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RESEARCH ARTICLE

Effect of halo priming on germination and growth parameters of finger millet, little millet, and barnyard millet under osmotic stress

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ABSTRACT

Seed priming has gained attention as a sustainable approach to improve crop performance. Halopriming, in particular, has shown promise in enhancing germination, growth, and stress tolerance. This study aimed to assess the effect of halopriming on the growth of finger millet (*Eleusine coracana*) variety CO(Ra) 15, little millet (*Panicum sumatrense*) variety ATL 1, and barnyard millet (*Echinochloa frumentacea*) variety CO(KV) 2 in osmotic conditions. The experiment used different salt solutions with 0, 1, and 1.8 g/L NaCl concentrations. In Hp1 (halo priming 1) finger millet variety CO(Ra) 15, 100% seed germination was observed in normal conditions. In Hp2, the little millet variety ATL 1 has shown 100% seed germination under osmotic stress with a concentration of 1 g/L NaCl (S1). In Hp1, barnyard millet variety CO(KV)2 showed 100% seed germination under osmotic stress with a concentration of 1.8 g/L NaCl (S2). Various growth parameters were recorded, and results showed that the barnyard millet variety Co (KV)2 and the little millet variety ATL 1, both under the Hp1 category, demonstrated the longest shoot lengths of 7.3 cm and 5.85 cm respectively under salt stress (S1). Furthermore, In Hp 1 finger millet variety CO(Ra) 15 recorded the highest root length of 12.25 under salt stress (S2). Vigour Index showed that Hp1 showed positive results regarding the germination percentage and seedling growth of finger millet variety CO(Ra) 15 and little millet variety ATL 1. This study suggested halopriming is an effective technique for promoting sustainable agriculture, especially in areas affected by salinity stress.

Keywords: millet, halopriming, germination, osmotic stress, shoot and root length and vigour index

INTRODUCTION

Among the oldest cultivated grains, millets may have been the first cereal grains used for domestic purposes. In India, they constitute a staple diet for nearly one-third of the global population (Paschapur et al., 2021). Millets exhibit remarkable adaptability to marginal soils and diverse environmental conditions, making them resilient crops. Little millet, scientifically known as *Panicum sumatrense*, is a tiny, gluten-free cereal grain that has been cultivated for centuries in India and other regions of Asia and Africa. This crop belongs to the family Poaceae (Ashokkumar et al., 2019). Notably, little millet demonstrates adaptability to diverse climates and soils, making it a valuable resource for sustainable agriculture (Kheya et al., 2023). Little millet, in particular, stands out as a rich source of antioxidants, which enhance immunity and provide protection against infections and diseases (Kaur et al., 2019). Additionally, it is abundant in essential minerals, including magnesium, which is crucial for maintaining healthy bones, muscles, and nerve function.

Barnyard millet (*Echinochloa frumentacea*) is indigenous to Eurasia. In India, it holds significant importance as a dryland crop. It is cultivated across a diverse range of environmental conditions and poor soils; it is primarily found in tribal belts of various parts of India (Dutta et al., 2023). In Tamil Nadu, tribal farmers cultivate barnyard millet in drylands and hill areas, specifically in districts such as Ramanathapuram, Madurai, Salem, Namakkal, Dindigul, Coimbatore, and Erode. This versatile crop serves dual purposes: as a grain and as fodder. Its grains are nutritious, and the straw provides valuable fodder. Barnyard millet is nutritionally significant due to its high protein content (11.8%) and crude fiber (9.8%). It comprises 16.6% of the amino acid leucine, which is twice the amount present in rice (Kumar et al., 2018). Breeding efforts increased production by creating a high-yielding barnyard millet variety CO (KV) 2 (Nirmalakumari et al., 2009).

Finger millet, scientifically known as *Eleusine coracana*, is a member of the Poaceae family. An annual herbaceous plant is grown as a cereal crop in arid and semi-arid locations in Africa and Asia (Kayastha et al., 2024). It is notable for being tetraploid, capable of self-pollination, and having evolved from its wild relative, *Eleusine africana*. exhibits a short-day photoperiodic response, which enhances its ability to withstand dry conditions and resist diseases and pests. The chromosomal number of the species is 2n = 4x = 36 (Ashokkumar et al., 2019). Finger millet is a crop that is environmentally friendly and may grow well in areas with poor soil quality and high elevations. It exhibits high resistance to drought and salinity environments, using just minimal irrigation and inputs. Finger millet is a common crop grown in India during the dry season, known as rabi. The amazing adaptability of finger millet to a wide range of soil pH values is seen. The plant can flourish in moderate acidity (pH 5) and alkaline soils (pH 8.2). The adaptability of this crop makes it highly important for areas with diverse soil conditions (Chandrashekar, 2010).

Seed priming is a method that involves partially hydrating seeds, which enables the initiation of germination processes without developing the radicle. The efficacy of this economical approach has been effectively proven in improving the percentage and rate of germination, as well as the overall emergence of diverse crop seeds (Johnson et al., 2021). There are several seed priming techniques available, including osmopriming (which involves soaking seeds in osmotic solutions), hydropriming (where seeds are soaked in water), halopriming (which uses saline solutions like NaCl), thermopriming (seeds are exposed to temperature fluctuations), and hormone priming (which involves applying plant growth regulators) (Hassanpouraghdam et al., 2009; Iqbal & Ashraf, 2013; Zhang et al., 2016; Damalas et al., 2019; Farooq et al., 2020). Seed priming leads to prompt and consistent seedling emergence, which can be especially advantageous under unfavourable soil conditions.

Halopriming is a technique that involves immersing seeds in salt solutions. This process improves the process of germination and the emergence of seedlings, even in difficult environmental conditions (Nawaz et al., 2011). The primed seeds demonstrate an increased ability to undergo osmotic adjustment due to certain metabolic modifications. Plants that come from primed seeds have a higher concentration of Na^+ and Cl^- ions in their roots and higher levels of sugars and organic acids in their leaves compared to plants that do not come from primed seeds (Hussain et al., 2019). Considering these benefits, halopriming demonstrates potential as a way to improve the salt tolerance of crops. Researchers have noted that halopriming leads to fast and coordinated germination, believed to be caused by the activation of pre-germinative processes. These mechanisms cause changes in the quantity and quality of biological compounds in the seeds (Gour et al., 2022). Hence, this study aimed to assess halopriming's influence on three different millet varieties: CO 15 for finger millet, ATL 1 for little millet, and CO(KV)-2 for Barnyard millet.

MATERIALS AND METHODS

Collection and extraction of plant material

The plant materials used in this study included finger millet (*Eleusine coracana*) variety CO(Ra) 15, little millet (*Panicum sumatrense*) variety ATL 1, and Barnyard millet (*Echinochloa frumentacea*) variety CO(KV)-2. All the varieties were obtained from ICAR-KVK, Gandhigram, Dindigul, Tamil Nadu, India. To investigate the effects of "Halopriming," saline solutions with concentrations of 0 (unprimed), 1, and 1.8 g/L of NaCl were used.

Methods

Initially, the seeds were disinfected using sodium hypochlorite (6%) for 3 minutes, then rinsed with distilled water to remove excess chlorine. It was then divided into three groups for each crop, each group having 20 seeds, each group soaked in different NaCl solutions of (S0=0 g/L) and S1 = 1 g/L and S2 = 1.8 g/L) for 24 hours in total darkness (Khadraji et al., 2020). The experiment was conducted by a completely randomised block design. Following the priming process, the seeds were thoroughly washed with distilled water multiple times (4-5 times) to remove any remaining sodium chloride. Subsequently, the seeds were left at room temperature for 12-14 hours.

For each crop 20 primed seeds were placed in Petri dishes containing a layer of germination paper soaked with distilled water, 1 g/L NaCl, and 1.8 g/L NaCl were used each day. Each crop and experiment were conducted in triplicate. The germinated seeds were counted daily for 7 days after sown (DAS). The germination percentage (GP) was determined using the following formula: $GP=Nn\times100$, where (N) represent the total number of seeds and (n) represents the number of seeds that have sprouted (Ashokkumar et al., 2023; Vivekanandhan et al., 2024).

The shoot length (cm) was quantified by measuring the distance from the seed to the tip of the leaf blade, as shown in Figure 1B. The root length (cm) and the total seedling length (cm) were measured following the method described by Ashokkumar et al. (2023). The vigour index (VI) was calculated using the formula VI = germination x total seedling length (Abdul-Baki & Anderson, 1973).

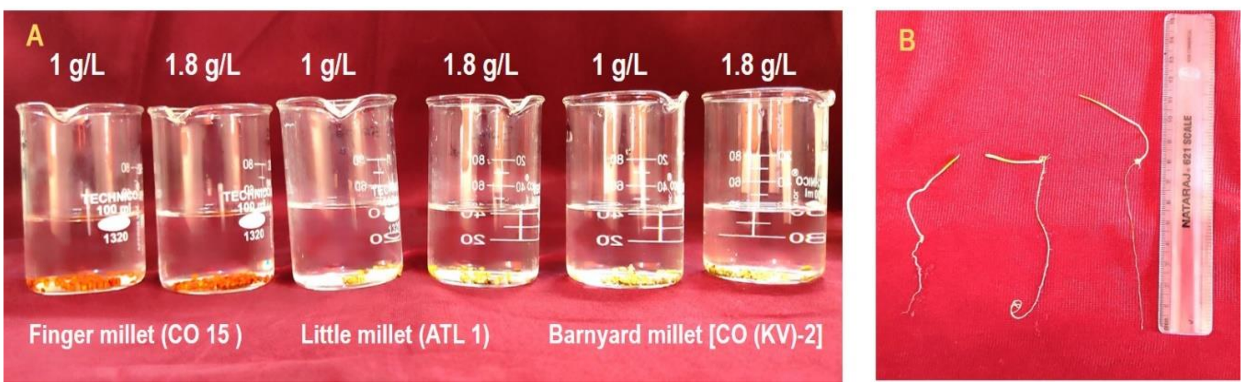

Figure 1. A) Seed priming with 1 g and 1.8 g of NaCl for three millet crops. B) Growth parameters measurement

Data analysis

The statistical analysis was performed using the WASP 2.0 software. A one-way analysis of variance (ANOVA) was conducted with three replicates per treatment for all the parameters examined. The critical difference was computed with a significance level of 5%.

RESULTS AND DISCUSSION

Effect of halopriming on Germination percentage

In Hp1 (halo priming 1), finger millet variety CO(Ra) 15 has shown 100% of seed germination in normal conditions (Table 1 - 3). In Hp2, the little millet variety ATL 1 has shown 100% seed germination under osmotic stress with a concentration of 1g/L NaCl (S1). In Hp1, barnyard millet variety CO (KV) 2 has also shown 100% seed germination under osmotic stress with a concentration of 1.8 g/L NaCl (S2).

The germination percentage has increased as a result of priming due to the accelerated rate of cell division in the primed seeds and the activation of various metabolic processes crucial for the initial stages of seed germination (Khadraji et al., 2017; Paul et al., 2022).

Effect of halopriming on shoot length and root length

The primed seeds exhibited a reduction in shoot length in comparison to their respective controls when exposed to salt stress. Despite this, the barnyard millet variety Co (KV)2 and the little millet variety ATL 1, both under the Hp1 category, demonstrated the longest shoot lengths of 7.3 cm and 5.85 cm respectively under salt stress (S1). The root length of the primed seeds was less than that of their respective controls under salt stress. The little millet variety ATL 1 had shown poor performance in root length compared to the other two varieties of little millet and barnyard millet. In Hp 1, finger millet variety CO(Ra) 15 recorded the highest root length of 12.25 under salt stress (S2). Similar results were observed in chickpea (Khadraji et al., 2020) and maize (Mahara et al., 2022). The technique of halopriming mitigates the detrimental effects of salinity on seed germination and the establishment of seedlings. This method is employed to enhance seed germination performance under both optimal and challenging conditions (Jisha et al., 2013).

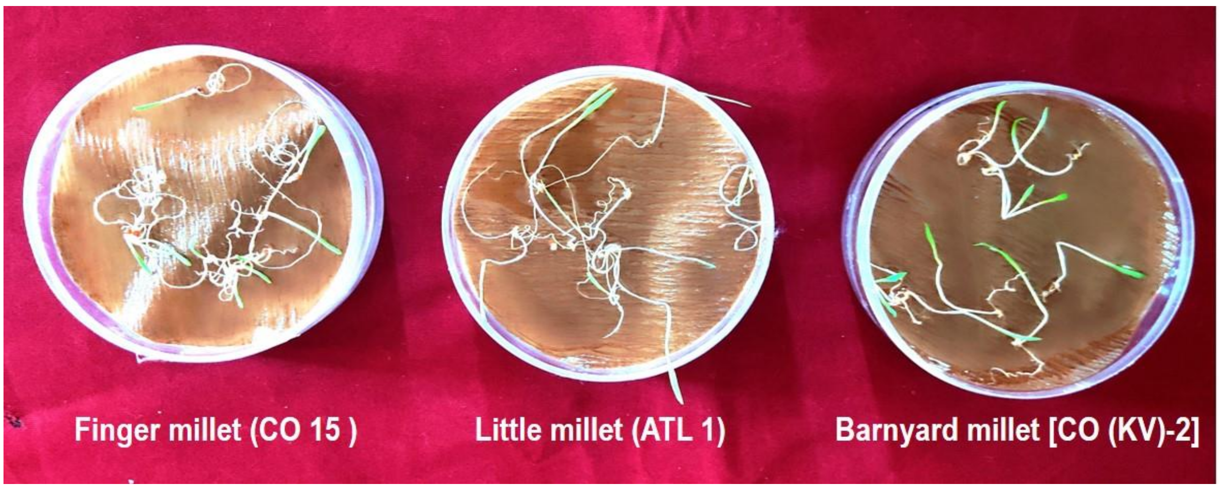

Figure 2. Effect of halopriming Hp1 (1 g / L NaCl) on finger millet, little millet and barnyard millet at 7 DAS.

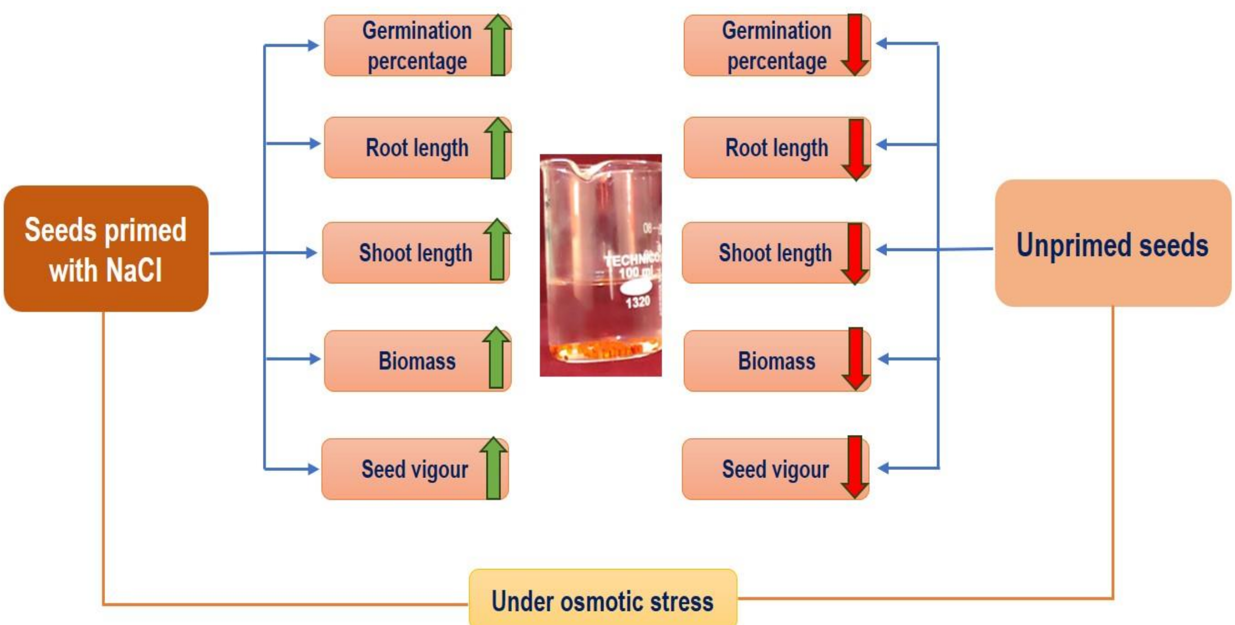

Figure 3. Graphical representation of halopriming effects in millet crops.

Table 1. Effect of halo priming on germination %, vigour index, and growth parameters at seedling stage finger millet variety CO(Ra) 15

Halopriming	Germination %			Shoot Length (Cm)			Root Length (Cm)			Seedling Length (Cm)			Vigour Index		
	0	S1	S2	0	S1	S2	0	S1	S2	0	S1	S2	0	S1	S2
0	82.5^{b}	70^{c}	100^{a}	2.7^{a}	2.5^{a}	2.2^{b}	9.55^{b}	7.1^{b}	7.4^{c}	12.25^{a}	9.6^{b}	9.6^{c}	1010.5^{b}	672^{b}	960^{b}
Hp1	100^{a}	92.5^{a}	87.5^{c}	2.3^{b}	1.85^{b}	2.25^{b}	10.05^{a}	4.7^{c}	8.7^{b}	12.35^{a}	6.22^{c}	10.95^{b}	1235^{a}	605.5^{b}	957.5^{b}
Hp2	87.5^{b}	82.5^{b}	97.5^{a}	2^{b}	2.4^{a}	2.4^{a}	5.85^{c}	11.65^{a}	12.25^{a}	7.85^{b}	14.35^{a}	14.65^{a}	686.5^{c}	1184.25^{a}	1428.25^{a}
CV	3.208	3.535	3.039	4.949	1.814	1.788	0.834	2.611	1.781	1.252	1.704	1.774	2.317	4.807	2.359
CD (0.05)	9.186	9.186	9.186	0.367	0.13	0.13	0.225	0.65	0.536	0.431	0.551	0.662	72.071	125.526	83.718

Table 2. Effect of hallo priming on germination %, vigour index, and growth parameters at seedling stage of little millet variety ATL 1

Halopriming	Germination %			Shoot Length (Cm)			Root Length (Cm)			Seedling Length (Cm)			Vigour Index (Cm)		
	0	S1	S2	0	S1	S2	0	S1	S2	0	S1	S2	0	S1	S2
0	82.5^{b}	90^{b}	100^{a}	2.85^{b}	5.6^{a}	3.55^{b}	2.35^{b}	3.5^{a}	2.4^{b}	5.2^{c}	9.1^{a}	5.95^{c}	429.25^{c}	819^{a}	595^{c}
Hp1	97.5^{a}	92.5^{b}	87.5^{b}	3.25^{ab}	5.85^{a}	3.1^{c}	2.7^{b}	3.165^{a}	4.55^{a}	5.95^{b}	9.015^{a}	7.65^{b}	580^{b}	834.35^{a}	668.5^{b}
Hp2	97.5^{a}	100^{a}	82.5^{b}	3.65^{a}	3.4^{b}	4.2^{a}	4.1^{a}	2.0^{b}	5.15^{a}	7.75^{a}	5.4^{b}	9.35^{a}	755.5^{a}	540^{b}	771.0^{a}
CV	3.822	2.168	3.208	4.166	3.401	3.386	4.016	3.816	5.161	1.587	2.838	4.099	3.831	4.959	2.008
CD (0.05)	11.25	6.495	9.186	0.431	0.536	0.39	0.39	0.351	0.662	0.318	0.708	0.998	71.71	115.378	43.338

Table 3. Effect of hallo priming on germination %, vigour index, and growth parameters at seedling stage of Barnyard millet variety CO (KV) 2

Halopriming	Germination %			Shoot Length (Cm)			Root Length (Cm)			Seedling Length (Cm)			Vigour Index		
	0	S1	S2	0	S1	S2	0	S1	S2	0	S1	S2	0	S1	S2
0	100^{a}	82.5^{b}	92.5^{a}	7.75^{b}	2.6^{c}	5.7^{b}	9.55^{a}	5.1^{b}	4.85^{c}	17.3^{b}	7.7^{c}	11.05^{b}	1730^{a}	635.25^{c}	1126.25^{b}
Hp1	87.5^{b}	90^{ab}	100^{a}	9.2^{a}	7.3^{a}	7.75^{a}	10.2^{a}	7.3^{a}	5.7^{b}	19.4^{a}	14.6^{a}	13.45^{a}	1697.75^{a}	1314^{a}	1345^{a}
Hp2	90^{b}	97.5^{a}	82.5^{b}	6.35^{c}	5.8^{b}	5.55^{b}	5.75^{b}	5.75^{b}	8.65^{a}	12.1^{c}	11.55^{b}	14.2^{a}	1089^{b}	1125.5^{b}	1171.25^{b}
CV	2.201	3.208	3.149	2.879	2.206	3.287	3.902	4.527	3.253	2.559	3.413	3.876	4.061	3.272	3.367
CD (0.05)	6.495	9.186	9.186	0.712	0.367	0.662	1.055	0.871	0.662	1.325	1.226	1.591	194.537	106.706	130.077

Note, 0 -Unprimed; Hp1 - Halopriming 1 (1g/L); Hp2 - Halopriming 2 (1.8g/L); S1 = 1g/L; S2= 1.8g/L; 0 = 0g/L

Effect of halopriming on vigour index

Seed vigour, a complex agronomic characteristic encompassing seed lifetime, germination speed, seedling development, and early stress tolerance, plays a crucial role in determining the length and efficiency of the establishment period (Reed et al., 2022). After assessing the vigour Index, it was noted that Hp1 showed positive results regarding the germination percentage and seedling growth of finger millet variety CO(Ra) 15 and little millet variety ATL 1. Several studies have demonstrated that exposure to high levels of salt may significantly reduce the strength of seeds and impede the process of germination and early growth in various species, such as *Oryza sativa* (Ashokkumar et al., 2013; Ashokkumar et al., 2023; Vivekanandan et al., 2024), *Lavandula stoechas* (Benadjaoud et al., 2022), *Suaeda vermiculata* (Al-Shamsi et al., 2020) and Cicer arietinum (Khadraji et al., 2020) and *Zea mays* (Mahara et al., 2022). Previous studies in wheat yielded comparable findings to our present study results (Kumar et al., 2017). In addition, Hp1 had positive effects on the growth of barnyard millet seedlings and the percentage of germination, specifically for the Co (KV) 2 variety, when exposed to osmotic stress conditions. Figure 3 displayed graphical depictions of the impacts of halo priming on millet crops.

CONCLUSION

The impact of halopriming resulted in enhancements in germination, seedling establishment, and notably, the augmentation of seedlings' tolerance capacity to water stress in all three studied millet crops. Consequently, the application of this technique to seeds can render the variety resistant to osmotic stress. Moreover, halopriming offers additional benefits such as practicality and cost-effectiveness. The technological challenge of enhancing seed performance presents an opportunity for more comprehensive investigations into the physiological and biochemical alterations that transpire during seed treatments.

CONFLICTS OF INTERESTS

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All the authors contributed equally to this work.

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