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Priming of Linseed with ZnO Nanoparticles Enhances Seedling-stage Salinity Tolerance

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ABSTRACT

Linseed (*Linum usitatissimum* L.) is a nutritionally important oilseed crop that is frequently cultivated on marginal lands affected by salinity stress, resulting in poor germination and weak early seedling establishment. The present laboratory study evaluated forty linseed accessions differing in salinity tolerance to assess the effectiveness of zinc oxide (ZnO) nanoparticle seed priming in enhancing germination and seedling performance under saline conditions. Seeds were subjected to two salinity regimes (control and 10 dS m^{-1} NaCl) with and without ZnO nanoparticle priming (500 ppm). Germination percentage, seedling vigor index, root/shoot ratio, and Na^+ and K^+ ion contents were recorded. Salinity stress significantly reduced germination, vigor index, root/shoot ratio, and K^+ content, with greater reductions observed in salt-sensitive genotypes. ZnO nanoparticle priming alleviated the adverse effects of salinity by improving germination, maintaining higher seedling vigor and root/shoot ratio, reducing Na^+ accumulation, and sustaining a favorable K^+/Na^+ balance in both tolerant and sensitive accessions. The results demonstrate that ZnO nanoparticle seed priming is an effective, low-cost strategy to enhance early-stage salinity tolerance in linseed and may be useful for screening and managing salinity stress under controlled conditions.

Keywords Linseed, Salinity stress, Nano-priming, ZnO nanoparticles, Seedling vigor.

INTRODUCTION

Linseed or flax (*Linum usitatissimum L.), belonging to the family Linaceae, is an importan*t dual-purpose crop cultivated for its oil and fibre since prehistoric times. Linseed types are generally short-statured and branched, grown mainly for oil-rich seeds, whereas flax types are tall and unbranched, cultivated for fiber (Zuk *et al.* 2015). Linseed seeds are recognized as a 'superfood' owing to their high nutritional value and health-promoting properties, particularly due to their richness in omega-3 fatty acids such as α-linolenic acid (Bernacchia *et al,* 2014, Nowak and Jeziorek, 2023). Globally, the linseed crop is cultivated on 4.53 lakh ha area, producing 3.97 MT with a mean productivity of 991 kg ha (FAOSTAT 2024). In India, linseed is cultivated on approximately 0.2 m ha with a production of 0.12 MT and a mean productivity of 605 kg/ha.

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Despite increasing global demand, linseed productivity remains low as the crop is largely grown on marginal and degraded lands prone to salinity and drought stress. Globally, more than 1100 million hectares of land are affected by salinity (GOI 2025), and the affected area is expected to increase substantially by 2050 (Dubey *et al.* 2020). Salinity stress disrupts physiological, biochemical, and metabolic processes in plants, leading to poor germination, reduced seedling growth, and yield losses (Rahneshan *et al.* 2018, Tarchoun *et al.* 2022). Germination and early seedling growth are among the most sensitive stages to salinity stress, as high salt concentrations impose osmotic stress and ionic toxicity (Ashkan and Jalal, 2013, Abido and Zsombik 2019, Mustafa 2023).

Seed priming has emerged as an effective technique to improve seed performance under stress conditions. In recent years, nano-priming using metal oxide nanoparticles has gained attention due to its ability to enhance germination, seedling vigor, antioxidant defense, and ion homeostasis under abiotic stress (Paparella *et al*. 2015, Lee and Kasote 2024). Zinc oxide nanoparticles, in particular, play a crucial role in enzyme activation and membrane stability. The present study was therefore undertaken to evaluate the salinity tolerance of diverse linseed accessions at the seedling stage and to assess the potential of ZnO nanoparticle seed priming in mitigating salinity stress.

MATERIALS AND METHODS

Experimental site and plant material

The present investigation was conducted during the winter season of 2022–23 in the experimental laboratory of the Department of Botany and Plant Physiology, CCS Haryana Agricultural University, Hisar, India. The experimental material consisted of forty linseed (*Linum usitatissimum* L.) accessions representing diverse genetic backgrounds. Based on prior characterization, thirty accessions were categorized as salt-tolerant, while ten accessions were identified as salt-sensitive. The selected germplasm included both indigenous and exotic accessions (Table 1), thereby ensuring sufficient genetic variability for assessing salinity tolerance at the seedling stage.

Seed priming and salinity treatments

The experiment involved two factors: seed priming and salinity stress. Seed priming was performed using zinc oxide (ZnO) nanoparticles at a concentration of 500 ppm. Seeds were soaked in the nanoparticle suspension for 12 hours at room temperature and subsequently air-dried to their original moisture content before sowing. Non-primed seeds served as the control for priming treatments

Salinity stress was imposed using sodium chloride (NaCl) solution to achieve an electrical conductivity of 10 dS m^{-1}, representing moderate to high salinity stress. Accordingly, four treatment combinations were established:

(i) Control (C): Unprimed seeds under non-saline conditions,
(ii) Salt stress (S): Unprimed seeds under 10 dS m^{-1} salinity
(iii) Control + priming (CT): ZnO nanoparti cle-primed seeds under non-saline conditions, and
(iv) Salt stress + priming (ST): ZnO nanoparti cle-

Table 1. List of the linseed accession used for the experiment.

Sl. No.	Accession	Sl. No.	Accession	Sl. No.	Accession	Sl. No.	Accession
1	IC0498949	11	IC0499050	21	IC0499174	31	IC0376229
2	IC113107	12	IC0499140	22	IC0276956	32	IC0526375
3	IC0498747	13	IC0498726	23	IC0567357	33	IC0339613
4	IC0585322	14	IC0510948	24	IC0510929	34	IC0096538
5	IC0499081	15	IC0054977	25	IC0564605	35	IC0564641
6	IC0521452	16	IC0249011	26	IC0054969	36	IC0498419
7	EC0541222	17	IC0096637	27	EC0718842	37	IC0585353
8	IC0384566	18	IC0564596	28	EC244634	38	IC0096746
9	IC0498650	19	IC0564639	29	EC0041528	39	IC0498869
10	IC0096725	20	IC0498493	30	IC0510940	40	IC0564689

primed seeds under 10 dS m^{-1} salinity.

Experimental design and germination conditions

The experiment was laid out in a completely randomized design (CRD) with three replications per treatment. Germination tests were conducted using sterilized Petri plates lined with filter paper. Ten healthy and uniform seeds of each accession were placed in each Petri plate and moistened with the respective treatment solution. Plates were maintained at room temperature under laboratory conditions without additional light or temperature control. Moisture levels were maintained by periodic addition of the respective solutions as required.

Observations recorded

Germination percentage

Seed germination was recorded seven days after sowing. Seeds were considered germinated when visible radicle emergence was observed. Germination percentage was calculated using the formula suggested by Fang *et al.* (2006):

G% = n/ N×100 Eq. 1

Where, n is the sum of seeds germinated and N is the total number of seeds tested.

Seedling vigor index

The seedling vigor index-I and seedling vigor index-II of each genotype was determined 20 days after sowing by using the formula:

Seedling vigor index I = Germination percentage × mean dry weight

Seedling vigor index II = Germination percentage × mean seedling length

Root/shoot ratio

Root and shoot lengths were measured for randomly selected seedlings from each treatment, and the root/shoot ratio was calculated by dividing root length by shoot length.

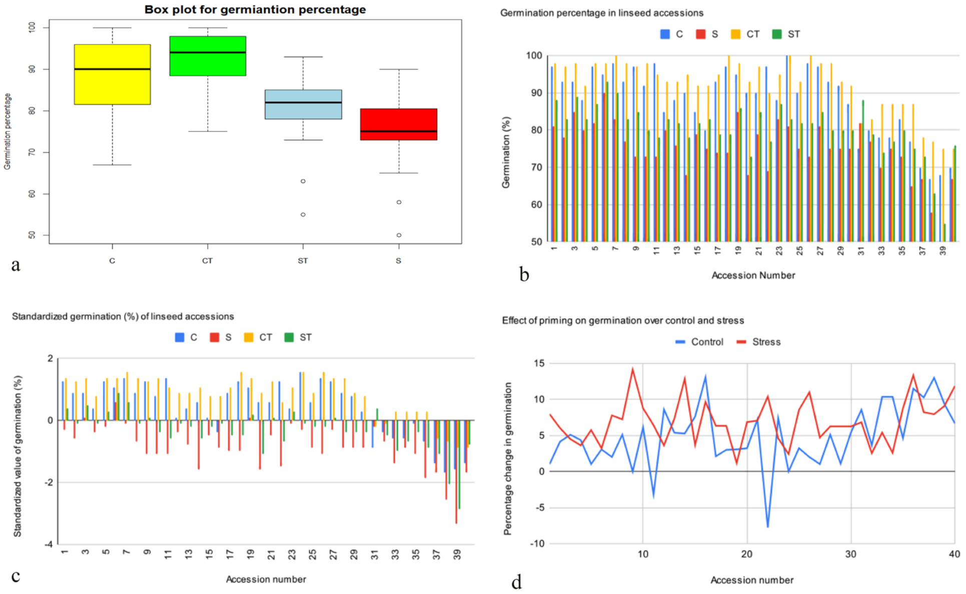

Fig. 1. Description of germination percentage in linseed accessions under different treatments. **(a)** Box plot showing range and mean of germination percentage **(b)** Mean germination percentage depicted in the form of bar chart **(c)** Comparative bar chart depicting normalized germination percentage over overall mean **(d)** Line chart depicting effect of priming on germination in comparison to control (no priming and no salt stress) and under salt stress condition. C: Control; S: Salt stress without priming: CT: Control + seed priming; ST: Salt stress + seed priming.

Na$^+$ and K$^+$ content

Seedlings were oven-dried and ground to a fine powder. Sodium (Na$^+$) and potassium (K$^+$) contents were estimated using a flame photometer following standard analytical procedures as described by Kacar and Inal (2008). Ion concentrations were expressed on a dry weight basis (mg g^{-1} DW).

RESULTS

Germination percentage

Salinity stress significantly reduced germination percentage across all linseed accessions compared to non-saline conditions (Fig. 1). Under control conditions, germination percentage ranged from 67% to 100%, with an overall mean of 88%. ZnO nanoparticle priming improved germination under control conditions, increasing the mean germination percentage to 92% and reducing variability among accessions.

Under salinity stress (10 dS m^{-1}), germination percentage declined in all accessions, with a more pronounced reduction observed in salt-sensitive genotypes. The minimum germination under salinity stress was recorded in accessions IC0498869 and IC0096746. However, ZnO nanoparticle priming partially alleviated the adverse effect of salinity, resulting in a higher mean germination percentage (81%) compared to unprimed seeds under stress (75%). Salt-tolerant accessions such as IC0521452 exhibited minimal reduction in germination under saline conditions.

Seedling vigor index

Seedling vigor index showed a marked decline under salinity stress relative to control conditions (Fig. 2). The reduction was more severe in salt-sensitive genotypes, indicating restricted seedling growth under saline conditions. Among all accessions, IC0510929 exhibited the highest seedling vigor index under

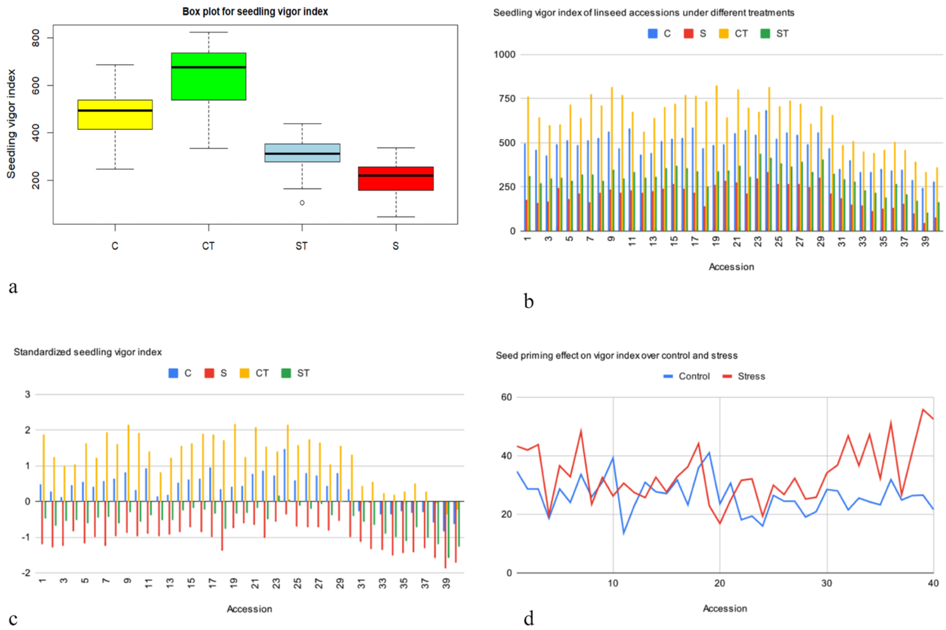

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Fig. 2. Description of seedling vigor index in linseed accessions under different treatments. **(a)** Box plot showing range and mean for seedling vigor index. **(b)** Average vigor index of different accessions depicted in the form of bar chart. **(c)** Comparative bar chart depicting normalized seedling vigor index over overall mean. **(d)** Line chart depicting effect of priming on vigor index in comparison to control (no priming and no salt stress) and under salt stress condition. C: Control; S: Salt stress without priming: CT: Control + seed priming; ST: Salt stress + seed priming.

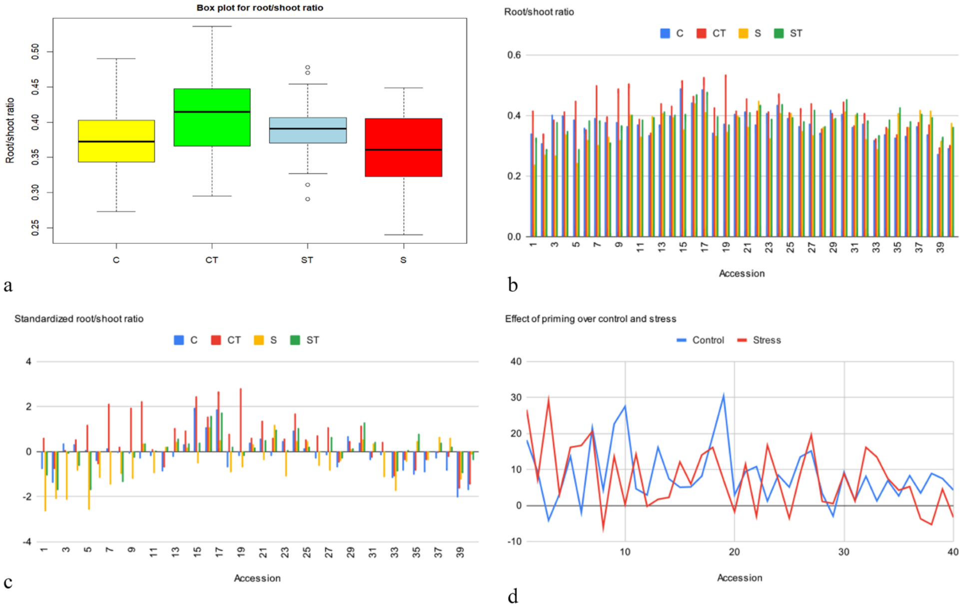

Fig. 3. Description of root/shoot ratio in linseed accessions under different treatments. **(a)** Box plot showing range and mean for root/shoot ratio. **(b)** Average root/shoot ratio of different accessions depicted in the form of bar chart. **(c)** Comparative bar chart depicting normalized root/shoot ratio over overall mean. **(d)** Line chart depicting effect of priming on root/shoot ratio in comparison to control (no priming and no salt stress) and under salt stress condition. C: Control; S: Salt stress without priming: CT: Control + seed priming; ST: Salt stress + seed priming.

both control and salinity stress conditions, indicating superior tolerance at the seedling stage.

ZnO nanoparticle priming significantly enhanced seedling vigor index under both control and saline environments. Under non-saline conditions, the highest vigor index was recorded in accession IC0564639, while under salinity stress, accession IC0567357 showed the maximum vigor index following priming. The lowest seedling vigor index across treatments

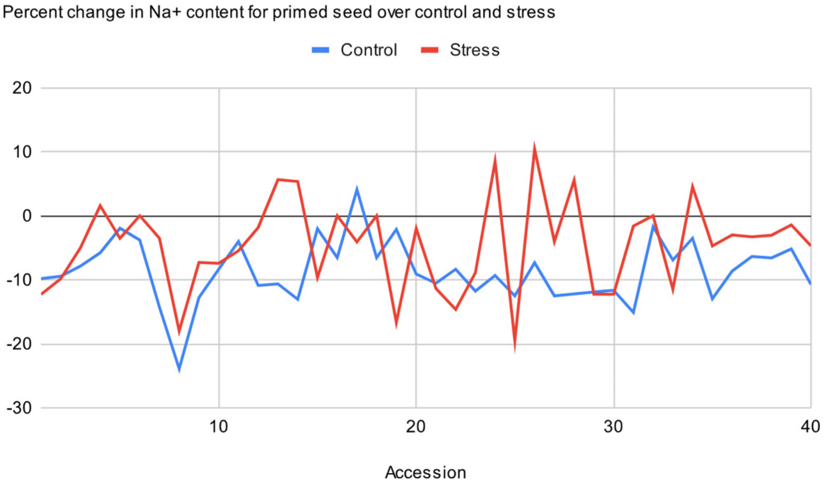

Fig. 4. Effect of change in Na^+ content when seed is primed with ZnO over control and stress condition.

Table 2. Na^+ and K^+ content (mg g^{-1} DW) in different linseed genotypes treated with ZnO nanoparticle as seed priming under saline and non-saline conditions.

	Na⁺ content					K⁺ content			
Accession No.	C	S	CT	ST	Accession No.	C	CT	S	ST
IC0498949	0.56	0.64	0.51	0.57	IC0498949	2.07	2.28	1.17	1.43
IC113107	0.58	0.67	0.53	0.61	IC113107	1.87	2.12	1.56	1.32
IC0498747	0.55	0.63	0.51	0.60	IC0498747	1.65	1.99	1.28	1.42
IC0585322	0.55	0.62	0.52	0.63	IC0585322	1.96	2.35	1.35	1.53
IC0499081	0.52	0.59	0.51	0.57	IC0499081	2.18	2.47	1.86	2.01
IC0521452	0.55	0.57	0.53	0.57	IC0521452	1.75	2.03	1.24	1.45
EC0541222	0.56	0.59	0.49	0.57	EC0541222	1.92	2.26	1.15	1.37
IC0384566	0.57	0.59	0.46	0.50	IC0384566	1.83	2.14	1.23	1.48
IC0498650	0.53	0.59	0.47	0.55	IC0498650	1.69	1.92	1.03	1.46
IC0096725	0.52	0.58	0.48	0.54	IC0096725	1.55	1.88	1.17	1.27
IC0499050	0.52	0.58	0.50	0.55	IC0499050	1.76	1.97	1.53	1.69
IC0499140	0.51	0.55	0.46	0.54	IC0499140	2.25	2.52	1.83	2.01
IC0498726	0.52	0.50	0.47	0.53	IC0498726	2.16	2.43	1.79	1.95
IC0510948	0.52	0.53	0.46	0.56	IC0510948	2.58	2.24	1.95	2.25
IC0054977	0.50	0.57	0.49	0.52	IC0054977	2.37	2.54	1.92	2.17
IC0249011	0.49	0.54	0.46	0.54	IC0249011	2.28	2.73	1.71	1.98
IC0096637	0.47	0.51	0.49	0.49	IC0096637	2.46	2.84	1.95	2.22
IC0564596	0.49	0.52	0.46	0.52	IC0564596	2.34	2.75	1.86	2.19
IC0564639	0.48	0.56	0.47	0.48	IC0564639	2.26	2.21	1.79	2.06
IC0498493	0.48	0.51	0.44	0.50	IC0498493	2.15	2.38	1.87	1.97
IC0499174	0.42	0.49	0.38	0.44	IC0499174	2.57	2.19	2.86	2.22
IC0276956	0.39	0.47	0.36	0.41	IC0276956	2.36	1.63	2.78	1.83
IC0567357	0.38	0.49	0.34	0.45	IC0567357	2.27	1.78	2.71	1.96
IC0510929	0.47	0.43	0.43	0.47	IC0510929	2.17	1.92	2.85	2.01
IC0564605	0.45	0.49	0.40	0.41	IC0564605	1.76	1.13	2.25	1.33
IC0054969	0.44	0.43	0.41	0.48	IC0054969	1.82	1.25	2.16	1.67
EC0718842	0.45	0.52	0.40	0.50	EC0718842	1.67	1.18	1.95	1.31
EC244634	0.46	0.51	0.41	0.54	EC244634	1.12	0.95	1.68	1.02
EC0041528	0.47	0.55	0.42	0.49	EC0041528	1.27	0.97	1.86	1.13
IC0510940	0.48	0.55	0.43	0.49	IC0510940	1.56	1.12	1.72	1.45
IC0376229	0.61	0.63	0.53	0.62	IC0376229	1.47	1.28	1.91	1.37
IC0526375	0.59	0.62	0.58	0.62	IC0526375	1.83	1.63	2.25	1.79
IC0339613	0.62	0.68	0.58	0.61	IC0339613	1.16	1.03	1.92	1.07
IC0096538	0.59	0.63	0.57	0.66	IC0096538	1.27	1.19	1.82	1.23
IC0564641	0.61	0.67	0.54	0.64	IC0564641	1.38	0.98	1.87	1.05
IC0498419	0.63	0.69	0.58	0.67	IC0498419	1.82	1.35	2.15	1.64
IC0585353	0.67	0.63	0.63	0.61	IC0585353	1.75	1.26	2.06	1.52
IC0096746	0.65	0.68	0.61	0.66	IC0096746	1.48	1.15	1.97	1.27
IC0498869	0.61	0.71	0.58	0.70	IC0498869	0.97	1.00	1.52	1.04
IC0564689	0.62	0.67	0.56	0.64	IC0564689	1.12	1.05	1.65	1.09
Mean	**0.53**	**0.58**	**0.49**	**0.55**	Mean	**1.85**	**1.43**	**2.20**	**1.61**

was consistently observed in accession IC0498869. Normalized vigor index trends indicated that the beneficial effect of ZnO priming was more pronounced under salinity stress than under control conditions.

Root/shoot ratio

Root/shoot ratio was significantly influenced by both salinity stress and seed priming. Salinity stress reduced the root/shoot ratio in most accessions, with salt-sensitive genotypes showing the greatest decline. Under control conditions, the highest root/shoot ratios were observed in accessions IC0054977 and IC0276956.

ZnO nanoparticle priming increased the root/shoot ratio under both control and saline conditions (Fig. 3). The highest ratio under control conditions

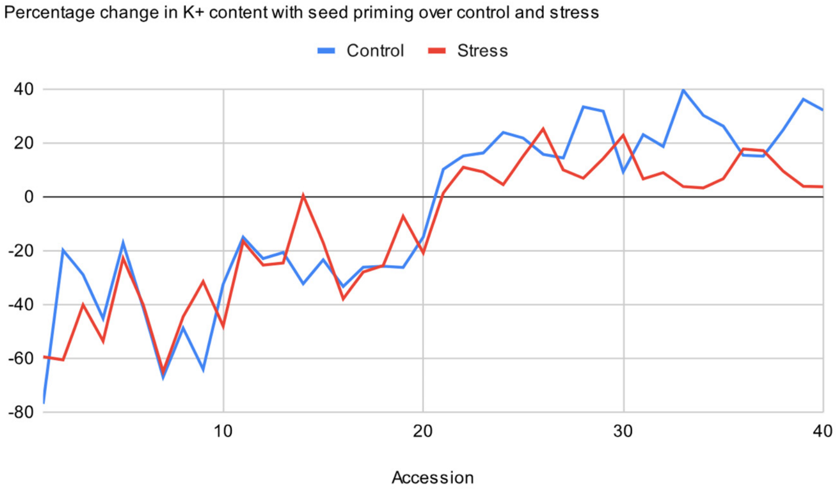

Fig. 5. Effect of change in K^+ content when seed is primed with ZnO over control and stress condition.

was recorded in accession IC0564639, whereas under salinity stress, accession IC0096637 exhibited the highest root/shoot ratio following priming. Accession IC0498869 consistently recorded the lowest root/shoot ratio across all treatments. Overall trends indicated that ZnO priming enhanced root development relative to shoot growth, particularly under saline conditions.

Na^+ and K^+ content

Salinity stress resulted in a significant increase in Na^+ content and a concomitant reduction in K^+ content in linseed seedlings (Table 2). Salt-sensitive genotypes accumulated higher Na^+ levels compared to tolerant genotypes under both saline and non-saline conditions. The highest Na^+ content under salinity stress was recorded in accession IC0498869, whereas the lowest Na^+ accumulation was observed in tolerant accessions such as IC0567357 and IC0276956.

ZnO nanoparticle priming reduced Na^+ accumulation in both tolerant and sensitive genotypes under saline conditions (Fig. 4). Several accessions showed a substantial reduction in Na^+ content following priming, indicating improved ionic regulation. Conversely, K^+ content was generally higher in salt-tolerant genotypes than in sensitive ones. Salinity stress reduced K^+ content across all accessions; however, ZnO priming helped maintain higher K^+ levels (Fig. 5), particularly in tolerant genotypes such as IC0096637 and IC0510948.

DISCUSSION

The reduction in germination and seedling growth under salinity stress observed in the present study is consistent with earlier reports in linseed and other crops (Qayyum *et al.* 2019, Bayram *et al.* 2024). ZnO nanoparticle priming enhanced germination and seedling vigor, likely by improving metabolic activity and ion regulation during early growth stages. Improved K^+ retention and reduced Na^+ accumulation in primed seedlings suggest better membrane integrity and ion homeostasis, which are key mechanisms of salinity tolerance (Kaya *et al*. 2012, Wu *et al.* 2015).

Salinity stress is a major abiotic constraint limiting crop establishment and productivity, particularly in marginal and degraded lands where linseed is predominantly cultivated (Moghaddam *et al.* 2018). The present study clearly demonstrates that salinity at the seedling stage adversely affects germination, seedling vigor, root–shoot growth balance, and ionic homeostasis in linseed, with pronounced differences among genotypes (Kandil *et al.* 2017). These findings reinforce the notion that early growth stages are highly sensitive to salinity stress and play a decisive role in determining subsequent plant performance and yield potential.

The reduction in germination percentage observed under salinity stress can be attributed to osmotic stress and ionic toxicity, which restrict water uptake by seeds and interfere with enzymatic and hormonal activities essential for germination (Nikolai *et al.* 2016). High external salt concentrations lower the water potential of the germination medium, delaying or inhibiting radicle protrusion. Similar reductions in linseed germination under saline conditions have been reported earlier, confirming the salt-sensitive nature of the crop (Qayyum *et al.* 2019, Bayram *et al.* 2024). The relatively higher germination recorded in tolerant genotypes indicates inherent genetic mechanisms that enable better osmotic adjustment and metabolic stability under stress.

Seed priming with ZnO nanoparticles significantly improved germination under both control and salinity stress conditions (Karim *et al.* 2023). This enhancement may be attributed to accelerated metabolic activation during imbibition, improved membrane integrity, and enhanced enzyme activity associated with reserve mobilization. Zinc is an essential micronutrient involved in the regulation of auxin synthesis, antioxidant enzyme activation, and protein metabolism. Nano-sized ZnO particles offer higher surface area and bioavailability, facilitating efficient uptake and utilization during early seedling growth. Similar positive effects of ZnO nano-priming on germination and early seedling establishment under abiotic stress have been reported in wheat, rice, and other crops (Mazhar *et al.* 2023, Lee and Kasote 2024).

Seedling vigor index, an integrative indicator of seedling growth potential, declined substantially under salinity stress, particularly in sensitive genotypes. This decline reflects the combined inhibitory effects of salinity on root and shoots elongation due to impaired cell division, reduced cell expansion, and metabolic disturbances (Saeid 2015). However, ZnO nanoparticle priming markedly improved seedling vigor under saline conditions, suggesting that nano-priming enhances stress resilience by maintaining cellular metabolism and growth processes. The improved vigor observed in tolerant accessions further highlights the role of genetic variability in salinity response, as also documented in earlier studies on linseed and other crops (Kaya *et al.* 2012, Mustafa 2023).

The root/shoot ratio is a critical adaptive trait under stress conditions, reflecting biomass allocation strategies that favor survival. In the present study, salinity stress reduced the root/shoot ratio, particularly in sensitive genotypes, indicating restricted root development and compromised nutrient acquisition. ZnO nanoparticle priming significantly enhanced the root/shoot ratio under saline conditions, suggesting preferential stimulation of root growth. Enhanced root development under stress is advantageous as it improves water and nutrient uptake efficiency, thereby supporting shoot growth and overall plant establishment (Sarita *et al.* 2022). Similar enhancements in root growth following nano-priming or beneficial microbial inoculation under salinity stress have been reported in several crops (Vázquez-Glaría *et al.* 2021, Khan *et al.* 2023, Gupta *et al.* 2024).

Ionic imbalance, particularly excessive Na^+ accumulation and reduced K^+ uptake, is a hallmark of salinity stress and a key determinant of plant tolerance. In the present study, salt-sensitive genotypes accumulated higher Na^+ and exhibited lower K^+ content compared to tolerant ones. Excess Na^+ disrupts membrane integrity, enzyme activity, and cellular metabolism, while K^+ plays a crucial role in osmoregulation, stomatal function, and enzyme activation. The ability of tolerant genotypes to restrict Na^+ uptake and maintain higher K^+ levels reflects efficient ion exclusion and selective uptake mechanisms. These observations are consistent with earlier reports in linseed and other crops, emphasizing the importance of ionic homeostasis in salinity tolerance (Wu *et al.* 2015, Dubey *et al.* 2020).

ZnO nanoparticle priming significantly reduced Na^+ accumulation and helped maintain higher K^+ content under salinity stress in both tolerant and sensitive genotypes. This effect may be attributed to improved membrane stability, enhanced activity of ion transporters, and strengthened antioxidant defense systems that mitigate salt-induced oxidative damage. By maintaining a favorable K^+/Na^+ ratio, ZnO nano-priming contributes to better physiological functioning and stress adaptation. Such modulation of ion balance through nano-enabled seed treatments highlights their potential as a sustainable tool for stress management in saline agro-ecosystems.

Overall, the present findings underscore the combined influence of genetic variability and nano-priming in determining salinity tolerance at the seedling stage in linseed. The laboratory-based screening approach adopted in this study provides a rapid, cost-effective, and reliable method for identifying salt-tolerant genotypes and evaluating stress-mitigation strategies before field-level validation (Sarker *et al.* 2014).

CONCLUSION

The study demonstrates substantial variation among linseed accessions in response to salinity stress at the seedling stage. ZnO nanoparticle seed priming effectively mitigated the adverse effects of salinity by improving germination, seedling vigor, root/shoot ratio, and ionic balance. Nano-priming with ZnO nanoparticles can therefore be recommended as a practical approach for enhancing early-stage salinity tolerance and for laboratory-based screening of linseed genotypes.

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