EFFECTS OF HALOPRIMING AND HYDROPRIMING ON SEED GERMINATION AND SEEDLING EMERGENCE OF CHILLI *(Capsicum frutescens)*

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ABSTRACT:

Capsicum frutescens plants are exposed to a variety of environmental factors, including salinity, drought, high temperatures, and others, which have a detrimental effect on plant development and productivity. There exist several physiological and non-physiological techniques to enhance the germination process, which can help overcome environmental constraints and improve seed performance. Seed priming is a pre-sowing seed treatment that provides regulated hydration of seeds to absorb water and pass through the initial stage of germination, hence allowing seed to germinate more efficiently. This enhances the pre-germinative metabolic process and hastens germination. To evaluate the effect of hydropriming and halopriming methods on the seed germination and seedling emergence of Capsicum frutescens an experiment was carried out in Controlled Randomized Design (CRD) in the lab of College of Natural Resource Management, Bardibas, Mahottari. Seeds of capsicum was used with 7 treatments consisting of 3 replications in the experiment. Treatments includes T1: Halopriming with 1% KNO_3(Potassium Nitrate), T2: Halopriming with 2% KNO_3, T3: Halopriming with 3% KNO_3, T4: Halopriming with 1% NaCl(Sodium chloride), T5: Halopriming with 2% NaCl, T6: Halopriming with 3% NaCl, T7: Hydropriming with normal water. Seeds were soaked in prepared solution of KNO_3 and NaCl at different concentrations for 24 hours at lab temperature. The findings of this experiment suggests that halopriming treatments with KNO_3 may improve root and shoot emergence and 50% germination as compared to seed treated with NaCl. As evidenced by the slower growth of radicle and plumule in seed treated with NaCl and hydropriming, it was found that seed primed with 3% KNO_3 had these characteristics.

Keywords: *chilli, salinity, moisture, treatment, vigor*

INTRODUCTION

Capsicum frutescens commonly known as the bird's eye chilli, Thai chilli, or African birds eye chilli belongs to the Solanaceae family (Al-Snafi, 2015) . One important trait linked to capsicum genus members is pungency. It is also a crucial component of fruit quality. Capsicum fruit's level of pungency is correlated with the total amounts of the different vanillyl amides, or capsaicinoids (Jarret et al., 2007).

These peppers are rich in vitamins, especially vitamin A and vitamin C, as well as minerals like potassium. They are an excellent source of iron, protein, phosphorus, copper, riboflavin, thiamin, folate, and niacin. They also contain antioxidants, which contribute to their potential health benefits (Dhamodharan et al., 2022). Because it contains phytochemicals, it has been used in traditional medicine to treat a variety of conditions, including rheumatism, toothaches, sore throats, coughs, and parasitic infections, their qualities also include other effects like antibacterial and anticancer ones (Muthuswamy et al., 2021).

Environmental variables that negatively impact plant development and productivity are encountered by these plants, such as salinity, drought, high temperatures, and others (Robledo, 2020). The hazardous effects of salt on soil fertility and crop water intake have made it a major global issue (Yuvaraj et al., 2021). Field crops face significant challenges from environmental stressors including salinity in the soil and water, particularly in dry and semi-arid regions of the world and at the higher salt concentration strongest inhibition of germination occurred (M. El Naim et al., 2012). As a crucial planting stage, germination affects crop yield in a significant way. Economic losses arise from low and sluggish seed germination and seedling emergence in *Capsicum frutescens* cultivation (Robledo, 2020).

In order to overcome environmental limitations and improve seed performance, a variety of physiological and non-physiological methods are available to improve the germination process. One inexpensive and efficient way to promote seed germination is through seed priming. Priming involves regulated drying and hydration of seeds, which improves the pre-germinative metabolic process and speeds up germination (Rhaman et al., 2020).

A variety of methods exist for seed priming; one of these methods is halo-priming, which involves immersing seeds in an inorganic salt solution (NaCl, $CaCl_2$, KNO_3, etc.) to lower their water potential. This action is similar to the effects of drought stress on seeds, and it also sets off a physiological reaction in the seeds that activates their plant stress memory, causing the plants to react swiftly and forcefully to impending abiotic stress (El-Sanatawy et al., 2021). And another cost-effective and environmentally responsible method is hydro-priming which involves soaking seeds for a predetermined amount of time in either hot or cold water prior to planting them in any kind of growing or nutrient medium (Adhikari et al., 2021). This promotes water absorption in the seed and softens the seed coat just enough to allow for the improved, quick, and easy growth of the seed embryo. Furthermore, the efficient hydro-priming therapy initiates metabolic pathways that are activated during germination (Pandita & Anand., 2007).

Only two inorganic salts, NaCl and KNO3, were used in this study to examine the effects of halopriming on capsicum at different concentrations. The study did not include certain naturally occurring inorganic salts like $CaCl_2$, KCl, $NaNO_3$, $MnSO_4$, $MgCl_2$, etc. because of resource and time constraints.

The main objective of the study is to identify the effect of halopriming and hydropriming techniques on chilli seed germination and seedling vigor. Other specific objectives are :To study the effect of the effectiveness of priming techniques in chilli and to study the germination diversity including speed of germination percentage, mean germination time, seedling vigor index under different priming technique.

One of the biggest issues facing the world today is the salinity of the soil in coastal and tropical areas. According to studies, 7% of the world's soils are saline, and 3% of them are highly saline with little precipitation, huge evaporation, and saltwater drainage raising the salinity of the soil (Thornton & Powell, 1992). Strong ionic influences on the protoplasm and osmotic water retention are the main causes of the detrimental effects of high salt content on plants.

Salinity has an impact on the germination process of seeds through oxidative stress, ion-specific effects, and osmotic stress, as demonstrated by longer germination times and decreased germination rates (Munns, 2002). Due to the harmful effects of high salt and chloride ions on embryo viability, salinity can have an impact on seed germination. The harmful effects include altering the structure of other macromolecules and enzymes, damaging cell organelles and the plasma membrane, and interfering with protein synthesis, respiration, and photosynthesis (Uçarlı, 2020).

Salinity can have a negative effect on seed germination by raising ABA (Abscisic acid) levels, lowering the amount of GAs (Gibberellin), changing the seed's membrane permeability, and changing water behavior in the seed (Lee & Luan, 2012). The salinity of the soil causes poor germination and the poor formation of seedlings. It is a massive issue that negatively impacts crop plant growth and development and lowers agricultural productivity (Robledo, 2020). The causes of inhibition or delayed seed germination and seedling establishment are salt and osmotic stresses (Shanko et al., 2017).

Many of the metabolic processes involved in the early germination phases are stimulated by seed priming, and it has been observed that seedlings from primed seeds appear faster, develop quicker, and perform better under challenging conditions (Cano & Bolarin, 2015). Besides, priming influences enzymatic activity by boosting it and mitigating the effects of seed ageing (Lee & Kim, 2000).The hydropriming technique is inexpensive and environmentally friendly as no additional chemicals are used. One of the main drawbacks of this method is the uncontrollably high water intake by seeds. In certain species, rapid hydration can result in seed damage by causing vital nutrients to leak out of the seed during germination (Nawaz et al., 2013).

MATERIALS AND METHODS

Experiment design and treatments

An experiment was carried out in the lab of College of Natural Resource Management, Bardibas, Mahottari located at 27.0425° N latitude, and 85.8691°E longitude. On October 5, 2023 an experiment was carried out in Completely Randomized Design (CRD) to evaluate the effect of hydropriming and halopriming methods on the seed germination and seedling emergence of *Capsicum frutescens.* As a research material, seeds of capsicum was used with 7 treatments consisting of 3 replications in the experiment.

Table 1. Treatments and their concentration used in this research

	Treatment	Concentration
T1	KNO_3	1% KNO_3 (1g KNO_3 in 100ml of water)
T2	KNO_3	2% KNO_3 (2g KNO_3 in 100ml of water)
T3	KNO_3	3% KNO_3 (3g KNO_3 in 100ml of water)
T4	NaCl	1% NaCl (1g NaCl in 100ml of water)
T5	NaCl	2% NaCl (2g NaCl in 100ml of water)
T6	NaCl	3% NaCl (3g NaCl in 100ml of water)
T7	Hydropriming	100 ml of water

Halopriming Treatment

Seeds were soaked in prepared solution of KNO_3 and NaCl at different concentrations, as mentioned above for 24 hours at lab temperature. After that, the primed seeds were dried with filter paper to remove excess moisture content for 30 minutes. Then, the seed were placed on each petri-plates for further germination

Seed Germination Test

Three replicates of 15 seeds on each petri plate holding moist filter paper were used to sow the seeds. As a result, germination was tracked every day (Basra et al., 2005) . When a seed reached a length of 2 mm in its radicle, it was deemed to have germinate (Demir et al., 2005).

As described by the Association of Official Seed Analysis germination percentage were calculated by using formula (El-Sanatawy et al., 2021):

Germination Percentage = $\frac{Total\ seed\ germiated}{Total\ number\ of\ seed} \times 100$

Mean Germination Time (MGT) was calculated by using formula (Demir et al., 2019):

MGT=$\sum n.t / \sum n$, where, n = number of seeds newly germinated
t = days from planting,
$\sum$ n = Final germination

Germination Index is calculated by using formula (Rastegar et al., 2011):
GI=n/d where, n is the number of seed emerged in day d
d is day after planting

Germination Rate is calculated by using formula (Ranal & Santana, 2006):
GR = $\sum (Ni / Di)$ where , N is the number of seed germinated
D is the number of days after germination
i is i^{th} day

Seedling vigor index is calculated by using formula (Hyder et al., 2020)
SVI = % seed germination * total plant length

Data collection

As the seed start to germinate after 5-6 days. The germination of seed was regularly checked and monitored. Number of germinated seed were counted from each petri-plate and after 15 days data was taken. Out of 15 seeds, 5 germinated seeds were taken as sample from each treatment within each replication and root length and shoot length were measured using centimeter scale.

Data Analysis

In-order to ascertain the significance of variance ($p < 0.05$), the recorded data were collected for statistical analysis using Gen stat version 15 and MS excel software, Duncan's multiple range test was employed to evaluate variations in treatment means.

Table 2. Effect of halopriming and hydropriming on germination of Chilli seeds.

Treatments	Germination %	Germination Energy	Germination Index	Germination Rate	Mean Germination Time
T1	91.11 [a]	0.9111[a]	1.792[a]	2.124[a]	5.612[a]
T2	95.56[a]	0.9556[a]	1.662[a]	2.206[a]	6.897[a]

T3	95.56[a]	0.9556[a]	1.940[a]	2.669[a]	5.656[a]
T4	93.33[a]	0.9333[a]	1.763[a]	2.182[a]	7.535[a]
T5	95.56[a]	0.9556[a]	1.970[a]	2.680[a]	5.545[a]
T6	97.78[a]	0.9778[a]	2.012[a]	2.558[a]	5.836[a]
T7	93.33[a]	0.9333[a]	1.714[a]	2.048[a]	7.089[a]
SE_M (±)	4.26	0.0426	0.1759	0.2838	0.664
LSD (0.05)	13.11	0.1311	0.5420	0.6183	2.047
CV %	3.1%	3.1%	7.9%	4.3%	3.4%
Grand mean	94.6	0.946	1.836	2.353	6.31
F- value	0.945	0.945	0.723	0.174	0.236

Table 3. Effect of halopriming and hydropriming on the seedling emergence and T_{50} of chilli seeds

Treatments	Root length (cm)	Shoot length (cm)	Effect on time taken to reach 50% of germination (T_{50})	Seedling vigor Index
T1	2.800[b]	2.073[b]	6.667[a]	433.5[b]
T2	2.373[c]	1.953[b]	6.667[a]	414.3[bc]
T3	3.567[a]	2.900[a]	5.667[ab]	618.2[a]
T4	2.100[d]	1.710[c]	6.667[a]	356.1[cd]
T5	1.817[e]	1.400[d]	5.333[b]	307.4[d]
T6	2.467[c]	1.900[b]	5.333[b]	426.9[b]
T7	1.817[e]	1.517[d]	6.667[a]	311.3[d]
S_{EM} (±)	0.0707	0.0544	0.337	21.54
LSD (0.05)	0.2179	0.1676	1.039	66.37
CV %	1%	1.6%	7%	3.1%
Grand mean	2.420	1.922	6.14	411.1
F- value	<0.001	<0.001	0.022	<0.001

RESULTS & DISCUSSION

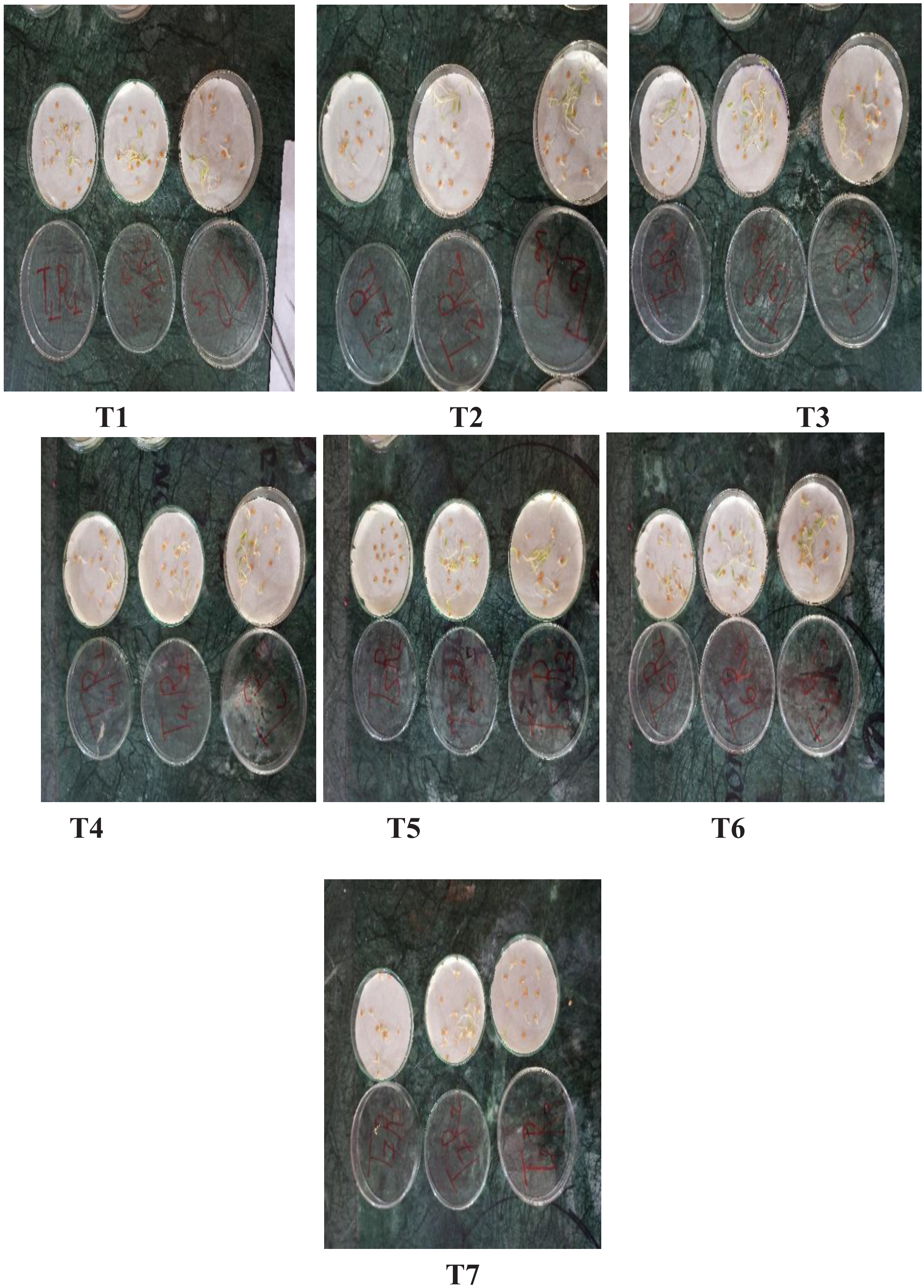

Figure 1. Treatment and replication of experiment

Effect on root length and shoot length

With the application of various priming treatment the root length and shoot length showed the significant variation with p value 0.05 (<0.001) (Table 3). In T3, larger root length (3.567cm) was observed and shorter root length (1.817cm) was observed in T7 (Hydropriming) and T5. Similarly, the larger (2.90cm) and shorter shoot length (1.40cm) was observed in T3 and T5. Therefore, T3 (3% KNO_3) gave better result than other halopriming techniques. Likewise, T4 (1% NaCl) and T7 (Hydropriming) didn't show better result. It was observed that the faster growth of radicle and plumule was observed in seed primed with 3% KNO_3 as proven by lesser growth of radicle and plumule in seed treated with NaCl and hydropriming. This phenomenon may be the consequence of increased embryo cell wall extensibility.

When compared to seed treatment with KNO_3, NaCl halopriming treatments didn't improve germination and seedling emergence. It's possible that this is because the *Capsicum f.* seeds treated with NaCl absorbed more Na+ and/or Cl- from the salt solution, which increased their toxicity (Sawant et al., 2013). NaCl halopriming may cause toxicity issues since ions have been shown to accumulate in tissues in a number of vegetable species (Robledo, 2020). Due to halopriming, enhancement in root and shoot length were observed by the researcher (Lamichhane et al., 2021). Different metabolic activities in the seed embryo may be induced, leading to the increased shoot and root length of seedlings observed in primed seed (Tania & Rhaman, 2020).

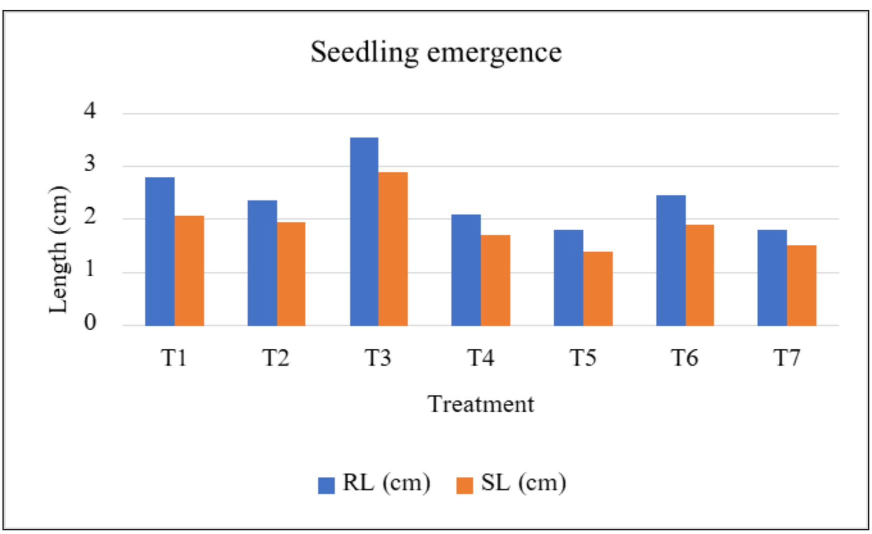

Figure 2. Effect of halopriming and hydropriming treatment on root length and shoot length on Chilli seedlings

Effect on Germination percentage, germination rate, germination Energy, germination index and MGT

It was notable that there was non-significant difference in treatment on germination percentage with p value (0.945) (Table 2). According to the statistical analysis, there was no role of halopriming treatment on the germination percentage of seedling. Hence, halopriming didn't enhance the germination potential of seedling. All the treatment gave the same results. A brief priming period will not provide the seed with the necessary amount of water to enter the lag phase of germination. However, if priming is left for too long, too much water may be allowed, which could be more than what is needed to start the lag phase of germination. Radicle protrusion will then occur, which will cause the seed to lose its ability to withstand desiccation and ultimately lose viability (Tania & Rhaman., 2022).

Likewise, it is notable that germination energy, germination index, MGT and germination rate also showed the same result (Table 2). As there was non-significant difference in treatment. The P value of germination energy (0.945), germination index (0.723), mean germination time (0.236) and germination rate (0.174). The reason behind non-significant result was due to the limited number of inorganic salts with equivalent concentrations used in the entire experiment.

Effect on SVI

According to the statistical analysis, with the application of various halopriming and hydropriming treatment the seedling vigor index showed the significant variation with p value (<0.001) (Table 3). As compared to other treatment, T3 showed the better result, it showed the greater influence on seed vigor index. In Treatment T3, maximum seed vigor index was observed which was 618.2. Thereafter, treatment T1, T6 showed significant variation following T2, T4, T7 and T5 doesn't show the better result. We can say that halopriming improve seed vigor and help to establish profound stand as compared to hydropriming. Due to the low membrane injury coupled with high enzyme activities (dehydrogenase and amylase) there is enhancement in seed vigor in primed seed (Lamichhane et al., 2021).

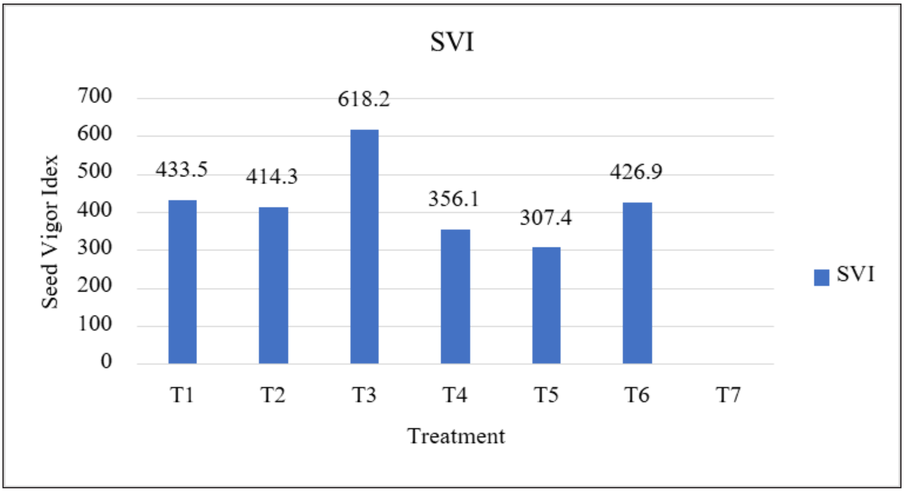

Figure 3. Effect of halopriming and hydropriming treatment on the Seed Vigor Index of chilli seeds

Effect on the time take to reach 50% of germination (T_{50})

According to our research, the significant effect was reported in the T_{50} value at 5% level of significance with p value (<0.001). T1, T2, T4, T7 showed the significant effect on the 50% germination of seedling (Table 3). The maximum T50 value was observed in T1, T2, T4, T7 (6.667) and minimum T_{50} value in T5 and T6 (5.333) respectively. The early disintegration of the reserve can be blamed for a notable time reduction to 50 % germination (Robledo, 2020).

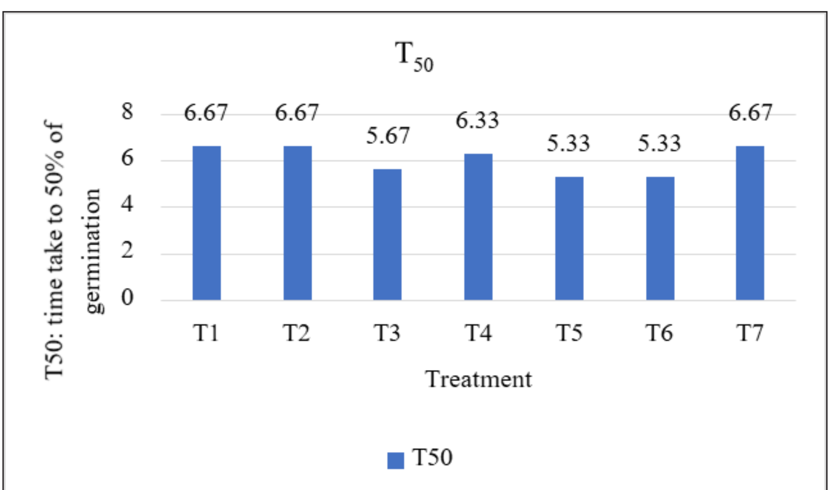

Figure 4. Effect of halopriming and hydropriming treatment on T_{50} of chilli seed

CONCLUSION

In a wide range of environmental condition, priming can lower the likelihood of poor seed establishment. Our research showed that halopriming is a simple technique for increasing the percentage of germination and seedling emergence. The findings of this investigation suggest that halopriming treatments with KNO_3 may improve root and shoot emergence and 50% germination. When compared to seed treated with KNO_3, NaCl halopriming treatments didn't quickly improve 50% germination and seedling emergence. Furthermore, it can be said that halopriming with NaCl may amplify the harmful impact caused by salt stress. All the things considered, 3% KNO_3 halopriming proved to be more successful than any other halopriming method.

REFERENCES

Adhikari, B., Dhital, P. R., 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), 0255258

Al-Snafi, A. E. (2015). The pharmacological importance of capsicum species (*capsicum annuum* and *capsicum frutescens*) grown in Iraq. *Journal of Pharmaceutical Biology, 5*(3), 124-142.

Basra, S. M. A., Farooq, M., Tabassam, R., & Ahmad, N. (2005). Physiological and biochemical aspects of pre-sowing seed treatments in fine rice (*Oryza sativa* L.). *Seed Science and Technology*, *33*(3), 623-628.

Cano, E. A., & Bolarin, M. C. (2015). Effect of NaCl priming on increased salt tolerance in Tomato. *Journal of Horticultural Science, 66*(5), 621-628.

Demir, I., Ermis, S., Okçu, G., & Matthews, S. (2005). Vigour tests for predicting seedling emergence of aubergine (*Solanum melongena* L.) seed lots. *Seed Science and Technology*, *33*(2), 481-484.

Demir, I., Kenanoglu, B. B., & Özden, E. (2019). Seed vigour tests to estimate seedling emergence in cress (*lepidium sativum* l.) seed lots. *Notulae Botanicae Horti -Agrobotanici Cluj-Napoca*, *47*(3).

Dhamodharan, K., Vengaimaran, M., Sankaran, M., Dhamodharan, K., Vengaimaran, M., & Sankaran, M. (2022). Pharmacological properties and health benefits of capsicum species: a comprehensive review. *Capsium Current Trend and Prespectives*. https://doi.org/10.5772/intechopen.104906

El-Sanatawy, A. M., Ash-Shormillesy, S. M. A. I., Qabil, N., Awad, M. F., & Mansour, E. (2021). Seed halo-priming improves seedling vigor, grain yield, and water use efficiency of maize under varying irrigation regimes. *Water*, *13*(15), 2115.

Hyder, S., Gondal, A. S., Rizvi, Z. F., Haider, M. I. S., & Inam-ul-Haq, M. (2020). Biological control of chili damping off disease, caused by *Pythium myriotylum. Frointers in Microbiology, 12*.

Jarret, R. L., Baldwin, E., Perkins, B., Bushway, R., & Guthrie, K. (2007). Diversity of fruit quality characteristics in capsicum frutescens. *HortScience*, *42*(1), 16-19.

Lamichhane, A., K.C., M., Shrestha, M., & Baral, B. (2021). effect of seed priming on germination of Okra (*Abelmoschus esculentus var. Arka Anamika*). *Malaysian Journal of Sustainable Agriculture*, *5*(2), 111-114.

Lee, S. C., & Luan, S. (2012). ABA signal transduction at the crossroad of biotic and abiotic stress responses. *Plant, Cell & Environment*, *35*(1), 53-60.

Lee, S., & Kim, J. H. (2000). Total sugars, alpha-amylase activity, and germination after priming of normal and aged rice seeds. *The Korean Journal of Crop Science, 45*(2), 108-111.

M. El Naim, A., E. Mohammed, K., A. Ibrahim, E., & N. Suleiman, N. (2012). Impact of salinity on seed germination and early seedling growth of three sorghum (S*orghum biolor* l. moench) cultivars. *Science and Technology*, *2*(2), 16-20.

Munns, R. (2002). Comparative physiology of salt and water stress. *Plant, Cell & Environment*, *25*(2), 239-250.

Muthuswamy, R., S, A., & Nison, M. (2021). Review on capsicum frutescens: A tribal herbal food used as Medicine. *Research Journal of Pharmacognosy and Phytochemistry*,*13*(4), 191-194.

Nawaz, J., Hussain, M., Jabbar, A., Nadeem, G. A., Sajid, M., Subtain, M., & Shabbir, I. (2013). *Seed Priming A Technique*.

Pandita, V., & Anand, A. (2007). Enhancement of seed germination in hot pepper following pre sowing treatments, *Seed Science and Technology, 3*(2), 282-290.

Ranal, M. A., & Santana, D. G. de. (2006). How and why to measure the germination process. *Brazilian Journal of Botany*, *29*(1), 1-11.

Rastegar, Z., Sedghi, M., & Khomari, S. (2011). Effects of accelerated aging on soybean seed germination indexes at laboratory conditions. *Notulae Scientia Biologicae*, *3*(3), 126-129.

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.

Robledo, D. A. R. (2020). Effects of halopriming on seed germination and seedling emergence of C*apsicum frutescens*. *Journal of Botany Research*, *3*(1), 114-118.

Sawant, S. A., Nene, A., Somani, S. P., Omanwar, S. K., & Somani, P. R. (2013). Simultaneous waste water purification via photocatalysis and seed germination. *Green and Sustainable Chemistry*, *3*(3), 129-133.

Shanko, D., Jateni, G., & Debela, A. (2017). effects of salinity on chickpea (*Cicer arietinum* l.) landraces during germination stage. *J Biochem Mol Bio, 3*(2), 9.

Tania, S. S., Rhaman, M. S., & Hossain, M. M. (2020). *Hydro-priming and halo-priming improve seed germination, yield and yield contributing characters of Okra (Abelmoschus esculentus L.)*. *Tropical Plant Research*, *7*(1), 86-93.

Thornton, J. M., & Powell, A. A. (1992). Short-term aerated hydration for the improvement of seed quality in Brassica oleracea L. *Seed Science Research*, *2*(1), 41-49.

Uçarlı, C. (2020). Effects of salinity on seed germination and early seedling stage. In F. Shah, S. Shah, Y. Chen, C. Wu, & D. Wang (eds.), *Abiotic Stress in plants*. Intech Open.

Yuvaraj, M., Subash Chandra Bose, K., Elavarasi, P., & Tawfik, E. (2021). Soil salinity and its management. In R. Swaroop Meena & R. Datta (Eds.), *Soil Moisture Importance*. Intech Open.