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ORIGINAL RESEARCH ARTICLE

Optimizing seed priming techniques: Impact of halo priming with sodium chloride on fenugreek (*Trigonella foenum* L.) in germination and stress acclimatization

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ABSTRACT

Germination is a critical stage in plant development that determines the crop yield. Seed priming acts as a pre-sowing treatment that stimulates germination and activates metabolic process. Hydro-priming is widely practiced system which enhances germination but reduces seed storability whereas halo-priming enhances self-life and induces salinity tolerance. This study investigated the effects of sodium chloride (NaCl) priming on the germination of fenugreek (*Trigonella foenum* L.) seeds under moisture stress conditions. Despite stress condition, seed priming can noticeably increase the germination. A lab experiment was therefore, performed to study the effect of NaCl priming on different levels of moisture stress. NaCl priming was done at 0 ppm, 2 ppm, 4 ppm and, 8 ppm subjected to priming duration of 12 hours, 24 hours and 36 hours. The result demonstrated 100% germination at 0 g for all durations (12, 24, and 36 hours), and similar outcomes were observed at 8 g for 12, 24, and 36 hours, and at 4 g and 6 g for 12 and 36 hours. While the mean germination time, coefficient of velocity of germination increased with increment in priming duration at 0 g and decrease with increment in priming duration in 8gm NaCl concentration. Halo-priming was found superior above hydro-priming for fenugreek germination as salinity induces changes in lipid metabolism towards accumulation of saturated and monounsaturated fatty acids in stressed plants. This research provides compelling evidence that NaCl priming can be used to promote germination and improves acclimatization of fenugreek seedlings under saline conditions.

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INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an important medicinal and culinary annual legume crop native to parts of Asia and the Mediterranean region (Acharya *et al*., 2008). The production of leaves of fenugreek in 2022/23 was 4,189 metric tonnes (Mt) where Banke, Saptari, Kailali, Bara, Kavre, and Bardiya are the main fenugreek-producing districts of Nepal (MOAD, 2022). Fresh fenugreek leaves are an edible herb that come from the fenugreek plant. The fenugreek leaves consumption has recently attracted a lot of attention for its ability to stabilize insulin, blood sugar, hemoglobin levels, and diabetes symptoms. The bioactive chemicals found in it has an anti-mutagenic, antioxidant, anti-inflammatory, anti-proliferative, and anti-atherogenic properties. As a result, these substances protect human health by neutralizing free radicals, which are linked to the production of a range of degenerative disorders (Shah *et al*., 2021). In Nepal, fenugreek is grown across diverse agroecological regions but remains a marginal crop (Ahmad *et al*., 2016). The subsistence farming practices, lack of quality seeds, and limited market access has attributed to low production of fenugreek. Abiotic stress like moisture deficiency also impede fenugreek cultivation, especially in the key production areas of western and mid-western Nepal (Bhatta *et al*., 2020; Wyss *et al*., 2018). Fenugreek production can improve small farm holders by increasing their incomes and nutrition even through low-cost

techniques (Bhatta *et al*., 2020).

Seed priming, a pre-sowing seed enhancement treatment, is a promising strategy for boosting fenugreek germination, growth and yields under stress (Hussain *et al*., 2022). Priming triggers pre-germinative metabolic processes enabling rapid and uniform germination (Johnson & Puthur, 2021). Among different priming methods, hydro-priming (soaking in water) improves fenugreek germination but reduces seed storability (Hussain *et al*., 2022; Soughir *et al*., 2012). In contrast, halo-priming with NaCl solutions enhances shelf life while conferring salt stress tolerance (Moghaddam *et al*., 2018). This is attributed to metabolic changes like osmotic adjustment, antioxidant accumulation, and cell membrane modifications induced by NaCl priming (Johnson & Puthur, 2021; Moghaddam *et al*., 2018). Paradoxically, fenugreek imports are rising in Nepal especially from India indicating domestic potential (Kumar *et al*., 2019). Seed priming techniques, which involve controlled pre-sowing hydration, can mitigate abiotic stress and improve fenugreek productivity (Hussain *et al*., 2022). NaCl priming boosts shelf-life and induces salinity tolerance (Biswas *et al*., 2023; Uçarlı, 2020). This study aimed to determine the ideal NaCl priming concentrations and duration to mitigate moisture stress effects on fenugreek germination. The findings will help establish halo-priming as a viable on-farm seed enhancement technique for resource constrained Nepali farmers to improve fenugreek productivity and incomes.

MATERIALS AND METHODS

Fenugreek seeds of variety - Methi No. 47 with 99% Genetic and 98% Physical Purity was collected from Kantipur Seed, Khumaltar, Kathmandu. To prepare the NaCl solution, all the beakers, glass rod, and petri dishes were sterilized at a temperature of 121°C for 30 minutes using 15psi pressure on an autoclave. 0 grams, 2 grams, 4 grams, and 8 grams of Laboratory NaCl were measured in a digital weighing machine and carefully placed in 4 respective beakers using a spatula. 1000 ml of distilled water was gently poured using a glass rod in each of the 4 beakers to avoid splashes and spills. Then, NaCl was completely dissolved in water after gentle swirl using a glass rod. A laboratory test was performed in Horticulture Division Laboratory, NARC, Khumaltar, using a Randomized Complete Block Design with five replications and four treatments. In total, 1200 seeds were used in the experiment of 4 treatments, with 300 seeds taken altogether in 1 treatment. The priming was allowed for 12 hours, 24 hours, and 36 hours, respectively. Afterwards, the seed was left to shade dry for the next 24 hours in papers. For the germination test, the petri-dish base was covered with moistened filter papers (12 mm) and labeled. 20 seeds were arranged in a rectangular order: 4 seeds in 5 columns. 60 petri-dishes were put in 7 trays inside incubators for germination tests. The temperature was set at 25°C and relative humidity of 80% was maintained in the germinator for 144 hours (6 Days). Once a day at 12 pm in the afternoon, germination was scored in the data sheet format, and scoring of each petri-dish was recorded. Germination was counted when sprouting occurred until the 2-leaf stage. The scoring was done for 6 days. At the beginning of the germination test period, all 20 samples seedling from each petri-dish was taken. The fresh weight was measured. Then, after priming in NaCl solutions for 12 hours, 24 hours, and 36 hours, the turgid weight of each sample of 20 seeds was measured.

Relative water content (RWC) was determined using the formula:

RWC = (Fresh weight – Dry weight) / (Turgid weight – Dry weight) × 100 González and González-Vilar (2001)

The germination data was used to evaluate final germination percentage (FGP), mean germination time (MGT), coefficient of velocity of germination (CVG), and germination index (GI) as follows:

FGP = (Final number of seeds germinated / Total seeds) × 100 (Scott *et al*., 1984)

MGT = ∑Dx Rx /∑Rx (Orchard, 1977)

CVG = ∑R1, R2, R3, Rn / 100xR1T1, R2T2,R3T3,RnTn (Jones *et al*., 1987)

GI = ∑(7xR1) + (6xR2) + (5xR3) + ... + (1xR7) (Benech Arnold *et al*., 1991)

Where, Rx - Number of seeds germinated on day x, Dx - Number of days from sowing, Tx - Day on which Rx occurred

Germination rate index (GRI): G1/1+G2/2+.... +Gi/I; where G1 is the germination percentage on day 1, G2 is the germination percentage at day 2; and so on. (Al-Ansari & Ksiksi, 2016)

Mean germination rate (MGR): MGR = CV/100 = 1/T; where T is mean germination time. (Al-Ansari & Ksiksi, 2016)

Data analysis was done using ANOVA and Tukey's test at 5% probability level on SPSS software.

RESULTS AND DISCUSSION

The effect of different sodium chloride (NaCl) priming concentrations and durations on fenugreek seed germination under induced moisture stress was evaluated by determining various germination parameters. Final germination percentage was 100% for control (0 ppm NaCl) across all priming durations, and at 6 g/L NaCl for 12 and 36 hours. At 4 g/L NaCl, 100% germination was observed only for 12- and 36-hour priming. Priming with 8 g/L NaCl resulted in 90-95% germination across durations. Increasing priming duration increased mean germination time at 0 ppm NaCl, while the reverse trend was observed at higher NaCl concentrations. The maximum mean germination time occurred at 4 g/L NaCl for 24 hours and 8 g/L NaCl for 12

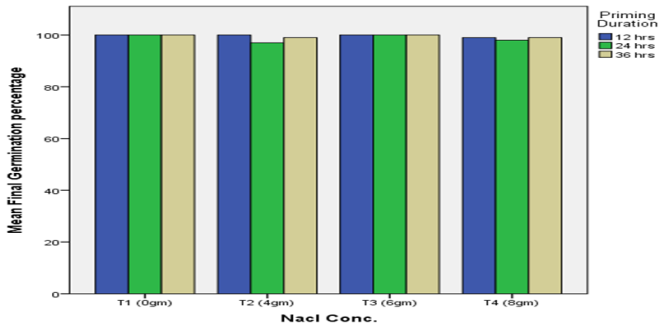

Figure 1. *Effect of NaCl priming on final germination percentage of fenugreek under different level of NaCl concentration.*

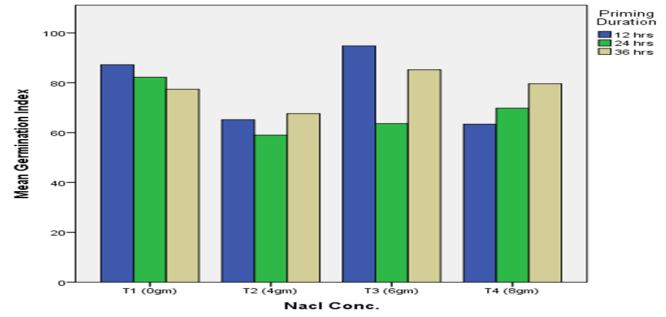

Figure 2. *Effect of NaCl priming on germination index of fenugreek under different level of NaCl concentration.*

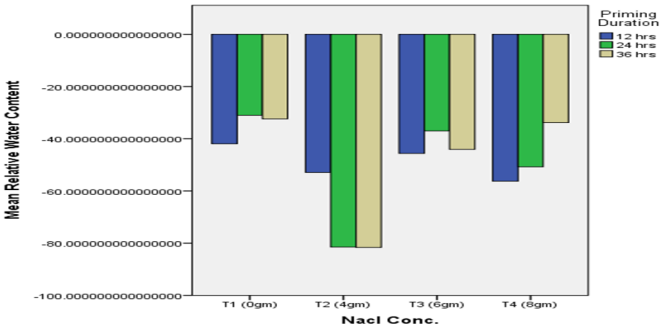

Figure 3. *Effect of NaCl priming on relative water content of fenugreek under different level of NaCl concentration.*

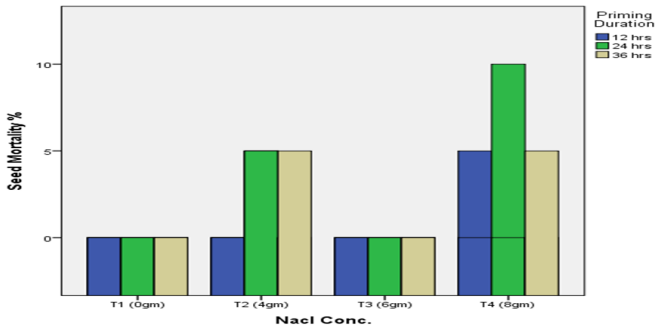

Figure 4. *Effect of NaCl priming on seed mortality percentage of fenugreek under different level of NaCl concentration.*

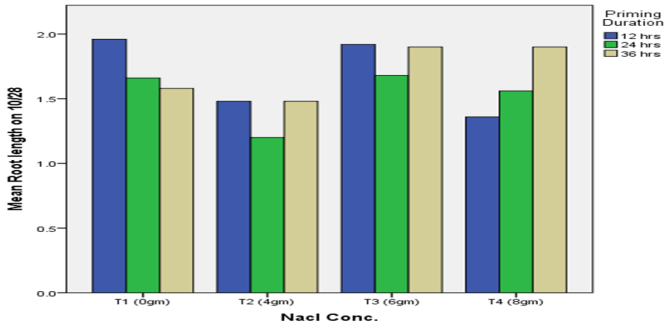

Figure 5. *Effect of NaCl priming on root length of fenugreek under different level of NaCl concentration.*

hours. Similarly, the coefficient of velocity of germination showed similar responses, increasing with duration at 0 ppm NaCl, and decreasing at higher concentrations. The minimum values were recorded at 4 g/L NaCl, 24 hours and 8 g/L NaCl, 12 hours. The germination index exhibited an opposite pattern compared to mean germination time. The maximum germination index occurred at 6 g/L NaCl for 12 hours priming and the minimum at 4 g/L NaCl for 24 hours. Moderate NaCl concentrations of 4-6 g/L for 12-24 hour priming durations can maximize final germination percentage, indicating that short-term priming with NaCl in this range can mitigate adverse moisture stress impacts during germination (Uçarlı, 2020).

Relative water content decreased with increasing NaCl concentration and duration, with significant differences between treatments. The lowest relative water content was measured at 4 g/L NaCl, with 24 and 36 hours priming. No seed mortality was observed at 0 ppm and 6 g/L NaCl across all priming durations, indicating absence of negative impacts at these levels. At 4 g/L NaCl concentration, longer duration shows increased moisture stress effects. Priming with 8 g/L NaCl resulted in 5% mortality at 12 and 36 hours, which rose to 10% at 24 hours duration. at higher NaCl levels (8 g/L) and longer durations (36 hours), germination percentage declined, implying the emergence of toxicity effects (Uçarlı, 2020). The results align with the findings of a study that showed high NaCl concentrations progressively decreased the germination percentage and the germination potential of black seeds (Guo *et al*., 2020). This signifies that 24-hour priming at 8 g/L imposed the maximum moisture stress leading to mortality. Overall, the results showed increased seed mortality with rising NaCl concentration beyond 6 g/L, and at extended 24 hours priming duration. However, mortality remained within 10% across treatments. (Guo *et al*., 2020). (Turhan *et al*., 2010) found that low salt concentration decreases the germination rate, and high salt concentration decreases germination percentage (Turhan *et al*., 2010). 12-24 hour priming with 4-6 g/L NaCl can lead to faster and more uniform germination, as well as higher germination index and coefficient of velocity of germination compared to unprimed seeds (Zammali *et al*., 2022). This demonstrates the stimulatory role of halo-priming in promoting germination even under stress. However, extended 36 hour priming or high 8 g/L NaCl can become counterproductive and reduce germination percentage (Zammali *et al*., 2022). These findings align with the results of a study that showed that seed priming can mitigate the adverse effects of high salinity on seed germination by improving carbohydrate and protein mobilization (Sghayar *et al*., 2023). Root length also declined with increasing NaCl concentration and duration. The longest roots were observed at 8 g/L NaCl, 36 hours while the shortest were at 4 g/L NaCl, 24 hours. The germination rate index declined progressively with increasing duration at 0 ppm NaCl concentration. An opposite trend of increasing germination rate index was evident at 8 g/L NaCl, where longer priming enhanced the rate. The maximum germination rate index was attained by seeds primed at 6 g/L NaCl for 12 hours duration. The results show that the relative water content, an indicator of

cellular hydration status, declined with rising NaCl concentration and duration, suggesting elevated moisture stress (Kaydan & Yagmur, 2008). The reduced relative water content aligned with increased seed mortality at higher NaCl levels and longer priming times (Kaydan & Yagmur, 2008). Root length also progressively declined with rising NaCl and duration, implying emerging toxicity (Atak *et al*., 2006). These findings align with the results of a study that showed that increasing salinity significantly reduces germination percentage and rate, root and shoot length, and fresh and dry weights of the seedlings (Rajabi Dehnavi *et al*., 2020).The mean germination rate showed a similar response as germination rate index. It decreased gradually with extended priming time at 0 ppm NaCl, while an incremental trend was observed at 8 g/L NaCl. The highest mean germination rate also occurred at 6 g/L NaCl for 12 hours priming. One study found that different NaCl priming concentrations and durations have significant effects on total germination percentage, and 8 g/L NaCl priming for 36 hours improved germination rate index and mean germination rate, indicative of recovery responses to counteract toxicity (Soughir *et al*., 2012). These findings align with the results of a study that showed that seed priming can improve seed germination and seedling growth under salt stress conditions by improving carbohydrate and protein mobilization (Johnson & Puthur, 2021).

Conclusion

The study demonstrated that sodium chloride (NaCl) priming can improve fenugreek seed germination and early seedling growth under moisture stress conditions, but only within an optimal priming concentration and duration range. Short-term (12-24 hour) priming with moderate NaCl levels (4-6 g/L) maximized germination percentage and rate, mitigating adverse effects of moisture stress. However, higher NaCl concentrations (8 g/L) and longer durations (36 hours) led to toxicity effects, declining germination and growth. The results highlight the importance of optimizing NaCl priming protocols to leverage benefits while avoiding toxicity. Careful titration of concentration and duration is essential to harness the potential of NaCl priming to improve fenugreek germination and vigor under moisture deficit conditions. It can be concluded that NaCl priming can have positive effects on seed germination and seedling growth, but the optimal concentration and duration of priming may vary depending on the plant species and environmental conditions.

DECLARATIONS

Author contribution statement

Conceptualization: YS.; Methodology: BT and YS; Software and validation: YS.; Formal analysis and investigation: YS.; Resources: YS and BT.; Data curation: BT and YS.; Writing—original draft preparation: YS and BT.; Writing—review and editing: BT.; Visualization: YS.; Supervision: YS.; Project administration: YS; Funding acquisition: YS and BT. All authors have read and agreed to the published version of the manuscript.

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