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# Greenhouse soil sowing of *Catharanthus roseus*: effects of potassium nitrate and salicylic acid priming on salinity tolerance in different sowing methods

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#### **ABSTRACT**

Purpose: This study aimed to evaluate the effects of seed priming on Madagascar periwinkle (Catharanthus roseus L.) seedlings under saline conditions, focusing on salicylic acid and potassium nitrate treatments. Research method: The research was conducted at Ferdowsi University of Mashhad in 2024. F<sub>1</sub> Seeds of the 'Sunstorm Red' were primed with salicylic acid (150, 250 and 350 mg/L) and potassium nitrate (50 and 100 mg/L) for 24 hours. A factorial experiment based on a completely randomized design with three replications assessed four salinity levels (0, 4, 6, and 8 dS/m) and two sowing methods: direct sowing in pots and indirect sowing via germination in petri dishes followed by transplantation. Traits such as relative water content, SPAD index, electrolyte leakage, were measured 65 days after sowing. Findings: Seed priming significantly reduced the negative effects of salinity. Potassium nitrate priming outperformed salicylic acid in enhancing relative water content, SPAD index, stem and root lengths, and biomass. Direct sowing showed better resistance to salinity compared to indirect sowing, particularly in improving stem length, root length, and SPAD index. Primed seeds exhibited higher germination rates and stronger seedlings with enhanced growth characteristics compared to untreated seeds. Research limitations: The study was limited to specific priming agents and salinity levels, suggesting further research to optimize potassium nitrate concentrations for improved seedling production. Originality/Value: This research recommends potassium nitrate priming for robust *C. roseus* seedling production in saline environments. The findings can guide growers in selecting cost-effective priming strategies to enhance plant resilience and productivity sustainably.



## **INTRODUCTION**

Water and soil salinity are major constraints on crop yields in dry and semi-dry areas. While urban populations are expanding, it has resulted in a decline in the availability of high-quality freshwater resources. Water shortages could make it necessary to use low-quality and salty water for irrigating plants in urban parks and gardens (Mousavi Bazaz et al., 2023). Furthermore, high salt levels in water can severely harm the growth of ornamental plants, especially when they are young, causing damage to their cells and disrupting their ability to carry out essential functions (Ellouzi et al., 2023). Exposure to saltwater can also reduce the size of flowers and how long they last in a vase life (Othman & Al-ajlouni, 2024). Changes in plant physiology resulting from increased salinity include decreased photosynthesis and lowered stomatal conductance, leading to reduced plant growth and lower flower quality (Othman & Al-ajlouni, 2024). Seedlings are much more susceptible to salt damage than plants in later stages of growth. This sensitivity necessitates further research to develop management techniques that can mitigate the adverse effects of salinity during this critical phase (Bezerra et al., 2020).

Catharanthus roseus, commonly known as the Madagascar periwinkle, is a prominent flowering plant within the Apocynaceae family. Its widespread cultivation primarily from its aesthetically pleasing and remarkably persistent floral displays (Hira et al., 2024). It is also a valuable medicinal plant used for high blood pressure and diabetes (Ababaf et al., 2021).

Comparative studies on ornamental species reveal diverse levels of salt tolerance, as seen in the case of *Zinnia elegans*, which experiences linear growth decline even at a salinity level of 1.5 dS m<sup>-1</sup>, whereas *Rudbeckia hirta* retains its germination rates under moderate salinity levels (Miladinov et al., 2020). Conversely, *Catharanthus roseus* is classified as sensitive to salinity levels above 1.5 dS m<sup>-1</sup>, whereas *Tagetes patula* and *Celosia argentea* are considered moderately sensitive, underscoring the importance of understanding irrigation management in saline environments (Bezerra et al., 2020).

A key agricultural approach, seed priming, has become essential for improving seed germination rates under saltwater conditions by improving physiological and biochemical functions (Khan et al., 2022; Nyandwi et al., 2024). Priming improves salt tolerance through three interrelated processes: maintaining ion balances, activating antioxidants, and reallocating resources (Hameed et al., 2025; Zulfiqar et al., 2022). Methods like hydropriming, osmopriming, and hormonal priming can reduce the negative effects of high salt levels on growing seeds by enhancing their ability to balance fluids and maintain stable ion levels (Khan et al., 2022). Seeds that have been primed demonstrate increased activity in antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), which neutralize reactive oxygen species, thereby reducing lipid peroxidation by 40-60% in comparison to non-primed seeds (Khan et al., 2022). Research has demonstrated that potassium nitrate (KNO<sub>3</sub>) priming can counteract the negative effects of salinity by increasing seedling vitality and encouraging stem growth in saline environments. A study on cumin found that using KNO<sub>3</sub> for priming decreased ascorbate peroxidase activity by 35% and lowered proline accumulation by 28% under 150 mM NaCl (Kalhor Monfared et al., 2022).

A promising approach to increasing plant resilience to salt-related stress involves using salicylic acid (SA) priming to modify antioxidant defenses, maintain cell membrane integrity, and regulate stress signaling pathways, as indicated in research (Ellouzi et al., 2023). Additionally, studies have found that SA priming at a concentration of 0.5 millimolar notably enhanced germination outcomes in *Cannabis sativa* under saline conditions, which suggests its potential for enhancing salt tolerance in agricultural crops (Gul et al., 2024). Research in other ornamental species, including *Limonium bicolor*, has demonstrated that SA application



can enhance seedling establishment under salt stress by improving germination (Liu et al., 2019).

A laboratory study on madagascar periwinkle seed germination was conducted, which found that seeds primed with potassium nitrate had a higher germination rate than control seeds and those treated with ascorbic acid (Mousavi Bazaz & Nemati, 2023). This greenhouse study aims to grow primed seeds in soil to achieve the following objectives:

- 1. Investigate early seedling development in *Catharanthus roseus*, focusing on priming and salt tolerance.
  - 2. Examine the impact of KNO<sub>3</sub> and salicylic acid on periwinkle seedling growth.
- 3. Compare direct and indirect sowing methods to determine their effects on seedling growth and quality under saline conditions.

## MATERIALS AND METHODS

## Plant materials and seed preparation

The research was carried out in 2024 to assess the impact of priming on the development of *Catharanthus roseus* seedlings in a greenhouse setting, under conditions of salinity stress, at the Ferdowsi University of Mashhad's Agricultural Research Greenhouse. Seeds from the F<sub>1</sub> generation of *Catharanthus roseus* 'Sunstorm Red' were obtained from Syngenta.

The experiment was structured as a factorial based on a completely randomized design, consisting of three replications. The variables examined consisted of four different levels of salinity (0, 4, 6, and 8 dS/m) and five seed priming concentrations: 150, 250, and 350 ppm of salicylic acid, as well as 50 and 100 ppm of KNO<sub>3</sub>. The control treatment consisted of directly sowing unprimed seeds. The seeds, initially treated with a 2.5% hypochlorite solution for a period of 10 minutes, followed by three deionized water rinses, were then exposed to various priming treatments for a duration of 24 hours (Ahmad et al., 2017). Following this, the seeds were then removed and assessed in two different sowing techniques. The first approach consisted of direct sowing, which entailed planting seeds in pots with a 12 cm diameter, topping them with coco peat, and then irrigating them with saline water over a period that allowed the water to reach the desired final salinity level. In the second approach, indirect seeding was employed, where seeds were immersed in petri dishes filled with deionized water to allow germination (the emergence of a 2 mm radicle) prior to being moved to the same pots utilized for direct seeding. The potting mix comprised 25% peat moss, 50% sand, and 25% agricultural soil (sandy loam), the soil electrical conductivity (EC) and pH were 4.5 dS/m and 7.24 respectively.

## **Procedure for imposing salt stress**

Salinity levels were applied gradually from the start of planting and reached the final salinity level. Sodium chloride (NaCl) from Scharlau Company was used as the salt source. Irrigation (100 ml for each pot) with the desired salinity levels was performed every other day, considering the soil's field capacity and greenhouse temperature. Leaching (irrigation without salinity) was done every 12 days to prevent excessive salt accumulation. The average greenhouse temperature was (27/20°C at day/night). The pots were maintained under these conditions for 65 days until the seedlings reached an appropriate stage for transplanting.

#### **Evaluated traits**

After 65 days, the desired traits were measured. These traits included the number of healthy seedlings reaching the transplant stage, chlorophyll index, electrolyte leakage, relative water content, root and stem length, fresh and dry weight of stem and root, and leaf number.



To measure dry weight, the fresh samples were dried in an oven at 72°C for 48 hours and then weighed.

## **SPAD** index

A chlorophyll meter was used to measure the SPAD index (SPAD-502 Konica, Minolta, Tokyo).

## Electrolyte leakage

To measure electrolyte leakage, leaf samples were washed and excess water was removed. Samples of similar size were placed in deionized water for 24 hours. Initial electrical conductivity was measured using an EC Meter. The samples were then autoclaved at 120°C for 20 minutes, and secondary electrical conductivity was measured. The formula for calculating electrolyte leakage is as follows (1) (Akrimi et al., 2023):

$$EL(\%) = (EC1/EC2) \times 100$$
 (1)

Where EL is the percentage of electrolyte leakage, EC1 is the initial electrical conductivity, and EC2 is the secondary electrical conductivity.

## **Relative water content**

Relative water content (RWC) was measured using the following method: Leaf samples were harvested from the greenhouse and weighed accurately (fresh weight). They were then immersed in distilled water for 18 hours and weighed again (saturated weight). Finally, they were dried in an oven at 72°C for 48 hours, and their dry weight was measured. The formula for calculating RWC is as follows (2) (Bayat et al., 2023):

RWC (%) = 
$$[(FW-DW)/(TW-DW)] \times 100$$
 (2)

Where FW is the fresh weight, DW is the dry weight, and TW is the saturated weight.

## Statistical analysis

Statistical analysis of the data was performed using Minitab software version 17, and mean comparisons were made using the LSD test at a 5% probability level. Excel software was used to create graphs.

#### **RESULTS**

## **Number of healthy seedlings**

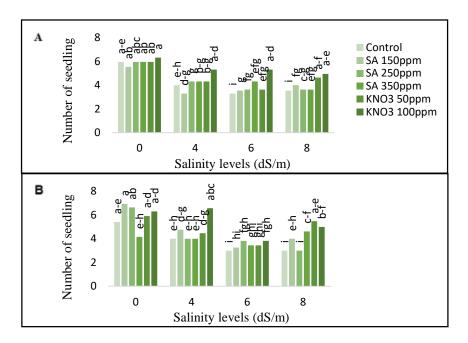
The results showed that increasing salinity reduced the number of thriving seedlings (Fig. 1, A and B). In the absence of salinity, priming had no significant effect on seedling growth in direct soil planting, while indirect planting showed a difference only at 250 ppm salicylic acid (Fig. 1). Priming significantly enhanced number of healthy seedlings at 4, 6, and 8 dS/m salinity levels, outperforming unprimed seeds in both sowing methods (Fig. 1). The highest number of healthy seedlings was observed at 4 and 6 dS/m with 100 ppm KNO<sub>3</sub>, regardless of sowing method. At 8 dS/m, 100 ppm KNO<sub>3</sub> yielded the highest trait values in direct sowing, while 50 ppm was optimal in indirect sowing (Fig. 1).

## Stem length

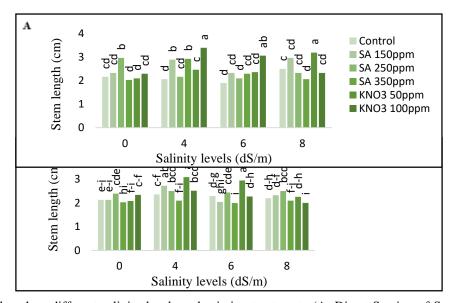
In both experiments, the employment of primed seeds led to greater stem lengths at different salinity levels than unprimed seeds. In soils with salinity levels of 4 and 6 dS/m, the



maximum stem length resulting from direct soil sowing was linked to the use of 100 ppm KNO<sub>3</sub>, whereas indirect sowing yielded the best results with 50 ppm KNO<sub>3</sub> (Fig. 2). At 8 dS/m salinity level, the longest stem lengths achieved in direct soil sowing and indirect sowing were associated with 50 ppm KNO<sub>3</sub> and 350 ppm salicylic acid, respectively .The stem length in direct sowing at salinity levels of 0, 4, 6, and 8 dS/m averaged 32.31, 26.65, 23.34, and 22.56 cm, respectively, whereas in indirect sowing, it averaged 22.18, 25.52, 23.32, and 22.32 cm, respectively, showing greater stem lengths in direct sowing conditions.



**Fig. 1.** Number of healthy seedlings at different salinity levels and priming treatments (A: Direct Sowing of Seeds, B: Indirect Sowing). Means followed by the same letter are not significantly different according to the LSD test at the 5% probability level.



**Fig. 2.** Stem length at different salinity levels and priming treatments (A: Direct Sowing of Seeds, B: Indirect Sowing). Means followed by the same letter are not significantly different according to the LSD test at the 5% probability level.

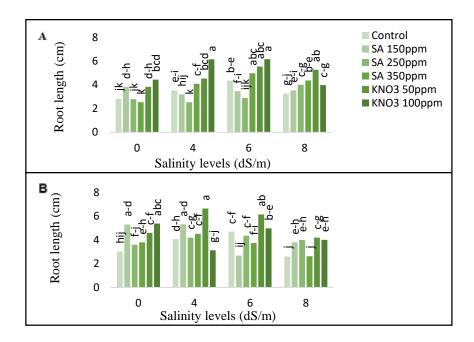


## **Root length**

Maximum root length in direct and indirect sowing was found with the application of KNO<sub>3</sub> at specific salinity levels. In direct soil sowing, this occurred at a salinity of 4-6 dS/m when using 100 ppm KNO<sub>3</sub> (Fig. 3), whereas in indirect sowing, it was achieved at the same salinity when applying 50 ppm KNO<sub>3</sub>. At 8 dS/m salinity level, the longest root lengths observed in both sowing methods were with the presence of 50 ppm KNO<sub>3</sub>. A statistically significant difference was found at each of the three salinity levels, between the priming treatment and the control, as depicted in Figure 3. In direct sowing, the mean root length at 0, 4, 6, and 8 dS/m salinity levels was 14.11, 14.09, 14.52, and 14.06 cm, respectively, whereas in indirect sowing, it was 14.28, 14.64, 14.43, and 13.53 cm, respectively.

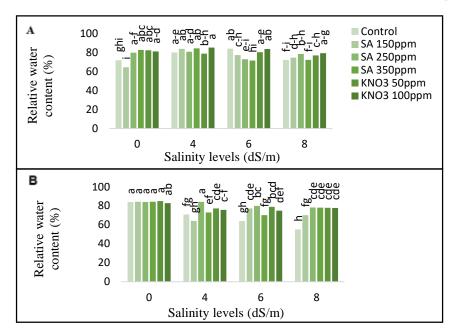
## Relative water content of leaves

Leaves from direct sowing experiments showed little to no impact from rising salinity levels, with corresponding mean RWC values of 77.1%, 82.16%, 78.32%, and 75.69% recorded at salinity levels of 0, 4, 6, and 8 dS/m, respectively (Fig. 4). In indirect sowing, the percentages achieved were 84.08%, 74.21%, 74.17%, and 72.84%, and direct sowing recorded higher mean values at three distinct levels of salinity. Upon reviewing both experiments, the greatest value for this characteristic in direct sowing was found at a salinity level of 4 dS/m with the application of 100 ppm KNO<sub>3</sub> (Fig. 4).



**Fig. 3.** Root length at different salinity levels and priming treatments (A: Direct Sowing of Seeds, B: Indirect Sowing). Means followed by the same letter are not significantly different according to the LSD test at the 5% probability level.





**Fig. 4.** Relative water content of leaves at different salinity levels and priming treatments (A: Direct Sowing of Seeds, B: Indirect Sowing. Means followed by the same letter are not significantly different according to the LSD test at the 5% probability level.

## Electrolyte leakage

The data revealed that at salinity levels of 4 and 8 dS/m, in both direct and indirect sowing methods, the application of KNO<sub>3</sub> priming at concentrations of 50 and 100 ppm led to a decrease in electrolyte leakage in seedlings compared to those from unprimed seeds (Fig. 5). In indirect sowing at a salinity level of 8 dS/m, various levels of salicylic acid proved to be effective in decreasing electrolyte leakage compared to seedlings from unprimed seeds, whereas this effect was not seen in direct sowing (Fig. 5). In direct sowing, no notable difference was observed in electrolyte leakage at salinity levels of 4 dS/m, whereas at other salinity levels, the difference was significant. Specifically, mean electrolyte leakage was found to be 35.36% at 0 dS/m, 40.53% at 4 dS/m, 34.69% at 6 dS/m, and 37.37% at 8 dS/m. Electrolyte leakage in indirect sowing increased with salinity levels, displaying an upward trend; the mean values at salinity levels of 0, 4, 6, and 8 dS/m were 24.10%, 34.86%, 36.80%, and 37.84%, respectively.

## **SPAD** index

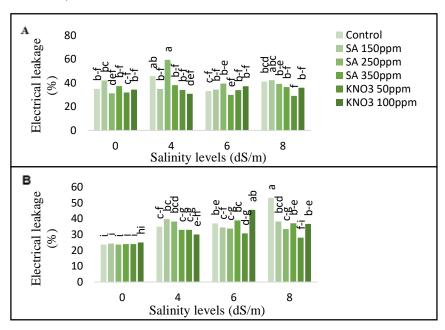
According to the data, the average chlorophyll content was 34.40 at 0 dS/m, 33.97 at 4 dS/m, 34.17 at 6 dS/m, and 35.27 at 8 dS/m for direct sowing, whereas for indirect sowing it was 35.98 at 0 dS/m, 35.36 at 4 dS/m, 34.93 at 6 dS/m, and 32.56 at 8 dS/m. In direct sowing, primed seeds exhibited significant differences at salinity levels of 6 and 8 dS/m compared to unprimed seeds, and priming enhanced the SPAD index more than unprimed seeds (Fig. 6-A). At salinity levels of 0 and 4 dS/m, the maximum trait values were obtained with 100 ppm KNO<sub>3</sub>. Conversely, at salinity levels of 6 and 8 dS/m, the highest trait values were observed at 150 ppm salicylic acid, as depicted in Figure 6-A.

The highest salinity tolerance values for indirect sowing were achieved with salt concentrations of 4, 6, and 8 dS/m when using 50 ppm KNO<sub>3</sub>, 250 ppm salicylic acid, and 50 ppm KNO<sub>3</sub>, respectively, as shown in Figure 6-B.

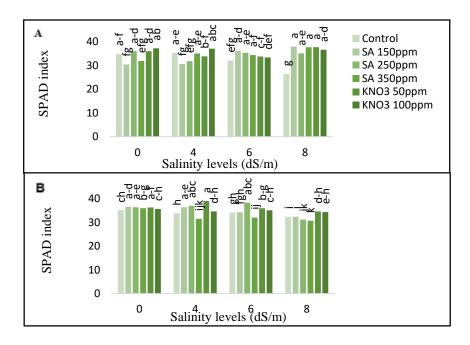


## Leaf number

In direct sowing, the highest leaf number was observed at a salinity of 6 dS/m with 100 ppm KNO<sub>3</sub>, whereas in indirect sowing, this level was attained at a salinity of 4 dS/m with 50 ppm KNO<sub>3</sub> (Tables 1 and 2).



**Fig. 5.** Electrical leakage of leaves at different salinity levels and priming treatments (A: Direct Sowing of Seeds, B: Indirect Sowing). Means followed by the same letter are not significantly different according to the LSD test at the 5% probability level.



**Fig. 6**. SPAD index of leaves at different salinity levels and priming treatments (A: Direct Sowing of Seeds, B: Indirect Sowing). Means followed by the same letter are not significantly different according to the LSD test at the 5% probability level.



## Fresh weight of stem and root

The results indicated that the highest fresh weight of stem in direct sowing at salinity levels of 4, 6, and 8 dS/m was achieved with 50, 100, and 50 ppm KNO<sub>3</sub>, respectively, and in indirect sowing, with 50, 50, and 100 ppm KNO<sub>3</sub>, respectively (Tables 1 and 2). The application of salicylic acid at different salinity levels, except at 8 dS/m in indirect sowing, did not have a significant effect compared with seedlings from unprimed seeds (Tables 1 and 2).

The highest fresh weight of the root in direct sowing was achieved at a salinity level of 6 dS/m with 100 ppm KNO<sub>3</sub> (Table 1), and in indirect sowing, at a salinity level of 6 dS/m with 50 ppm KNO<sub>3</sub> (Table 2).

**Table 1.** Interaction effect of seed priming and salinity stress on seedling growth of *Catharanthus roseus* (direct sowing).

Factors		Parameters				
Salinity (dS/m)	Priming (ppm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Number of leaves
0	No-prime	0.126 hi	0.0163 g	0.013 ef	0.0018 fg	10 b
0	SA-150	0.106 hi	0.0200 fg	0.011 f	0.0021 efg	10 b
0	SA-250	0.126 hi	0.0200 fg	0.016 def	0.0018 fg	10 b
0	SA-350	0.089 i	0.0130 g	0.068 a	0.0014 g	10 b
0	KNO3-50	0.159 gh	0.0163 g	0.029 bcd	0.0015 fg	10 b
0	KNO3-100	0.566 ab	0.0248 ef	0.018 def	0.0088 a	9.4 bc
4	No-prime	0.348 cde	0.0163 g	0.020 def	0.0035 cde	10.0 b
4	SA-150	0.483 abc	0.0497 b	0.049 abc	0.0071 ab	10.0 b
4	SA-250	0.119 hi	0.0130 g	0.013 ef	0.0077 a	8.0 c
4	SA-350	0.383 bcd	0.0465 bc	0.030 bcd	0.0077 a	10.0 b
4	KNO3-50	0.270 def	0.0332 de	0.018 def	0.0085 a	10.0 b
4	KNO3-100	0.614 a	0.0666 a	0.056 ab	0.0086 a	12.0 a
6	No-prime	0.271 def	0.0130 g	0.029 bcd	0.0033 de	10.0 b
6	SA-150	0.270 def	0.0332 de	0.023 def	0.0033 de	10.0 b
6	SA-250	0.203 fg	0.0163 g	0.016 def	0.0082 a	8.0 c
6	SA-350	0.299 def	0.0349 cd	0.018 def	0.0041 cd	10.0 b
6	KNO3-50	0.286 def	0.0400 bcd	0.030 bcd	0.0012 g	10.0 b
6	KNO3-100	0.512 abc	0.0792 a	0.031 bcd	0.0043 cd	11.4 a
8	No-prime	0.213 fg	0.0200 fg	0.013 ef	0.0051 bc	10.0 b
8	SA-150	0.241 efg	0.0200 fg	0.025 cde	0.0038 cd	9.1 bc
8	SA-250	0.233 efg	0.0200 fg	0.020 def	0.0065 ab	9.4 bc
8	SA-350	0.250 efg	0.0416 bcd	0.018 def	0.0041 cd	10.0 b
8	KNO3-50	0.637 a	0.0497 b	0.068 a	0.0027 def	11.4 a
8	KNO3-100	0.248 ef	0.0200 fg	0.020 def	0.0033 cde	10.0 b

In each column, averages that have at least one common letter are in statistically similar groups LSD range tests at the 5% possibility.

No-prime: seedling without seed priming, SA-150, SA-250, and SA-350: Seedlings whose seeds were primed with salicylic acid at concentrations of 150, 250, and 350 ppm, respectively. KNO<sub>3</sub>-50 and KNO<sub>3</sub>-100: Seedlings whose seeds were primed with KNO<sub>3</sub> at concentrations of 50 and 100 ppm, respectively.



## Dry weight of stem and root

The highest dry weight of the stem in direct sowing at salinity levels of 4, 6, and 8 dS/m was achieved with 100, 100, and 50 ppm KNO<sub>3</sub>, respectively, and in indirect sowing, with 50, 50, and 100 ppm KNO<sub>3</sub>, respectively (Tables 1 and 2). For the dry weight of the root, in direct sowing, the highest values were achieved with 100 ppm KNO<sub>3</sub> at a salinity level of 4 dS/m and with 250 ppm salicylic acid at salinity levels of 6 and 8 dS/m (Table 1). In indirect sowing, the application of 50 ppm KNO<sub>3</sub> at a salinity level of 4 dS/m, 150 ppm salicylic acid at 6 dS/m, and 250 ppm salicylic acid at 8 dS/m was more effective than the other levels (Table 2).

**Table 2.** Interaction effect of seed priming and salinity stress on seedling growth of *Catharanthus roseus* (indirect sowing).

Factors		Parameters						
Salinity (dS/m)	Priming (ppm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Number of leaves		
0	No-prime	0.365 ab	0.0218 d-g	0.0126 ef	0.0058 a-d	10.0 b		
0	SA-150	0.316 b-e	0.0300 b-e	0.0300 ab	0.0087 a	10.0 b		
0	SA-250	0.130 kl	0.0214 efg	0.0229 bcd	0.0087 a	10.0 b		
0	SA-350	0.163 i-l	0.0123 h	0.0229 bcd	0.0070 abc	10.0 b		
0	KNO3-50	0.191 g-k	0.0227 c-g	0.0262 abc	0.0083 ab	9.5 b		
0	KNO3-100	0.256 c-h	0.0346 abc	0.0159 c-f	0.0044 c-f	10.0 b		
4	No-prime	0.289 b-f	0.0283 c-f	0.0159 c-f	0.0010 j	10.0 b		
4	SA-150	0.243 d-i	0.0346 abc	0.0200 b-e	0.0029 e-i	10.0 b		
4	SA-250	0.360 abc	0.0300 b-e	0.0282 ab	0.0080 ab	10.0 b		
4	SA-350	0.333 a-d	0.0300 b-e	0.0200 b-e	0.0030 e-i	10.0 b		
4	KNO3-50	0.445 a	0.0487 ab	0.0400 a	0.0083 ab	10.0 b		
4	KNO3-100	0.283 b-f	0.0260 c-f	0.0252 abc	0.0042 c-g	10.0 b		
6	No-prime	0.160 i-l	0.0172 g	0.0100 f	0.0033 d-i	10.0 b		
6	SA-150	0.222 e-j	0.0100 h	0.0144 def	0.0072 abc	9.5 b		
6	SA-250	0.277 b-g	0.0316 b-e	0.0182 b-e	0.0051 b-e	10.0 b		
6	SA-350	0.145 jkl	0.0100 h	0.0100 f	0.0025 f-j	10.0 b		
6	KNO3-50	0.435 a	0.0565 a	0.0391 a	0.0020 g-j	11.1 a		
6	KNO3-100	0.229 e-i	0.0329 bcd	0.0144 def	0.0038 d-h	9.5 b		
8	No-prime	0.1001	0.0100 h	0.0100 f	0.0016 i	10.0 b		
8	SA-150	0.173 h-k	0.0100 h	0.0100 f	0.0053 a-e	9.2 bc		
8	SA-250	0.220 e-j	0.0100 h	0.0100 f	0.0070 abc	9.5 b		
8	SA-350	0.230 e-i	0.0100 h	0.0100 f	0.0030 e-i	9.5 b		
8	KNO3-50	0.206 f-k	0.0200 fg	0.0144 def	0.0019 hij	9.1 bc		
8	KNO3-100	0.231 e-i	0.0200 fg	0.0159 c-f	0.0019 hij	8.0 c		

In each column, averages that have at least one common letter are in statistically similar groups LSD range tests at the 5% possibility.

No-prime: seedling without seed priming, SA-150, SA-250, and SA-350: Seedlings whose seeds were primed with salicylic acid at concentrations of 150, 250, and 350 ppm, respectively.  $KNO_3$ -50, and  $KNO_3$ -100: Seedlings whose seeds were primed with  $KNO_3$  at concentrations of 50 and 100 ppm, respectively.



## **DISCUSSION**

Present study has shown that using KNO<sub>3</sub> and salicylic acid at specific concentrations can have a beneficial effect on certain plant characteristics and, in comparison to unprimed seedlings, exhibit improved resilience to salt stress.

Researches have shown that treating seeds with KNO3 can substantially boost seed germination rates and consistency across different environmental settings. For instance, a study on Cucumis metaliferus showed that KNO<sub>3</sub> priming at a concentration of 2.5% improved final germination percentage under salinity stress (Mutetwa et al., 2023). In Lycopersicum esculentum, varieties Mersa and Tekeze-1 responded differently to KNO<sub>3</sub> priming, with Mersa showing a 22% improvement in germination at 1.5% KNO<sub>3</sub> concentration, whereas Tekeze-1 performed better at 0.5%, indicating genotype-specific responses (Mebratu, 2022). The positive effect of KNO<sub>3</sub> in germination was consistence with our study. This improvement is attributed to the regulation of hormonal balance, particularly the ratio of gibberellic acid (GA) to abscisic acid (ABA), and the activation of enzymes involved in seed metabolism (Mutetwa et al., 2023). Moreover, studies confirm that KNO<sub>3</sub> maintains osmotic homeostasis under saline conditions by regulating the selective uptake of K<sup>+</sup> ions and reducing the accumulation of Na<sup>+</sup> ions, allowing plants to better manage water uptake under saline conditions This mechanism is critical for improving salt tolerance in plants, as it prevents ionic imbalance and osmotic stress (Li & Yang, 2023; Rehman et al., 2024; Sayed et al., 2019).

In this study, the use of salicylic acid and KNO<sub>3</sub> at specific salinity levels resulted in a positive effect on increasing root and stem length, as well as root and shoot fresh and dry weight, in *Catharanthus roseus*. Research findings suggest that KNO<sub>3</sub> priming results in enhanced leaf area, increased plant height, and greater dry weight that are essential for achieving desirable ornamental quality (Miladinov et al., 2020).

Studies have demonstrated that treating seeds with salicylic acid can substantially induced stem elongation in ornamental plants exposed to salt stress, for example, increasing shoot growth in *Limonium bicolor* as reported by Liu et al. (2019). Preliminary SA treatment resulted in increased shoot dry matter and overall plant growth in *Saccharum officinarum* (Apon et al., 2023). In addition, seedlings treated with SA showed enhanced mineral nutrition, specifically higher concentrations of calcium and magnesium, which are necessary for maintaining cell wall stability and facilitating elongation processes during periods of salt stress (Ben Youssef et al., 2024). Improving potassium uptake and decreasing sodium accumulation may stabilize osmotic balance and enhance turgor pressure required for stem growth, while also modulating hormonal balance by increasing gibberellic acid (GA) levels and decreasing abscisic acid (ABA) levels, thus facilitating cell elongation and stem development under saline conditions (Apon et al., 2023; Liu et al., 2019).

In this study, benefits of KNO<sub>3</sub> were recoded for shoot and root length. KNO<sub>3</sub> priming not only enhances root length but also improves root architecture, including root number and root dry weight. In rice, priming with KNO<sub>3</sub> improved root growth, leading to better water and nutrient uptake, which is essential for crop productivity (Dhillon et al., 2021). In sesame, priming with 15 mM KNO<sub>3</sub> improved root length, fresh weight, and dry weight of seedlings. This treatment also enhanced catalase, peroxidase, and proline content, indicating improved stress tolerance (Kumar et al., 2014). KNO<sub>3</sub> priming also influences the activity of nitrate reductase (NR), an enzyme involved in nitrate metabolism. Increased NR activity in primed seeds enhances nitric oxide production, which acts as a signaling molecule promoting germination and root growth (Lara et al., 2014).



The current research findings revealed a higher impact of priming on the SPAD index in direct sowing compared to indirect sowing, and KNO<sub>3</sub> priming was found to be more effective than SA treatment. However, applying SA also showed a better effect on this trait compared to untreated seeds. KNO<sub>3</sub> is known to promote chlorophyll production, directly influencing the SPAD index and enhancing the visual quality of ornamental plants (Saraf et al., 2018). Increased potassium levels from KNO<sub>3</sub> enhance chlorophyll production, directly improving the SPAD index, which measures leaf greenness and health (Ahmadvand et al., 2012). Also, KNO<sub>3</sub> priming enhances antioxidant enzyme activity, reducing oxidative stress caused by salinity leads to increased leaf area (Miladinov et al., 2020; Nazir et al., 2014). KNO<sub>3</sub> provides nitrate (NO<sub>3</sub><sup>-</sup>) as a nitrogen source for chlorophyll biosynthesis, while potassium (K<sup>+</sup>) enhances photosynthetic efficiency by activating RuBisCO and stabilizing stomatal conductance (Khan et al., 2022; Zulfiqar et al., 2022).

Research on various ornamental plants, including *Dianthus superbus* and Chrysanthemum, has found a 20%–30% rise in the SPAD index under salinity stress due to increased ROS scavenging, better ion homeostasis, and enhanced chlorophyll biosynthesis resulting from the application of SA (Ellouzi et al., 2023; Oliveira et al., 2023; Zulfiqar et al., 2022). Chlorophyll synthesis and photosynthetic activity in SA, resulting in enhanced growth and more efficient water use under stress conditions (Shahmoradi & Naderi, 2018). Furthermore, SA helps safeguard cell membranes by minimising lipid peroxidation, thereby maintaining effective water retention (Arruda et al., 2023).

Disruption of membrane integrity caused by high salt levels results in water loss (Zulfiqar et al., 2022). KNO<sub>3</sub> stimulates root development and hydraulic conductivity, thus allowing for improved water absorption from saline soils. Research on decorative plants exposed to long-term salt stress revealed that plants treated with KNO<sub>3</sub> showed enhanced root water uptake, leading to greater relative water content compared to untreated test subjects (Bandurska et al., 2023). The nitrate part of KNO<sub>3</sub> acts as a source of nitrogen for the synthesis of amino acids, which is essential for osmotic regulation and protein stability when the plant is under stress (Beyk-Khormizi et al., 2023).

#### **CONCLUSION**

The findings from this experiment indicated that priming can reduce the negative impact of saltwater on the Catharanthus roseus. Generally, KNO<sub>3</sub> treatments performed better than salicylic acid in lowering the detrimental effects of salinity for the majority of the evaluated characteristics. Direct sowing of pre-treated seeds proved to be a more efficient method than indirect sowing for reducing the detrimental effects of salinity on seedling growth, including stem length, root length, and the SPAD index. Values for other characteristics showed remarkable consistency across different levels of salinity. We observed that treating seeds, particularly with KNO<sub>3</sub>, resulted in a higher survival rate of plants across various salt concentrations compared to untreated seeds, and it also promoted subsequent growth stages in young seedlings. Improved growth of stems, root development, biomass accumulation, and SPAD index assessments were carried out throughout the seedling phase, until the seedlings reached a suitable stage for transfer to the main field. The enhanced vegetative traits can primarily be attributed to increased germination rates in primed seeds compared to those without treatment, ultimately yielding more vigorous seedlings. For enhanced seedling production in Catharanthus roseus, seeds primed with KNO3 are recommended for application. Additional research in the form of supplementary experiments is suggested to accurately determine the optimal KNO<sub>3</sub> concentrations.



## **Conflict of interest**

The authors declare that there is no conflict of interest.

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