1.86±0.1^{b}	182.3±9.9^{a}
NaCl 5%	58.33±13.3^{ab}	8.75±0.7^{b}	15.67±6.8^{b}	1.06±0.0^{b}	2.29±0.2^{b}	1.85±0.2^{b}	1.87±0.2^{b}	157.08±7.7^{ab}
NaCl 10%	76.67±8.8$_{a}$	6.91±0.5^{a}	31.08±4.0^{a}	1.12±0.1^{a}	2.68±0.1^{a}	3.13±0.1^{a}	3.4±0.1^{a}	229.5±5.2^{ab}
NaCl 15%	59.17±9.9^{ab}	8.17±0.9^{b}	18.00±5.8^{b}	1.09±0.1^{ab}	2.38±0.1^{b}	2.10±0.2^{ab}	2.15±0.1^{b}	156±7.4^{ab}
Water	51.33±5.7^{b}	9.30±0.9^{b}	9.25±3.4^{b}	1.03±0.1^{b}	2.01±0.1^{b}	1.12±0.1^{b}	1.2±0.1^{b}	84.25±4.3^{b}

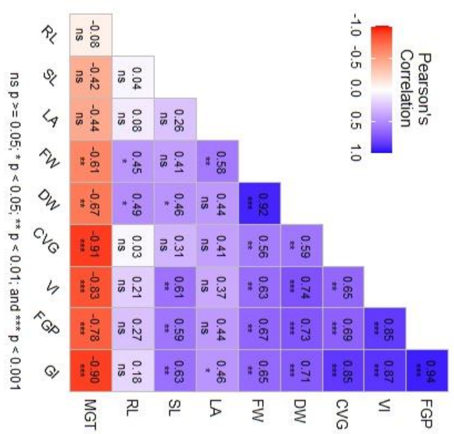

Figure 1: Correlation heat map among the growth parameters. FGP-final germination parameter; VI-vigor index; CVG-coefficient of germination velocity; GI-germination index; MGT-mean germination time; RL-root length; SL-shoot length; FW-fresh weight; DW-dry weight; LA-leaf area

Table 2: Initial growth parameters of chili plants after exposed to water and different NaCl concentrations

Priming treatment	Measured parameters				
	Root length (cm)	Shoot length (cm)	Leaf area (cm^2)	Fresh weight (mg)	Dry weight (mg)
NaCl 1%	4.37±0.2^{b}	4.17±0.1^{b}	1.08±0.1^{a}	96.5±3.0^{a}	73.16±2.5^{ab}
NaCl 5%	5.92±0.1^{a}	4.43±0.1^{ab}	1.07±0.1^{a}	86.25±2.6^{a}	68.6±2.8^{ab}
NaCl 10%	5.29±0.1^{ab}	5.85±0.1^{a}	1.01±0.0^{a}	111.6±1.7^{a}	105.9±1.7^{a}
NaCl 15%	5.20±0.1^{ab}	3.95±0.1^{b}	0.69±0.0^{ab}	85.41±1.4^{a}	77.33±1.5^{a}
Water	4.55±0.1^{ab}	4.25±0.1^{ab}	0.51±0.1^{b}	29.45±1.9^{b}	24.25±2.0^{b}

According to the statistical analysis, NaCl treatments exhibited varied effects on germination performance and seedling growth parameters compared to hydropriming. NaCl 1% (53.33%) and NaCl 5% (58.33%) slightly improved FGP over hydropriming (51.33%), though the differences were not statistically significant. NaCl 15% showed a marginal improvement in FGP (59.17%) compared to water but fell short of the highest germination performance achieved with NaCl 10%. NaCl 1%, NaCl 5%, and NaCl 15% all accelerated germination relative to water, suggesting enhanced germination dynamics through salt priming. The GI increased across all NaCl treatments, with NaCl 15% achieving double the GI of water. NaCl 1% and NaCl 5% demonstrated moderate improvements in GI compared to water. Salt priming moderately improved VI compared to water, with NaCl 1% (2.28), NaCl 5% (2.29), and NaCl 15% (2.38) surpassing the results of hydropriming. Among these treatments, seed priming with 10% NaCl resulted in the highest final germination percentage (FGP) compared to other treatments (Table 1). In contrast, the lowest germination percentage was observed in the hydro-priming treatment. Specifically, the 10% NaCl solution increased germination percentage by approximately 25% compared to hydro-priming. Similarly, mean germination time (MGT), germination index (GI), and vigor index (VI) values differed significantly among the treatments ($p < 0.05$) (Table 1). The 10% NaCl treatment significantly reduced the mean germination time, indicating earlier germination. A similar trend was observed for the GI and VI parameters, with the highest values recorded in the 10% NaCl-treated seeds, while the lowest values were found in the hydro-priming treatment. Compared to hydro-priming, GI and VI values in the 10% NaCl treatment were approximately 236% and 33%. .higher, respectively. However, increasing NaCl concentration beyond 10% did not further impact GI or VI. Additionally, seed priming with 10% NaCl enhanced the coefficient of velocity of germination (CVG), though this effect was not statistically significant (Table 1). A similar pattern was observed for plant height, where significant plant height was recorded in the 10% NaCl treatment compared to the other treatments. While root length and fresh weight were highest in the 10% NaCl-treated plants, these differences were not statistically significant.

According to the statistical analysis of the pot experiment, NaCl treatment significantly impacted on early plant growth compared to hydro-priming. Root and shoot lengths increased by approximately 50% under NaCl 1%, NaCl 5%, and NaCl 15% compared to water, indicating enhanced seedling development. Additionally, fresh weight was significantly influenced by salt priming. NaCl 1% exhibited a dramatic increase in fresh weight (~116% higher than water), while NaCl 5% and NaCl 15% achieved ~86% increases. However, among the treatments, plants treated with 10% NaCl exhibited remarkable growth in leaf area, fresh weight, and dry weight (Table 2). For instance, the leaf area of plants treated with 10% NaCl was nearly double that of those in the water treatment. Additionally, fresh and dry weights increased approximately fourfold compared to the hydro-priming treatment.

The correlation analysis revealed both significant positive and negative correlations (Figure 1). The results indicated that root length, shoot length, fresh weight, dry weight, leaf area, final germination percentage, vigor index, coefficient of germination velocity, and germination index were all negatively correlated with mean germination time. Additionally, shoot length showed a positive correlation with vigor index (R = 0.61) and germination index (R = 0.63). A strong positive correlation was observed between fresh weight and dry weight (R = 0.92). Root length was also positively correlated with fresh weight (R = 0.45), dry weight (R = 0.49), shoot length (R = 0.04), and leaf area (R = 0.08).

Initial plant growth and development depend largely on the germination performance of the seeds (Nawaz et al., 2011). Seed germination can be influenced by priming techniques, which enhance both the rate and uniformity of germination (Nimac et al., 2018; Galahitigama et al., 2021). In the current study, seed priming with NaCl significantly affected chili seedling emergence and germination rate. Among the NaCl concentrations tested, 10% NaCl yielded the highest germination and vegetative growth performance.

As shown in Table 1, seed priming with 10% NaCl resulted in superior performance across various parameters, including final germination percentage, mean germination time, coefficient of germination velocity, germination index, and vigor index, compared to other treatments. The early germination observed in NaCl primed seeds may be due to rapid water uptake (Nawaz et al., 2011). The reduction in germination time can be attributed to early reserve breakdown, enhanced mobilization of stored nutrients, and the early activation or synthesis of enzymes involved in cell wall degradation (Nawaz et al., 2011).

Interestingly, as NaCl concentration increased, the germination rate declined. This finding aligns with Aymen et al. (2012), who reported that priming fenugreek seeds with more than 4 gL^{-1} NaCl reduced seed emergence. Similarly, Youssef et al. (2021) found that exceeding a certain threshold of NaCl concentration may not promote germination, as toxic levels of sodium and chloride ions negatively affect embryo viability. In addition, Tester and Davenport (2003) explained that increased sodium in roots has a toxic effect that causes physical damage and reduces their capacity to absorb water and nutrients. This results in a decrease in protein synthesis, photosynthesis, and enzymatic processes. Additionally, toxic levels of Na^+ and Cl^- accumulate in leaves, interfering with metabolic processes and causing stunted growth and reduced leaf area. Furthermore, accumulation of these toxic ions impedes nutrient transport and reduces the efficiency of seed reserve mobilization during germination (Youssef et al., 2021).

In terms of vegetative growth, the 10% NaCl halo-priming treatment significantly improved shoot weight, root length, leaf area, fresh weight, and dry weight of chili plants. These results are consistent with Biswas et al. (2019), who reported enhanced vegetative growth in tomatoes following NaCl seed priming. In addition, according to the literature, high concentrations of NaCl may enhance seed germination by triggering a hormetic response. This might be associated with the activation of antioxidat enzymes, the accumulation of osmo-protectants such as proline, soluble sugars, and betaines, and the upregulation of genes encoding dehydrins, heat shock proteins, and other protective molecules (Cao et al., 2018; Jatana et al., 2024). However, increasing NaCl concentrations beyond optimal levels has been shown to progressively inhibit plant performance, likely due to salinity-induced physiological stress from water and nutrient imbalances (Aymen et al., 2012). This stress can disrupt plant metabolism, leading to reduced growth and productivity.

CONCLUSION

This study concludes that NaCl seed priming significantly improved the germination and early growth attributes of chili plants compared to hydro-priming. However, the duration of seed priming had no observable effect on germination. Among the tested NaCl concentrations, 10% NaCl (w/v) priming notably enhanced germination rates and early growth stages. The correlation matrix reveals strong positive relationships among FGP, VI, CVG, DW, and FW, indicating their association with germination performance and vigor. MGT shows a significant negative correlation with FGP, GI, and VI, highlighting its inverse link to germination efficiency. RL and SL have weak or nonsignificant correlations with other parameters, suggesting limited impact on germination or vigor. However, further research is needed to understand the physiological and biochemical mechanisms underlying the effects of NaCl seed priming.

REFERENCES

Aloui, H., Mohamed Aymen, E., Chérif, H. (2017). Seed priming to improve seedling growth of pepper cultivars exposed to salt concentrations. *International Journal of Vegetable Science*, *23*(6), 489-507.

Aymen, E. M., and Hannachi, C. (2012). Effect of NaCl priming duration and concentration on germination behavior of Tunisian safflower. *Journal of Stress Physiology & Biochemistry*, *8*(3), 30-36.

Bajehbaj, A. A. (2010). The effects of NaCl priming on salt tolerance in sunflower germination and seedling grown under salinity conditions. *African Journal of Biotechnology*, *9*(12).

Ben Youssef, R., Jelali, N., Boukari, N., Albacete, A., Martinez, C., Alfocea, F. P., Abdelly, C. (2021). The efficiency of different priming agents for improving germination and early seedling growth of local Tunisian barley under salinity stress. *Plants*, *10*(11), 2264.

Biswas, S., Rasal-Monir, M., Islam, M., Modak, S., Kabir, M. H. (2019). Induction of salt tolerance in tomato through seed priming. *J. Plants*, *7*, 47-56.

Cao, Y., Liang, L., Cheng, B., Dong, Y., Wei, J., Tian, X., Peng, Y., Li, Z. (2018). Pretreatment with NaCl promotes the seed germination of white clover by affecting endogenous phytohormones, metabolic regulation, and dehydrin-encoded genes expression under water stress. International Journal of Molecular Sciences.19, 3570.

Galahitigama, G. A. H., Wathugala, D. L. (2016). Pre-sowing seed treatments improves the growth and drought tolerance of rice (*Oryza sativa* L.). *Imperial Journal of Interdisciplinary Research*, *2*(9), 1074-1077.

Galahitigama, G. A. H., Tharindi, P. W. M., Kaushalya, K. C. (2021). Different halo-primed seed treatments on growth and yield of rice (*Oryza sativa* L.). *Journal of Agriculture Research and Life Sciences*, *2*(1), 25-28.

Ibrahim, E. A. (2016). Seed priming to alleviate salinity stress in germinating seeds. *Journal of plant physiology*, *192*, 38-46.

Indurugalla, Y., Manimekala, D., Galahitigama, G. A. H. (2023). Biostimulatory Impacts of Seed Priming through Botanical Extracts on Crop Production: A Critical Review. *Asian Journal of Agricultural and Horticultural Research*, *10*(4), 559-579.

Jatana, B.S., Grover, S., Ram, H., Baath, G.S. (2024). Seed priming: Molecular and physiological mechanisms underlying biotic and abiotic stress tolerance. Agronomy, 14, 2901.

Kader, M. A. (2005). A comparison of seed germination calculation formulae and the associated interpretation of resulting data. *Journal and Proceeding of the Royal Society of New South Wales*, *138*, 65-75.

Lakitan, B., Susilawati, S., Wijaya, A., Ria, R. P., Putri, H. H. (2022). Non-destructive leaf area estimation in habanero chili (*Capsicum chinense Jacq*.). International Journal of Agricultural Technology.18(2):633-650.

Quintero Castellanos, M. F., Guillen Castillo, O., Delgado Sánchez, P., Marín-Sanchez, J., Guzmán Carrasco, A. I., Sánchez Prados, A., Guzmán Palomino, J. M. (2018). Relieving dormancy and improving germination of Piquín chili pepper (*Capsicum annuum var. glabriusculum*) by priming techniques.

Nawaz, A., Amjad, M., Pervez, M. A., Afzal, I. (2011). Effect of halopriming on germination and seedling vigor of tomato. *African Journal of Agricultural Research*, *6*(15), 3551-3559.

Nimac, A., Lazarević, B., Petek, M., Vidak, M., Šatović, Z., Carović-Stanko, K. (2018). Effects of salinity and seed priming on germination of sea fennel (Crithmum maritimum L.). *Agriculturae Conspectus Scientificus*, *83*(2), 181-185.

Rhaman, M. S., Rauf, F., Tania, S. S., Khatun, M. (2020). Seed priming methods: Application in field crops and future perspectives. *Asian Journal of Research in Crop Science*, *5*(2), 8-19.

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 Journal of Agricultural and Biological Science*, *6*(1), 39-43.

Tester, M., Davenport, R. (2003). Na+ tolerance and Na+ transport in higher plants. *Annals of botany*, *91*(5), 503-527.

Yadav, P. V., Kumari, M., Ahmed, Z. (2011). Chemical seed priming as a simple technique to impart cold and salt stress tolerance in capsicum. *Journal of Crop Improvement*, *25*(5), 497-503.