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Improving the Germination Performance of Kalmegh (*Andrographis paniculata* Wall. ex Nees) through Hydropriming, Halo-Priming and Osmopriming Under Salinity Condition

Saumya Shruti [a*], Ashwin Trivedi [b], Kalyanrao Patil [c] and Kamlesh Chaudhary [a]

[a] Department of Plant Physiology, B. A. College of Agriculture, Anand Agricultural University, Anand-388 110, Gujarat, India.
[b] ICAR-Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand-388110, Gujarat, India.
[c] Department of Seed Science and Technology, B. A. College of Agriculture, Anand Agricultural University, Anand-388 110, Gujarat, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**Corresponding author: E-mail: shrutisaumya82@gmail.com;*

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ABSTRACT

Salinity is a significant global issue that adversely affects seed germination and seedling vigour across a wide range of crops. Priming, however, is considered a basic method to enhance seed performance (germination and vigour) under saline conditions. The aim of this study is to evaluate the effects of different priming treatments on seedlings under varying salinity levels (0, 4, 6, 8, and 10 dS m^{-1}) in Kalmegh, a traditionally valuable medicinal plant. Seeds were primed through four methods *viz*., T_0- Non-primed (control), T_1- Hydro primed (distilled water), T_2- halo priming with NaCl (0.8%), T_3- halo priming with $CaCl_2$ (0.8%) and T_4- osmo priming with KNO_3 (1%) for 6 hours at 25ºC in the dark. This experiment was arranged in a factorial design based on a CRD (Completely Randomized Design) with three replications. The results demonstrated that priming treatments significantly improved germination percentage, seedling length (cm), seedling fresh weight (mg), seedling dry weight (mg), and vigour indices. Notably, seeds primed with KNO_3 showed the highest germination performance at all salinity levels. Even at the highest salinity level, they achieved a germination percentage of 94% and a Seedling Vigour Index I of 506, compared to the control group, which exhibited a germination percentage of 88% and a Seedling Vigour Index I of 298. KNO_3 priming at 0 dS/m produced the highest seedling length (8.5 cm), root length (2.7 cm), shoot length (6.4 cm), fresh weight (26.32 mg), and dry weight (10.3 mg).

Keywords: Germination percentage; kalmegh; priming; salinity; seed vigour indices.

1. INTRODUCTION

Salinity is a broad term that refers to the existence of increased concentrations of various salts like sodium chloride, magnesium, calcium sulfates, and bicarbonates in both soil and water [1]. Soil salinity is considered a global concern that affects more or less 20% of irrigated land and reduces crop productivity significantly [2]. Elevated salt levels have adverse impacts on both the quantity and quality of crops, including slowing down plant growth and development, hindering enzymatic functions, and diminishing the rate of photosynthesis [3].

Numerous research findings suggested that elevated stress leads to a delay in seed germination and a decrease in germination rates [4].

Andrographis paniculata, also referred to as "King of Bitters," is known as 'kalmegh' in India and “kariatu” as in local name in the Gujarat region. This herb is a highly esteemed medicinal plant and is prevalent in various Asian nations, including Malaysia, Indonesia, Pakistan, India, and Sri Lanka. It is extensively grown in regions such as Thailand, China, Mauritius, and the East and West Indies [5]. This medicinal plant contains active compounds like andrographolide, DDAG, DAG, and 14-deoxy-11, with potential for treating serious illnesses like cancer, hepatitis, and HIV [6].

Andrographis paniculata typically reproduces naturally through seeds, but seed dormancy poses a challenge, causing low germination rates due to physical dormancy and the seeds' innate characteristics [7]. The ability of seeds to endure salinity during germination and the initial stages of seedling growth is essential for the successful establishment of plant growth in saline soils [8]. Salt concentration impact seed germination, delaying it in both low and high concentrations. Low salt (below optimum) induces dormancy, while high salt hinders sprouting and reduces germination due to water loss around roots and increased transpiration [9].

Globally, numerous research efforts have been undertaken to address salinity stress, and among these, seed priming stands out as a promising strategy with the potential to mitigate the adverse effects of salinity and enhance both crop yield and quality [10]. Seed priming is an inexpensive and straightforward method that improves germination and the establishment of seedlings by stimulating a range of physiological and metabolic processes [7]. This method is employed to enhance the speed of germination, seed vigour, seed establishment and crop yield [11].

Hydro priming, an eco-friendly method, involves soaking seeds in water and allowing them to re-dry to their original moisture level before planting. This process enhances water absorption and seed hydration under such conditions [12]. Cis-priming refers to a type of priming in which the stimulus and stress are identical., Halopriming is the prevalent form of cis-priming and helps plants develop tolerance to salinity stress. The process

of priming with salt solutions is commonly known as "halopriming." NaCl priming of seeds led to better germination and seedling vigour by breaking dormancy compared to untreated seeds. These seed treatments not only accelerated the germination rate and reduced germination time but also boosted seedling vigor, as evidenced by longer plumule and radicle lengths, as well as higher fresh and dry seedling weights [13]. Priming seeds with inorganic salts enhances the activity of many enzymes engaged in seed germination and modifies the distribution of organic substances within the embryo [14]. Priming of seeds through some agents like KNO_3 or PEG 8000 or 6000 known to be “osmopriming”.

The aim of this research was to evaluate the effects of different priming methods like, hydopriming, halopriming and osmoprimng on the germination performance of kalmegh (*Andrographis paniculata*) seedlings under salinity condition.

2. MATERIALS AND METHODS

Andrographis paniculata seeds were collected from the Medicinal and Aromatic Plants Research Station, Anand Agricultural University, Anand, Gujarat, India. The seeds were surface sterilized by immersing them in a 5% sodium hypochlorite (NaClO) solution for 2 minutes [15] followed by thorough rinsing with distilled water. The seeds were then soaked in NaCl (0.8%), $CaCl_2$ (0.8%), KNO_3 (1%), or distilled water for 6 hours in a dark environment at room temperature (25°C). After soaking, the seeds were rinsed in sterilized distilled water for 2 minutes. They were subsequently air-dried in darkness at 25°C for 24 hours, until they regained their initial fresh weight and colour before sowing [16]. The primed seeds were then sown in 15 cm diameter Petri dishes lined with 90 mm Whatman No. 1 filter paper, which was moistened with a consistent amount of sterile water. The experiment was conducted in a controlled growth chamber set to a temperature of 25±4°C and a relative humidity of 75% for 14 days. Germination was defined as the point when the radicle (embryonic root) reached a minimum length of 2 millimeters. All germination parameters were observed and recorded on the 14th day after sowing. Ten seedlings from each treatment and replication were used for observations related to seedling growth parameters.

For the experiment five salinity levels consisted of Electrical conductivity (0, 4, 6, 8 and 10). Salinity was introduced by the addition of salts *viz*., Calcium chloride ($CaCl_2$), Magnesium chloride ($MgCl_2$), Magnesium sulphate ($MgSO_4$) and Sodium chloride (NaCl) in the ratio of 5:8:2:25 respectively (Iqbal et al., 1998). A stock solution was prepared and then diluted to achieve the desired salinity concentrations.

2.1 Germination Percentage (%)

The laboratory germination test was conducted by using the top-of-paper method. One hundred seeds from each treatment were randomly selected from the seed lot, freshly harvested and processed, and placed evenly on germination paper in three replications. The germination paper was then placed in a germinator set to a temperature of 20 ± 0.5ºC, specific to each treatment, and a relative humidity of 95 ± 1 per cent. The final count was recorded on the fourteenth day of the germination test to determine the percentage of normal seedlings, representing the germination rate.

2.2 Shoot Length (cm)

Ten normal seedlings were selected for measuring shoot length. The measurement was taken from the base of the primary leaf to the base of the hypocotyl, and the average shoot length was expressed in centimeters.

2.3 Root Length (cm)

Ten healthy seedlings were chosen to assess root length. The average root length in centimeters was determined by measuring from the base of the hypocotyl to the tip of the longest root.

2.4 Seedling Length (cm)

Ten normal seedlings were randomly chosen from the germination test. The distance from the collar region to the tip of the primary shoot was measured as the shoot length in centimeters, while the distance from the collar region to the tip of the primary root was measured as the root length in centimeters. The total length of the seedling was calculated using the formula:

Seedling length (cm) = Shoot length (cm) + Root length (cm)

2.5 Seedling Fresh Weight (mg)

Ten seedlings were chosen, separated from their cotyledons, and weighed while they were still

damp to record the fresh weight of the seedlings. The weights were recorded in milligrams.

2.6 Seedling Dry Weight (mg)

Ten randomly selected normal seedlings from the germination test were placed in a hot air oven at 80°C for 24 hours. The weight of the dried seedlings was recorded, and the dry weight of the seedlings was calculated and expressed in milligrams.

2.7 Seedling Vigour Index

It was calculated by the method of Abdul-Baki and Anderson [17] was expressed in whole number:

Seedling Vigour Index-1 = Germination (%) x Total seedling length (cm)

Seedling Vigour Index-II = Germination (%) x Seedling dry weight (mg)

2.8 Data Analysis

The observed data were analysed using the statistical methods recommended by Steel and Torrie [18] for Completely Randomized Design (Factorial) for seed quality parameters. The standard error of the mean (S.Em.) and the critical difference (CD) at the 5% significance level were calculated and included in the relevant tables for result interpretation.

3. RESULTS AND DISCUSSION

According to the statistical analysis based on completely randomized design (factorial), all seedling growth parameters showed significant effects, except for seedling fresh and dry weight. Salinity impacted all seedling growth parameters, and the interaction between salinity and priming significantly influenced seedling morphological growth. The highest germination percentage was observed in KNO_3-primed seeds across all salinity levels, with a maximum of 88% at 0 dS/m and a minimum of 78% at 10 dS/m. Increasing salinity levels significantly reduced germination percentages across all priming treatments, and the related data are presented in Table 1.

The decrease in germination percentage with increasing salt concentrations may be attributed to the accumulation of harmful ions, a reduction in the osmotic potential of the solution, or an imbalance in the remobilization of seed reserves. This decline in germination percentage due to higher salinity levels is consistent with the findings of [19]. Liu et al., [6] also observed that decreased germination was linked to reduced water potential and limited water availability to the seeds. Okello et al., [20] confirmed that seeds of medicinal plant *Aspilia africana* treated with KNO_3 displayed better germination parameters when primed for 6 and 12 hours.

Increasing salinity levels significantly affected the germination percentage of seedlings. Among the various priming techniques, KNO_3 consistently improved germination percentages across all salinity levels compared to other treatments. While NaCl and $CaCl_2$ had similar effects on seedling length, fresh weight, dry weight, shoot length, and root length, the highest values were recorded with KNO_3 priming, outperforming hydropriming (water primed seeds). Our findings on the superior germination percentage with KNO3 are supported by studies from Abdullahi et al., [21] Lara et al., [22], Shim et al., [23] and Nawaz et al., [24].

Seedling length significantly increased with KNO_3 treatment (8.2 cm), followed by $CaCl_2$ (7.6 cm), NaCl (6.36 cm), and the shortest length was observed in hydroprimed seeds (4.8 cm). Higher salinity levels also had a negative impact on seedling length. The interaction between priming and salinity showed significant differences in seedling length across all salinity levels. KNO_3 treatment led to the highest shoot length (4.35 cm) and root length (2.2 cm) compared to the other treatments, followed by $CaCl_2$, NaCl, and hydropriming. Salinity stress significantly reduced both shoot and root lengths in growing seedlings. The control group (0 dS/m) exhibited the greatest shoot and root lengths. Seedling shoot and root length showed significnat similar result for NaCl and $CaCl_2$ priming treatment but the highest was observed at KNO_3 treatment and reduction in shoot and root length was obserbed at increasing salinity levels. This result was in accordance with Gholami et al., [25] Following KNO_3, $CaCl_2$ emerged as a more effective alternative priming agent for enhancing both the fresh and dry matter of seedlings. This trend is consistent with observations made by Basra et al., [26] in wheat. The positive effects of $CaCl_2$ may be attributed to calcium's role as a second messenger within plant cells, its protective function against unfavorable environmental stress, and its influence on hormonal balance, as suggested by Iqbal et al., [27].

Table 1. Effect of priming treatments on the seedling germination and growth parameters under salinity condition

Treatment		Seedling growth parameters							
EC levels (dS/m)	**Priming**	**Germination (%)**	**Length (cm)**	**Shoot length (cm)**	**Root length (cm)**	**Fresh weight (mg)**	**Dry weight (mg)**	**Vigour Index-I**	**Vigour Index-II**
0	Control (water)	80	8.20	5.96	2.20	19.56	6.60	656	528
	NaCl	87	8.30	6.00	2.30	23.20	7.30	722	638
	$CaCl_2$	90	8.40	6.20	2.40	24.10	8.80	756	797
	KNO_3	94	8.50	6.40	2.70	25.50	10.30	805	968
4	Control (water)	78	7.50	4.90	2.60	15.13	6.10	585	476
	NaCl	83.60	7.60	4.80	2.60	17.63	7.03	634	588
	$CaCl_2$	88.30	7.70	4.66	2.80	21.93	8.50	680	756
	KNO_3	91.30	7.80	4.60	2.90	23.30	9.50	721	909
6	Control (water)	73.60	6.20	3.43	2.30	14.20	5.50	454	409
	NaCl	77	6.28	3.60	2.40	16.30	6.40	484	493
	$CaCl_2$	87.60	6.36	3.73	2.50	18.66	8.06	557	706
	KNO_3	91	6.50	3.93	2.63	21.06	5.20	591	840
8	Control (water)	69.30	4.50	2.73	1.30	13.30	4.90	312	342
	NaCl	76	4.60	2.98	1.40	15.50	6.10	349	464
	$CaCl_2$	86.30	4.63	3.12	1.76	18.60	7.90	400	684
	KNO_3	88.30	4.80	3.23	1.90	20.84	9.30	443	824
10	Control (water)	68	3.16	1.98	1.10	10.46	4.03	224	274
	NaCl	74	3.26	2.01	1.20	11.43	5.20	241	389
	$CaCl_2$	85	3.30	2.10	1.26	13.30	7.70	280	657
	KNO_3	86	3.46	2.20	1.50	14.50	8.90	298	767
Interaction Treatment A (EC) × Treatment B (Priming)									
	C.D	3.047	0.192	0.236	0.194	0.877	0.289	4.021	3.257
	S.E.(m)	1.062	0.067	0.082	0.067	0.306	0.101	1.402	1.135

Note:Interaction effect signified the salinity stress (EC levels) and with different priming treatment based on a completely randomized design (factorial)

A higher percentage of dry matter was observed at lower salinity levels compared to higher salinity levels. The highest seedling fresh weight (25.5 mg) and dry weight (10.3 mg) were recorded in seeds primed with KNO_3, followed by $CaCl_2$. Increasing salinity levels had a negative impact on both seedling fresh and dry weight. Except for KNO_3, there were no significant differences among the various priming treatments in terms of their effects on seedling fresh and dry weight under Petri dish conditions. Verma and Solanki [28] evaluated the effects of various seed priming agents on seedling growth of Isabgol (*Plantago ovata* Forsk) Variety GI-3 under salinity levels (0 and 5.3 dS/m). The study compared control (unprimed seeds) with KNO_3, assessing traits like germination percentage, seed vigour, plumule and root length, fresh weight. It was suggested that KNO_3 emerged as the most effective treatment, significantly enhancing overall fresh and dry matter in the plant.

Seedling Vigour Index-I (SVI-I) and Seedling Vigour Index-II (SVI-II) significantly decreased with increasing salinity levels, with the highest values observed at 0 dS/m. At every salinity level, KNO_3 priming resulted in higher SVI-I (805) and SVI-II (968) compared to the other treatments, while the lowest values were observed in hydropriming (656). Similar results were obtained from the study of Mukherjee, [29] in sweet chirayita when treated with KNO_3 (2%), produced the highest Seedling Vigour Index-II of 54.18 compared to the control treatment. Ibrahim [30] suggested that the negative impact of salinity on both seed germination and seedling growth can be mitigated through KNO_3 priming. This improvement occurs through various mechanisms like enhanced activity of germination enzymes, modification of the mobilization of organic molecules in different seed parts, elevation in the levels of proteins, free amino acids, and soluble sugars, increased activity of acid phosphatase and phytase in both seeds and seedlings, and a reduction in peroxidase activity. Seed priming with KNO_3 influenced seedling emergence and the rate of germination. According to other studies on priming, key events include metabolic changes like DNA repair, increased RNA biosynthesis, and improved seed respiration. This highlights the significance of the seed imbibition period during priming [31].

The seedling vigour index of wheat showed a significant decline as salinity levels increased. Priming the seeds with KNO_3 resulted in the highest seedling vigour index values at the 0 dS/m level, but higher salinity levels led to a reduction in this index. Seedling vigour serves as an indicator of the cumulative damage incurred as seed viability decreases. This damage accumulates within the seeds, rendering them incapable of germination and ultimately leading to their demise [32]. The decline in seedling vigour index associated with increased salinity levels is due to salt stress hindering initial growth, particularly of the shoots. The decrease in seedling vigour index under water-restricted conditions is a common observation reported in other studies [33-35].

4. CONCLUSION

Priming with KNO_3 (osmo priming) resulted in highest germination percentage. Higher level of salinity produced significant increased amount of seedling fresh and dry matter as compared to only hydro-primed seeds. Seedling Vigour Index (I and II) showed significant interaction of salinity and priming techniques with the highest observed index at first level of salinity and minimum value observed at fifth level of salinity. We can recommend this simple and cost-effective strategy to farmers to improve the rate and uniformity of medicinal plant emergence under environmental stress.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTEREST

Authors have declared that no competing interests exist.

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