STANDARDIZING THE APPROPRIATE DOSES OF ZINC OXIDE NANOPARTICLES FOR ENHANCING THE QUALITY OF TOMATO (*SOLANUM LYCOPERSICUM* L.) SEEDS GROWN UNDER SALINE CONDITIONS

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Abstract. Tomato is one of the most widely grown horticultural crop species worldwide because of its high demand, nutritional benefits, and economic significance. Abiotic stress, particularly salinity stress, results in poor plant stand establishment, leading to decreased yield and quality. To address this problem, in this study, zinc oxide (ZnO) nanoparticles (NPs) were used as a seed priming agent to increase tomato seed quality under salinity stress. For standardization, three different concentrations of ZnO NPs (250, 500, and 750 ppm) and three soaking durations (3, 6, and 8 h) were tested, along with hydroprimed and unprimed controls. Among these, tomato seeds primed with ZnO NPs at 750 ppm for 6 h presented significantly greater germination (92%), seed vigour index I (1399) and seed vigour index II (1813) values. The standardized treatment was further evaluated for seed quality and biochemical parameters under 50 mM and 100 mM NaCl. Salinity stress (50 mM and 100 mM NaCl) markedly affected the seed quality parameters as well as the biochemical traits of 14-day-old tomato seedlings. Under salinity stress (50 mM and 100 mM NaCl), a significant decrease in the germination percentage (2–11%), seedling dry weight (17–29%), total seedling length (17–26%), seed vigour index I (19–32%), seed vigour index II (18–35%), and chlorophyll content (24–55%) was detected, whereas a significant increase in the proline content (84–131%), superoxide dismutase (SOD) activity (20–32%), and catalase (CAT) activity (19-39%) was detected compared with those in the control (no stress conditions). Overall, nanopriming with ZnO NPs at 750 ppm for 6 h significantly increased chlorophyll production, antioxidative mechanisms, and osmotic regulation, which reduced the accumulation of ROS and lipid peroxidation in the cell membrane. These effects may help alleviate the detrimental impacts of salinity stress on tomato plants, which ultimately increases their productivity even under saline conditions.
Keywords: *antioxidants, nanopriming, osmotic adjustment, proline, salinity stress, seed vigour*

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Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most significant and extensively grown vegetable crops in the world (Toni et al., 2021). It is also an important cash crop and ranks second only to potatoes in terms of consumption globally (Gulati et al., 2022). Tomatoes are excellent sources of lycopene, antioxidants, phenolic compounds, vitamin A and vitamin C in the human diet (Kaboré et al., 2022). However, tomato production and yield are severely impacted by various environmental stressors, such as drought, salinity, acidity, alkalinity, heavy metal toxicity, low temperature, heat, and flooding, as well as pests and diseases (Narayanankutty et al., 2019; Atta et al., 2023). Salinity is one of the most important environmental stresses and severely affects plant growth, fruit production, seed quality, and seedling vigour (Chanthini et al., 2022; Ghosh et al., 2024). Owing to restricted root cell proliferation and increased root injury, salinity decreases the tomato root elongation rate and lateral root growth (Zhang et al., 2016; Atta et al., 2021). Under salinity stress, tomato leaves, shoots, and stems have relatively small diameters due to reduced leaf chlorophyll content, damaged photosynthetic machinery, reduced tissue growth, and decreased cell division (Acosta-Motos et al., 2017; Atta et al., 2021). At salinity levels equivalent to or above 5 dS m-1, tomato yield is considerably reduced, with a 7.2% yield reduction for each unit increase in salinity (Zhang et al., 2016). Therefore, it is necessary to address the effects of salinity stress on seed quality, seedling vigour, and seedling biochemical parameters for improved planting value as well as increased production.

Priming before sowing is a seed enhancement approach to soak seeds in value-added solutions for a particular duration, which increases the metabolic reactions of the seeds but restricts seedling root emergence (Sundaria et al., 2019). Priming leads to increased emergence rates, robust seedling growth, and faster stand establishment rates under stressful and optimal conditions (Hussain et al., 2016). Amylases, proteases, and lipases, which breakdown macromolecules for the growth and enlargement of the embryo, become more active as a result of priming (Sghayar et al., 2023). Farmers eventually gain from these biological consequences since they take less time and money to reseed, add irrigation, fertilize, and control weeds on poor plants.

Recently, nanotechnology has become a cutting-edge seed priming tool for smart and sustainable agriculture (Shweta et al., 2021). Owing to the significant and distinctive characteristics of nanoparticles, such as their nanosize (less than 100 nm), easier penetration inside tissue, larger surface area, higher surface-to-mass ratio and increased chemical reactivity, they can effectively improve catalysis as well as adsorb and deliver chemicals of interest (Mitchell et al., 2021). NP priming, or nanopriming, has the potential to activate metabolic pathways during the early stages of germination, particularly under stress conditions (Imtiaz et al., 2023; Ghosh et al., 2024). Zinc (Zn), an essential micronutrient for plant metabolism, serves as a cofactor for enzymes that drive critical metabolic processes (Kumar et al., 2020; Roy Chowdhury et al., 2024).

Given the unique properties of nanoparticles and their ability to deliver substances effectively within biological systems, we hypothesized that priming tomato seeds with zinc oxide (ZnO) nanoparticles would improve germination, seedling vigor, seed quality, and biochemical characteristics under salt stress. Therefore, this study first aimed to determine the optimal ZnO nanoparticle concentration and treatment duration for tomato seed priming on the basis of germination percentage and vigour indices. This optimized nanopriming treatment was then compared with hydropriming and no

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priming (control) by assessing seed quality and biochemical parameters under varying levels of salt stress.

Materials and methods

Seed material collection and preparation

This study used seeds from two tomato genotypes, QA001 and QA002. The standardization experiment was conducted using genotype QA001, whereas the validation of the standardized treatment was carried out with genotype QA002. The standardized treatment, along with hydroprimed and unprimed seeds, was further evaluated for both seed quality and biochemical parameters under different concentrations of NaCl salt stress. Initially, the seeds were surface sterilized with a 0.1% $HgCl_2$ solution for 5 minutes, followed by thorough rinsing with distilled water. The initial weight and moisture content of the seeds were measured before the experiment commenced.

Seed nanopriming treatment

For the standardization experiment (*Fig. 1*), three different concentrations of ZnO NP solutions, i.e., 250, 500 and 750 ppm, and three different soaking durations, i.e., 3, 6 and 8 h, were used. In addition to the nanopriming treatment, hydroprimed and unprimed treatments were included as controls. For the nanopriming process, sterilized seeds were placed in test tubes containing a ZnO nanoparticle (ZnO-NP) solution, maintaining a 1:1 (g/ml) ratio of seed weight to solution volume. The test tubes were covered with aluminum foil and kept at 25°C in the dark for specific soaking durations (3, 6, and 8 hours). After each treatment, the seeds were rinsed with distilled water, placed on Petri dishes, and covered with dry filter paper to air-dry at room temperature until they returned to their original moisture content. For the hydropriming treatment, the seeds were soaked in distilled water, whereas the control seeds remained untreated and unsoaked in any solution (*Fig. 1*).

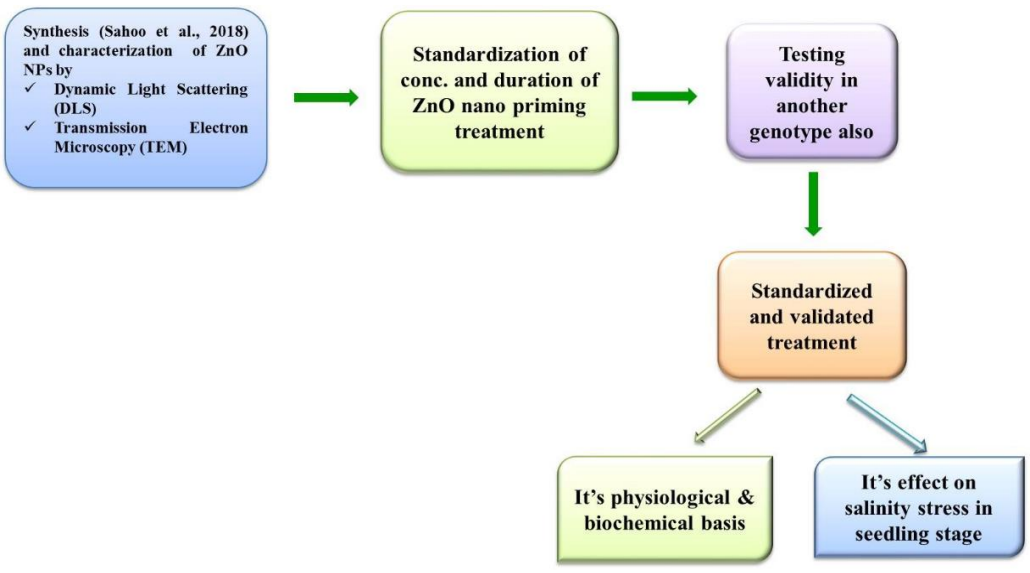

Figure 1. *Methodologies used during seed nanopriming*

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Salinity stress treatment

Tomato seeds were germinated under salt stress conditions by placing them on filter paper in Petri dishes. The filter paper was soaked with either a 50 mM NaCl solution or a 100 mM NaCl solution to create different stress levels.

Evaluation of physiological parameters of the seeds

The germination test was conducted via the top-of-the-paper (TP) method (ISTA 2022). Fifty seeds were randomly and evenly distributed across double-layered moist filter paper in Petri dishes (15×15 cm), with three replications for each treatment. The Petri dishes were incubated in a germination oven at a constant temperature of 25°C and 90% relative humidity in complete darkness. Germination counts were recorded on the 5th day (first count) and the 14th day (final count). Seedling vigour was assessed following Abdul-Baki and Anderson (1973). Fifty seeds were placed on moist filter paper in Petri dishes and incubated at 25°C. The percentage of normal seedlings was recorded. On day 14, the roots, shoots, and total length (cm) of ten randomly selected normal seedlings per replicate were measured. These seedlings were then dried at 70±10°C for 48 hours to determine the seedling dry weight, as well as the root length, shoot length, and total fresh and dry weights.

$$\text{Vigour index I} = \text{Germination (\%)} \times \text{Total seedling length (cm)} \quad \text{(Eq.1)}$$

$$\text{Vigour index II} = \text{Germination (\%)} \times \text{Seedling dry weight (mg)} \quad \text{(Eq.2)}$$

Evaluation of seedling biochemical parameters

The chlorophyll content of 14-day-old seedlings was measured following the methods of Arnon (1949). Fresh leaf samples (250 mg) were homogenized, and chlorophyll was extracted via 80% acetone at 0.4°C. The extracts were subsequently centrifuged at 10,000 rpm for 5 minutes. The absorbance was measured at 663, 652, 645, and 470 nm via a spectrophotometer with 80% acetone as a blank. The results are expressed as mg/g fresh weight (FW).

The proline content of 14-day-old seedlings was determined via the methods of Bates et al. (1973). Seedlings (500 mg) were ground with 3 ml of 3% sulfosalicylic acid and centrifuged at 10,000 rpm for 30 minutes. The supernatant (1 ml) was mixed with 1 ml of glacial acetic acid and 1 ml of acid ninhydrin reagent, and the mixture was incubated at 95°C for 1 hour. The reaction was stopped by placing the sample in an ice bath. Toluene (4 ml) was added, and the mixture was vortexed. The absorbance of the toluene fraction was measured at 520 nm via a spectrophotometer with toluene as a blank.

Evaluation of enzymatic antioxidant activities

The catalase activity of the seedlings was measured following the method described by Sinha (1972). Fresh seedling tissue (approximately 500 mg) was ground in a chilled mortar and pestle with 3 ml of 0.1 M potassium phosphate buffer (pH 7.8). The homogenate was subsequently centrifuged at 15000 rpm for 20 minutes. The reaction mixture consisted of 1.5 ml of 0.1 M potassium phosphate buffer (pH 7), 0.3 ml of 30 mM hydrogen peroxide, 0.2 ml of the enzyme extract, and 1 ml of distilled water. The absorbance at 240 nm was measured every 30 seconds for 3 minutes.

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Superoxide dismutase (SOD) activity was measured following the methods of Kumar et al. (2012). Fresh seedling tissue (500 mg) was ground in 50 mM potassium phosphate buffer (pH 7) and centrifuged at 13000 rpm for 30 minutes. The reaction mixture contained 50 µl of extract, 2.15 ml of 50 mM potassium phosphate buffer (pH 7.8), 0.2 ml of 200 mM methionine, 2.25 mM nitro blue tetrazolium, and 1.5 mM EDTA disodium salt. Then, 0.2 ml of 150 mM riboflavin solution was added, and the tubes were placed 30 cm below two 15 W fluorescent tubes. The light was turned on for 10 minutes, the samples were then removed, and the tubes were covered. The absorbance was measured at 560 nm. SOD activity is expressed as units/ml/g fresh weight.

Statistical analysis

The data from three replicate experiments were averaged, and these mean values were statistically analysed via a completely randomized design (CRD) in SPSS version 17. Significant differences between treatment means were determined at a significance level of $p \leq 0.05$. Principal component analysis (PCA) was also conducted via R software to further explore the data.

Results and discussion

Standardization of the ZnO nanopriming treatment

The results of seed priming by using various concentrations of ZnO nanoparticle solutions for different soaking durations with the tomato genotype QA001 (*Fig. 2*) revealed significant differences among the various treatments in terms of seed quality parameters such as germination percentage, the seed vigour index I and the seed vigour index II (*Table 1*).

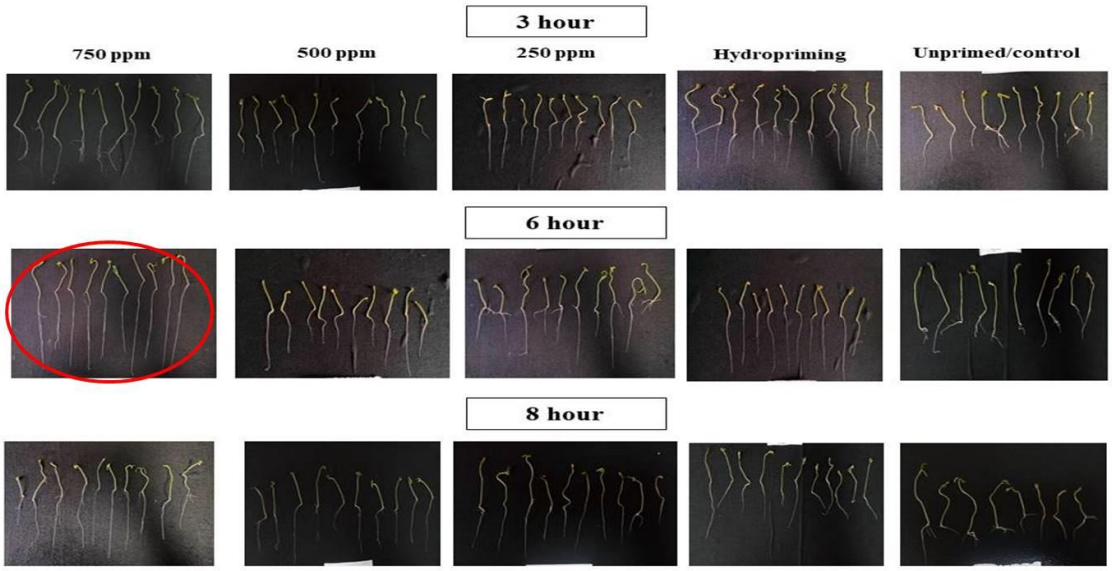

Figure 2. *Standardization of different concentrations and durations of nanopriming treatment*

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Table 1. *Standardization of the ZnO NP concentration and duration of germination (%), the seed vigour index I and the seed vigour index II in the tomato genotype QA001*

Treatment	Germination (%)				Seed vigour index I				Seed vigour index II			
	3 h	6 h	8 h	Mean	3 h	6 h	8 h	Mean	3 h	6 h	8 h	Mean
Control	84(66)	85(67)	87(69)	85^{d} (67)	838	833	863	845^{c}	821	940	767	842^{c}
Hydro priming	88(70)	89(71)	89(71)	89^{b} (71)	940	1266	692	966^{b}	896	1442	873	1070^{b}
NP (ZnO@250 ppm)	85(66)	90(72)	87(69)	87^{c} (69)	1004	1091	853	983^{b}	906	1167	891	988^{b}
NP (ZnO@500 ppm)	85(67)	88(70)	85(66)	85^{d} (67)	875	1218	618	904^{c}	847	1068	869	928^{b}
NP (ZnO@750 ppm)	90(72)	92(74)	91(73)	91^{a} (73)	1206	1399	960	1188^{a}	1083	1813	1085	1327^{a}
Mean	86^{c}(68)	90^{a}(71)	88^{b}(70)	87(69)	973^{b}	1161^{a}	797^{c}	977	911^{b}	1286^{a}	897^{b}	1038
ANOVA												
CD ($p \leq 0.05$)												
Treatment (T)	1.17				54.96				70.95			
Duration (D)	0.91				42.57				54.96			
Treatment × Duration (T×D)	2.03				95.19				122.90			

Growth parameters

Germination percentage

In the present study, among all the treatments, the QA001 genotype resulted in a significantly greater (91%) germination percentage in the nanopriming treatment with ZnO @ 750 ppm, whereas the lowest (85%) germination percentage was observed in the unprimed or control seeds, which was on par with the results of the ZnO@500 ppm treatment. The germination percentage of the plants subjected to the nanopriming treatment with 250 ppm ZnO@ (87%) was significantly lower than that of the plants subjected to the nanopriming treatment with 750 ppm ZnO@ and the hydropriming treatment (89%). However, a significantly greater (89%) germination percentage was reported for the 6 h duration than for the other durations, and a significantly lower (86%) germination percentage was observed for the 3 h duration. Among the interactions between priming treatments and soaking durations (T×D), the significantly highest (92%) germination percentage was reported in the nanopriming treatment with ZnO @ 750 ppm for a 6 h soaking duration, whereas the significantly lowest (84%) germination percentage was perceived in the unprimed or control seeds.

ZnO@750 ppm likely provided an optimal level of zinc, which is an essential micronutrient for various physiological and biochemical processes in seeds, leading to improved germination. Compared with the control, the hydropriming treatment improved germination, likely due to enhanced water absorption, but it was still less effective than the optimal ZnO nanopriming (750 ppm). The control seeds did not receive any priming treatment, so they might not have the same enhanced nutrient availability or stress protection as the primed seeds did, resulting in lower germination rates. Tymoszuk et al. (2020) reported similar findings in *Allium cepa* seeds: the optimal concentration of ZnO nanoparticles (750 ppm) provided the best conditions for seed germination by increasing nutrient availability, increasing water uptake, and providing protection against stress, leading to significantly greater germination percentages than

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lower concentrations or unprimed seeds. Sherpa et al. (2024) reported that, in germinated seeds of mung bean, ZnO nanoparticles enhance seed germination by increasing nutrient availability, water uptake, enzyme activation, antioxidant defense, protection against pathogens, and overall metabolic activity. These effects contribute to more robust and efficient germination, leading to better seedling establishment and growth. The optimal 6-hour soaking duration provided sufficient water uptake to activate metabolic processes effectively, while the synergistic effect of ZnO nanopriming at 750 ppm further enhanced germination by supplying essential nutrients and improving stress resistance (Salam et al., 2022).

Seed vigour indices I and II (SV I and II)

Like the germination percentage, a significantly greater percentage (1188) of SV I was reported in the nanopriming treatment with ZnO @ 750 ppm, whereas a significantly lower percentage (845) of SV I was observed in the unprimed or control seeds, which was on par with the results of the ZnO NP@500 ppm (904) treatment (*Table 1*). The SV I after nanopriming treatment with ZnO@250 ppm (983) was significantly lower than that after nanopriming treatment with ZnO@750 ppm but was similar to that after hydropriming treatment (966). In contrast, a significantly greater (1161) SV I was observed in the samples soaked for 6 h, followed by those soaked for 3 h (973), whereas a significantly lower (797) SV I was observed after 8 h. Among the interactions between priming treatments and soaking durations (T×D), the significantly highest (1399) SV I was reported when seeds were primed with a ZnO NP solution@750 ppm for a 6 h soaking duration; in contrast, the significantly lowest (618) value was observed in the nanopriming treatment with ZnO @500 ppm for an 8 h soaking duration. Similar results were obtained in the case of SV II. A significantly greater (1327) SV II was detected in the nanopriming treatment with ZnO @ 750 ppm, whereas a significantly lower (842) SV II was detected in the unprimed or control seeds. The SV II in the hydropriming treatment (1070) was significantly lower than that in the nanopriming treatment with ZnO @ 750 ppm but was on par with the SV II in the nanopriming treatment with ZnO @ 250 ppm (988) and the nanopriming treatment with ZnO @500 ppm (928). Additionally, a significantly greater (1286) SV II was reported in the case of the 6 h soaking duration. The significantly lowest (897) SV II was found in the 8 h soaking duration, which was on par with the 3 h duration (911). Among the interactions between priming treatments and soaking durations (T×D), the significantly highest (1813) SV II was perceived when the seeds were primed with ZnO NP solution @ 750 ppm for a 6-hr soaking duration; in contrast, the significantly lowest (767) value was observed in unprimed seeds.

Zinc plays a key role in enzyme activation and metabolic processes, contributing to the development of more robust seedlings, as reflected by the higher SV I (Tanwar et al., 2023). The 6-hour soaking duration provided sufficient water absorption, activating metabolic processes essential for seedling vigour. This 6-hour duration allowed seeds to achieve optimal hydration without the negative effects of oversoaking, which could lead to oxygen deficiency and lower vigor (Narish et al., 2012; Dutta, 2018). ZnO nanopriming likely improves seed quality by increasing the production and activity of enzymes that breakdown stored compounds during early germination. This leads to better mobilization of seed reserves, resulting in faster seedling emergence and growth (Imtiaz et al., 2023). The enhanced chemical reactivity of NPs as cofactors of germination and seedling growth-related enzymes may account for their overall

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beneficial effects on seedling growth. ZnO nanopriming treatments have also been shown to improve the uptake of essential nutrients, enabling better nutrient assimilation by seedlings, which contributes to overall growth and development. Similar findings were reported by Itroutwar et al. (2020) in seaweed-based biogenic ZnO nanoprimed seeds (100 ppm), where the greatest improvement in the seed germination rate and vigour index (2931.9) was observed compared with those of the control (hydropriming).

Validation of standardized treatment for another genotype

The results of previous standardization experiments clearly revealed that seeds primed with ZnO NP@ 750 ppm for a 6-h soaking duration presented the significantly highest values of germination%, SV I and SV II among all the treatments. Therefore, for genotype QA001, it was the best treatment (*Table 2*).

Table 2. *Validation of standardized ZnO NP concentration and duration of germination (%), seed vigour index I and seed vigour index II in the tomato genotype QA002*

Treatments	Germination %	SV I	SV II
Control	$88^{b}(70)$	850^{c}	949^{c}
Hydropriming (6 h)	$90^{b}(72)$	1232^{b}	1236^{b}
NP (ZnO @ 750 ppm) (6 h)	$93^{a}(75)$	1477^{a}	1611^{a}
Mean	90.33	1186	1266
ANOVA			
CD($p \leq 0.05$)			
Treatment (T)	2.35	129.24	161.23

However, to investigate the effectiveness of this treatment (750 ppm concentration and 6 h duration) compared with that of hydroprimed (6 h duration) and unprimed (control) treatment in another genotype, QA002, a validation experiment was performed. The germination %, SV I and SV II quality parameters (*Table 2*) were also evaluated.

Germination percentage

The ZnO nanopriming treatment at 750 ppm resulted in the highest germination rate (93%), which was significantly greater than that of the untreated control (88%) and similar to that of the hydropriming treatment (90%) (*Table 2*). This increase is likely due to the influence of zinc on key germination processes, such as breaking seed dormancy, deactivating or breaking down germination inhibitors, facilitating water absorption, and activating necessary enzymes (Harris et al., 2007; Samad et al., 2014).

Seed vigour indices I and II (SVI and II)

A significantly greater (1477) SV I was detected in the nanopriming treatment with ZnO@750 ppm, followed by the hydropriming treatment (1232), whereas the SV I was significantly lower (850) in the unprimed control seeds. Similar observations were recorded for SV II (*Table 2*).

Shoot length (cm)

The shoot length (cm) of 14-day-old seedlings of the two genotypes, QA001 and QA002, was measured after different treatments, i.e., unprimed (control), hydroprimed

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and nanoprimed with ZnO@750 ppm under control (no stress), 50 mM NaCl, and 100 mM NaCl salt stress conditions (*Tables 3* and *4*). There were statistically significant differences in shoot length among the genotypes, treatments, and stress conditions. Among the two genotypes used in this study, a significantly greater (4.35 cm) shoot length was reported for the QA001 genotype than for the QA002 genotype (3.85 cm). Among all the treatments, the ZnO nanopriming treatment resulted in a significantly greater (4.59 cm) shoot length, followed by the shoot length in the hydropriming treatment (4.15 cm), and a significantly shorter (3.57 cm) shoot length was reported in the case of unprimed/control seeds (*Tables 3* and *4*).

Table 3. *Effects of different treatments on the shoot length (cm) of tomato seedlings under control and salinity stress conditions*

Shoot length (cm)									
Conditions	**QA001**				**QA002**				**Grand mean**
	Treatments				**Treatments**				
	Control/ unprimed	**Hydro priming**	**NP (ZnO@750 ppm)**	**Mean**	**Control/ unprimed**	**Hydro priming**	**NP (ZnO@750 ppm)**	**Mean**	
Control (no-stress)	5.23	5.08	5.87	5.39	4.34	4.48	4.98	4.60	5.00
50 mM NaCl	3.73	4.49	4.22	4.15	2.78	3.61	4.25	3.55	3.85
100 mM NaCl	2.76	3.71	4.09	3.52	2.57	3.53	4.16	3.42	3.47
Mean	3.91	4.43	4.72	4.35	3.23	3.87	4.46	3.85	4.10
ANOVA									
CD ($p \leq 0.05$)									
Condition (C)	0.318		C×V	NS	C×V×T		NS		
Genotype (V)	0.261		C×T	NS					
Treatment (T)	0.318		V×T	NS					

Table 4. *Mean values of shoot length (cm) under different treatments and conditions*

Shoot length (cm)			
Treatments	**Mean**	**Conditions**	**Mean**
Control (unprimed)	3.57^{c}	Control (no-stress)	5.00^{a}
Hydropriming	4.15^{b}	50 mM NaCl	3.85^{b}
NP (ZnO@750 ppm)	4.59^{a}	100 mM NaCl	3.47^{c}
Mean	4.10	Mean	4.10

Similar to findings in wheat (Kalal and Jajoo, 2021), this study revealed increased shoot and root lengths in tomato seedlings primed with ZnO nanoparticles. This growth promotion is likely linked to the essential role of zinc in the synthesis of plant hormones such as auxins and gibberellins, which stimulate root and shoot elongation (Cakmak, 2008; Prasad et al., 2012). Furthermore, the involvement of zinc in carbohydrate and protein metabolism may contribute to the improved and more uniform germination observed in ZnO-NP-primed seeds (Broadley et al., 2007). A significantly greater (5.00 cm) shoot length was found in the control (no stress) treatment, followed by the 50 mM NaCl treatment (3.85 cm), whereas a significantly shorter (3.47 cm) shoot length was reported in the 100 mM NaCl treatment. The shoot length measured under

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50 mM NaCl and 100 mM NaCl stress conditions was reported to be 23% and 30.60% lower than that measured under the control (no stress) conditions, respectively.

Root length

After the seeds were subjected to different treatments, i.e., unprimed (control), hydroprimed and nanoprimed with ZnO @ 750 ppm, the root length (cm) was measured in 14-day-old seedlings of two genotypes, QA001 and QA002, under control (no stress) and salinity stress conditions with 50 mM NaCl and 100 mM NaCl concentrations (*Tables 5* and *6*).

Table 5. *Effects of different treatments on the root length (cm) of tomato seedlings under control and salinity stress conditions*

Root length (cm)									
Conditions	**QA001**				**QA002**				**Grand mean**
	Treatments				**Treatments**				
	Control/ unprimed	**Hydro priming**	**NP (ZnO@750 ppm)**	**Mean**	**Control/ unprimed**	**Hydro priming**	**NP (ZnO@750 ppm)**	**Mean**	
Control (no-stress)	6.38	6.92	9.25	7.52	6.14	7.41	9.24	7.60	7.56
50 mM NaCl	5.82	6.52	7.67	6.67	5.31	5.96	7.68	6.32	6.49
100 mM NaCl	4.78	5.66	7.15	5.86	4.94	5.91	7.19	6.01	5.94
Mean	5.66	6.37	8.02	6.68	5.46	6.43	8.04	6.64	6.66
ANOVA									
CD ($p \leq 0.05$)									
Condition (C)	0.757		C×V	NS		C×V×T		NS	
Genotype (V)	NS		C×T	NS					
Treatment (T)	0.757		V×T	NS					

Table 6. *Mean values of root length (cm) under different treatments and conditions*

Root length (cm)			
Treatments	**Mean**	**Conditions**	**Mean**
Control(unprimed)	5.56^{c}	Control(no-stress)	7.56^{a}
Hydropriming	6.4^{b}	50 mM NaCl	6.49^{b}
NP (ZnO@750 ppm)	8.03^{a}	100 mM NaCl	5.94^{b}
Mean	6.66	Mean	6.66

Statistically significant differences were reported for root length among the treatments and stress conditions. Among all the treatments, the ZnO nanopriming treatment resulted in a significantly greater (8.03 cm) root length, followed by the hydropriming treatment (6.40 cm), and a significantly shorter (5.56 cm) root length was reported in the case of unprimed/control seeds. Our findings are consistent with earlier research, such as the study by Kalal and Jajoo (2021) on wheat, which showed that ZnO nanoparticle seed priming improved root and shoot growth. They also noted similar growth stimulation in wheat seedlings. This beneficial impact of zinc-enriched priming

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solutions on seedling emergence and development is likely due to the critical role of zinc in the early growth phases of the coleoptile and radicle (Ozturk et al., 2006).

Among the conditions applied in this study, significantly greater (7.56 cm) root length was found in the control (no stress) condition, whereas significantly lower (5.94 cm) root length was reported in the 100 mM NaCl salt stress condition, which was similar to the root length under the 50 mM NaCl salt stress condition (6.49 cm). The root length measured under 50 mM NaCl and 100 mM NaCl stress conditions was reported to be 14.15% and 21.43% lower than that measured under the control (no stress) conditions, respectively.

Total seedling length

The total seedling length (cm) of 14-day-old seedlings of the two genotypes, QA001 and QA002, was measured after different treatments, i.e., unprimed (control), hydroprimed and nanoprimed with ZnO@750 ppm under control (no stress) and 50 mM NaCl or 100 mM NaCl salt stress conditions (*Tables 7* and *8*).

Table 7. *Effects of different treatments on the total length (cm) of tomato seedlings under control and salinity stress conditions*

Total seedling length (cm)									
Conditions	**QA001**				**QA002**				**Grand mean**
	Treatments				**Treatments**				
	Control/ unprimed	**Hydro priming**	**NP (ZnO@750 ppm)**	**Mean**	**Control/ unprimed**	**Hydro priming**	**NP (ZnO @750 ppm)**	**Mean**	
Control (no-stress)	11.61	12.00	15.12	12.91	10.48	11.88	14.22	12.19	12.55
50 mM NaCl	9.54	11.01	11.89	10.81	8.10	9.56	11.92	9.86	10.34
100 mM NaCl	7.54	9.36	11.23	9.38	7.50	9.44	11.35	9.43	9.40
Mean	9.56	10.79	12.75	11.03	8.69	10.30	12.50	10.50	10.77
ANOVA									
CD ($p \leq 0.05$)									
Condition (C)	0.906		C×V	NS	C×V×T		NS		
Genotype (V)	NS		C×T	NS					
Treatment (T)	0.906		V×T	NS					

Table 8. *Mean values of total seedling length (cm) under different treatments and conditions*

Total seedling length (cm)			
Treatments	**Mean**	**Conditions**	**Mean**
Control(unprimed)	9.13^{c}	Control (no-stress)	12.55^{a}
Hydropriming	10.55^{b}	50 mM NaCl	10.34^{b}
NP (ZnO@750 ppm)	12.63^{a}	100 mM NaCl	9.40^{b}
Mean	10.77	Mean	10.77

Statistically significant differences were observed for total seedling length among the treatments and stress conditions. Among all the treatments, the ZnO nanopriming treatment resulted in a significantly greater (12.63 cm) total seedling length followed by the total seedling length in the hydropriming treatment (10.55 cm), whereas a

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significantly shorter (9.13 cm) total seedling length was reported in the case of unprimed/control seeds. Considering all the conditions in this study, a significantly greater (12.55 cm) total seedling length was found in the control (no stress) condition, whereas a significantly lower (9.40 cm) total seedling length was reported in the 100 mM NaCl salt stress condition, which was on par with the total seedling length under the 50 mM NaCl salt stress condition (10.34 cm). The total seedling length measured under 50 mM NaCl and 100 mM NaCl salt stress conditions was reported to be 17.61% and 25.10% lower than the total seedling length recorded under control (no stress) conditions, respectively (*Tables 7* and *8*).

Seedling fresh weight

The fresh weight (g) of 14-day-old seedlings of the two genotypes, QA001 and QA002, was measured after different treatments, i.e., unprimed (control), hydroprimed and nanoprimed with ZnO@750 ppm under the control (no stress), 50 mM NaCl, and 100 mM NaCl salt stress conditions (*Tables 9* and *10*).

Table 9. *Effects of different treatments on the fresh weight (g) of tomato seedlings under control and salinity stress conditions*

Fresh weight (g)									
Conditions	**QA001**				**QA002**				**Grand mean**
	Treatments				**Treatments**				
	Control/ unprimed	**Hydro priming**	**NP (ZnO@750 ppm)**	**Mean**	**Control/ unprimed**	**Hydro priming**	**NP (ZnO@750 ppm)**	**Mean**	
Control (no-stress)	0.366	0.398	0.430	0.398	0.368	0.401	0.407	0.392	0.395
50 mM NaCl	0.362	0.370	0.386	0.373	0.343	0.367	0.384	0.365	0.369
100 mM NaCl	0.361	0.362	0.375	0.366	0.339	0.360	0.382	0.360	0.363
Mean	0.363	0.377	0.397	0.379	0.350	0.376	0.391	0.372	0.376
ANOVA									
CD ($p \leq 0.05$)									
Condition (C)	0.011		C×V	NS		C×V×T		NS	
Genotype (V)	NS		C×T	NS					
Treatment (T)	0.011		V×T	NS					

Table 10. *Mean values of fresh weight (g) under different treatments and conditions*

Fresh weight (g)			
Treatments	**Mean**	**Conditions**	**Mean**
Control(unprimed)	0.357^{c}	Control (no-stress)	0.395^{a}
Hydropriming	0.377^{b}	50 mM NaCl	0.369^{b}
NP (ZnO@750 ppm)	0.394^{a}	100 mM NaCl	0.363^{b}
Mean	0.376	Mean	0.376

There were statistically significant differences observed for FW among the treatments and stress conditions themselves. Among all the treatments, the ZnO nanopriming treatment resulted in a significantly greater seedling fresh weight

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(0.394 g), followed by the fresh weight reported in the hydropriming treatment (0.377 g), whereas a significantly lower (0.357 g) seedling fresh weight was perceived in the case of unprimed/control seeds. Considering all the conditions of this experiment, the significantly highest (0.395 g) seedling fresh weight was recorded for the control (no stress) condition, whereas the significantly lowest (0.363 g) seedling fresh weight was reported under the 100 mM NaCl salt stress condition, which was statistically similar to the seedling fresh weight measured under the 50 mM NaCl salt stress condition (0.369 g). The fresh weights of the seedlings evaluated under 50 mM NaCl and 100 mM NaCl salt stress conditions were 6.58% and 8.1% lower than the fresh weights observed under the control (no stress) conditions, respectively (*Tables 9* and *10*).

Total dry weight

The dry weight (mg) of 14-day-old seedlings of the two genotypes, QA001 and QA002, was measured after different treatments, i.e., unprimed (control), hydroprimed and nanoprimed with ZnO@750 ppm under the control (no stress) and 50 mM NaCl or 100 mM NaCl salt stress conditions (*Tables 11* and *12*). There were statistically significant differences in dry weight among the genotypes, treatments, and stress conditions themselves.

Table 11. *Effects of different treatments on the dry weight (g) of tomato seedlings under control and salinity stress conditions*

Dry weight (mg)									
Conditions	**QA001**				**QA002**				
	Treatments				**Treatments**				
	Control/ unprimed	**Hydro priming**	**NP (ZnO@750 ppm)**	**Mean**	**Control/ unprimed**	**Hydro priming**	**NP (ZnO@750 ppm)**	**Mean**	**Grand mean**
Control (no-stress)	10.87	14.33	17.05	14.08	9.91	11.81	15.07	12.26	13.17
50 mM NaCl	9.48	11.46	14.13	11.69	8.04	10.29	12.00	10.11	10.9
100 mM NaCl	7.51	10.00	11.60	9.70	6.76	9.68	10.64	9.03	9.365
Mean	9.29	11.93	14.26	11.83	8.24	10.59	12.57	10.47	11.15

ANOVA					
CD ($p \leq 0.05$)					
Condition (C)	1.004	C×V	NS	C×V×T	NS
Genotype (V)	0.817	C×T	NS		
Treatment (T)	1.004	V×T	NS		

Table 12. *Mean dry weight (mg) under different treatments and conditions*

Dry weight (mg)			
Treatments	**Mean**	**Conditions**	**Mean**
Control(unprimed)	8.77^{c}	Control (no-stress)	13.17^{a}
Hydropriming	11.26^{b}	50 mM NaCl	10.9^{b}
NP (ZnO@750 ppm)	13.42^{a}	100 mM NaCl	9.37^{c}
Mean	11.15	Mean	11.15

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Among the two genotypes used in this study, a significantly greater (11.83 mg) seedling dry weight was reported for the QA001 genotype, whereas a significantly lower (10.47 mg) seedling dry weight was reported for the QA002 genotype. Among all the treatments, the ZnO nanopriming treatment resulted in a significantly greater dry weight (13.42 mg), followed by the dry weight reported in the hydropriming treatment (11.26 mg), whereas a significantly lower dry weight (8.77 mg) was detected in the unprimed/control seeds. Considering all the conditions of this study, the significantly highest (13.17 mg) dry weight was recorded for the control (no stress) condition; in contrast, the significantly lowest (9.37 mg) dry weight was perceived under the 100 mM NaCl salt stress condition, which was also significantly lower than the dry weight measured under the 50 mM NaCl salt stress condition (10.9 mg). The dry weights measured under the 50 mM NaCl and 100 mM NaCl salt stress conditions were 17.24% and 28.85% lower, respectively, than the dry weight recorded under the control (no stress) conditions. However, statistically nonsignificant differences were revealed for the dry weight of 14-day-old seedlings among the interactions among stress conditions × genotypes (C×V), stress conditions × treatments (C×T), genotypes × treatments (V×T) and stress conditions × genotypes × treatments (C×V×T) (*Table 12*).

Effects of standardized treatments on the physiological and biochemical parameters of seedlings under salt stress

The physiological and biochemical parameters of the two genotypes, QA001 and QA002, were assessed after different treatments, i.e., unprimed (control), hydropriming and nanopriming with ZnO@750 ppm under the control (no stress), 50 mM NaCl, and 100 mM NaCl salt stress conditions.

Estimation of total chlorophyll content

In a recent study, among all the treatments, the ZnO nanopriming treatment (@750 ppm) resulted in a significantly higher (10.44 mg/g FW) total chlorophyll content, followed by the hydropriming treatment (8.69 mg/g FW), and the lowest content was reported in unprimed/control seeds (7.91 mg/g FW) (*Table 13*). Moreover, a significantly greater (12.22 mg/g FW) total chlorophyll content was detected in the control (no stress) condition than in all the other conditions applied in this study, followed by the 50 mM NaCl salt stress condition (9.29 mg/g FW), whereas a significantly lower (5.52 mg/g FW) total chlorophyll content was detected in the 100 mM NaCl salt stress condition. Among the two genotypes, the QA001 genotype presented a significantly greater (9.81 mg/g FW) total chlorophyll content than did the QA002 genotype (8.21 mg/g FW) (Table 13). Considering the interactions between the treatments and stress conditions (C×T), the significantly highest (14.35 mg/g FW) total chlorophyll content was reported in the ZnO nanopriming treatment under no stress (control) conditions. However, the significantly lowest value (4.69 mg/g FW) was detected in unprimed (control) seeds under 100 mM NaCl salt stress conditions. Elevated amounts of salt cause an increase in the accumulation of Na^+ and Cl^- ions, which impedes the synthesis of chlorophyll by affecting the function of some Fe^{3+}-containing chlorophyll-synthesizing enzymes (Mbarki et al., 2018). Like in the present study, chlorophyll degradation was also observed in rice under salinity stress (Zhang et al., 2012). However, the chlorophyll content of seeds primed with ZnO NPs increased under salt stress conditions, which was corroborated with findings in chitosan-nanoprimed rice seeds under various NaCl stress conditions (Soni et al., 2023). The

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increase in chlorophyll levels due to ZnO nanopriming might be because zinc acts as an important cofactor for the amino levulinic acid dehydratase (ALA-D) enzyme, which is essential for the chlorophyll biosynthesis pathway (Pauza et al., 2005). In addition, plants with more chlorophyll tend to have a higher vigour index because chlorophyll is essential for photosynthesis, which gives them the energy to grow and develop. With more chlorophyll, plants can absorb more sunlight, produce more nutrients, and become stronger. This results in better seedling growth, increased biomass, and overall healthier plants, all of which help increase the vigour index.

Table 13. *Effects of different treatments on the total chlorophyll (mg/g FW) content in tomato seedlings under control and salinity stress conditions*

Total chlorophyll (mg/g FW)									
Conditions	**QA001**				**QA002**				**Grand mean**
	Treatments				**Treatments**				
	Control/ unprimed	**Hydro priming**	**NP (ZnO@750 ppm)**	**Mean**	**Control/ unprimed**	**Hydro priming**	**NP (ZnO@750 ppm)**	**Mean**	
Control (no-stress)	11.41	12.38	15.85	13.21	10.02	10.83	12.85	11.23	12.22
50 mM NaCl	6.80	9.60	13.42	9.94	7.82	8.09	10.00	8.64	9.29
100 mM NaCl	5.17	6.63	7.01	6.27	4.21	4.59	5.5	4.77	5.52
Mean	8.46	9.54	11.43	9.81	7.35	7.84	9.45	8.21	9.01
ANOVA									
CD ($p \leq 0.05$)									
Condition (C)	0.708		C×V	NS		C×V×T		1.732	
Genotype (V)	0.576		C×T	1.225					
Treatment (T)	0.708		V×T	NS					

Estimation of proline content

Among all the treatments, the ZnO nanopriming treatment resulted in a significantly greater proline content (0.38 µmol/g FW), followed by the hydropriming treatment (0.32 µmol/g FW) and the unprimed/control treatment (0.28 µmol/g FW) (*Table 14*).

Moreover, among the conditions applied here, a significantly greater proline content (0.44 µmoles/g FW) was recorded for the 100 mM NaCl salt stress condition, followed by the 50 mM NaCl salt stress condition (0.35 µmoles/g FW) and the control (no stress) condition (0.19 µmoles/g FW). Additionally, considering the interactions between conditions and genotype (C×V), a significantly greater (0.48 µmoles/g FW) proline content was detected in the QA001 genotype under the 100 mM NaCl salt stress condition, whereas it was significantly lower (0.17 µmoles/g FW) in the QA002 genotype under the control (no stress) condition. Similar to the TSS content, considering the interactions between the treatments and stress conditions (C×T), the significantly highest (0.51 µmol/g FW) proline content was reported in the ZnO nanopriming treatment under 100 mM NaCl salt stress, whereas the unprimed (control) seeds under no stress (control) conditions presented the lowest proline content (0.14 µmol/g FW) (*Table 14*). Proline is a multifunctional chemical that performs a number of essential tasks, including controlling the osmotic potential, scavenging free

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radicals, preserving membrane integrity, and responding adaptively to abiotic stressors such as salt by increasing the proline concentration and accumulation in plant cells (Kavi Kishor and Sreenivasulu, 2014). The increase in proline levels due to ZnO nanopriming in this study might be because zinc acts as an important cofactor for the pyrroline-5 phosphate carboxylate synthase (P5CS) enzyme, which is essential for the proline biosynthesis pathway (Garg and Neha, 2019). Similar to the results of the present study, an increase in proline content was reported in maize by priming seeds with TiO2 NPs under salinity stress (Shah et al., 2021). Proline helps cells retain water and protects them from damage caused by salinity stress conditions. It also safeguards proteins and cell membranes while supporting enzyme function. By minimizing stress damage and keeping plant processes steady, proline helps plants grow better, develop stronger seedlings, and remain healthy, which leads to a higher vigour index.

Table 14. *Effects of different treatments on the proline content (µm/g FW) of tomato seedlings under control and salinity stress conditions*

Proline (µmoles/g FW)									
Conditions	**QA001**				**QA002**				**Grand mean**
	Treatments				**Treatments**				
	Control/ unprimed	**Hydro priming**	**NP (ZnO@750 ppm)**	**Mean**	**Control/ unprimed**	**Hydro priming**	**NP (ZnO@750 ppm)**	**Mean**	
Control (no-stress)	0.16	0.19	0.28	0.21	0.11	0.18	0.21	0.17	0.19
50 mM NaCl	0.38	0.39	0.43	0.40	0.24	0.30	0.35	0.30	0.35
100 mM NaCl	0.41	0.46	0.56	0.48	0.38	0.39	0.45	0.41	0.44
Mean	0.32	0.35	0.42	0.36	0.24	0.29	0.34	0.29	0.33
ANOVA									
CD ($p \leq 0.05$)									
Condition (C)	0.020		C×V	0.026					
Genotype (V)	0.014		C×T	0.032		C×V×T		0.046	
Treatment (T)	0.020		V×T	NS					

Estimation of CAT activity

Among the two genotypes, significantly greater (28.81 units/ml/g FW) catalase activity (*Table 15*) was detected in the QA001 genotype than in the QA002 genotype (28.17 units/ml/g FW). Among all the treatments, the ZnO nanopriming treatment resulted in significantly greater catalase activity (31.52 units/ml/g FW), followed by the hydropriming (28.43 units/ml/g FW) treatment, and the significantly lowest catalase activity was reported in the case of unprimed/control (25.52 units/ml/g FW) seeds. A significant increase in catalase activity (38.63%) was detected under the 100 mM NaCl salt stress condition, followed by an increase of 19.44% under the 50 mM NaCl salt stress condition compared with the catalase activity under the control (no stress) condition (23.87 units/ml/g FW). Considering all the interactions between conditions and genotypes (C×V), a significantly greater (33.39 units/ml/g FW) value of catalase activity was recorded in the QA002 genotype under the 100 mM NaCl salt stress condition, whereas it was significantly lower (22.30 units/ml/g FW) in the QA002 genotype under the control (no stress) condition. The highest catalase activity among all

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the treatments tested under all the different stress conditions was found in the ZnO nanopriming treatment under 100 mM NaCl salt stress conditions (34.99 units/ml/g FW) (*Table 15*).

Table 15. *Effects of different treatments on catalase activity (units/ml/g FW) in tomato seedlings under control and salinity stress conditions*

Catalase (units/ml/g FW)									
Conditions	**QA001**				**QA002**				**Grand mean**
	Treatments				**Treatments**				
	Control/ unprimed	**Hydro priming**	**NP (ZnO@750 ppm)**	**Mean**	**Control/ unprimed**	**Hydro priming**	**NP (ZnO@750 ppm)**	**Mean**	
Control (no-stress)	21.75	25.69	28.86	25.43	18.09	21.24	27.57	22.30	23.87
50 mM NaCl	23.22	29.56	31.84	28.21	27.55	28.02	30.84	28.80	28.51
100 mM NaCl	29.72	33.69	34.98	32.80	32.76	32.40	35.00	33.39	33.09
Mean	24.90	29.64	31.90	28.81	26.14	27.22	31.14	28.17	28.49

ANOVA					
CD ($p \leq 0.05$)					
Condition (C)	0.637	C×V	0.900		
Genotype (V)	0.519	C×T	1.101	C×V×T	1.557
Treatment (T)	0.637	V×T	0.900		

Notably, the unprimed (control) seeds under control (no stress) conditions presented significantly lower catalase activity (19.92 units/ml/g FW). Among the interactions between genotype and treatment (V×T), significantly greater (31.90 units/ml/g FW) catalase activity was reported in the ZnO nanopriming treatment for genotype QA001, whereas significantly lower (24.90 units/ml/g FW) catalase activity was found in unprimed/control seeds (seedlings) for genotype QA001. The interactions among stress conditions, genotypes and treatments (C×V×T) also revealed significant results. When plants face stress, they produce reactive oxygen species (ROS), which can harm their cells. Antioxidants such as superoxide dismutase (SOD) and catalase (CAT) help remove these harmful molecules, keeping cells safe. This decreases stress damage, keeps plant processes steady, and supports healthy growth. As a result, plants with higher antioxidant activity grow stronger, have more biomass, and remain healthier, leading to a higher vigour index.

Estimation of SOD activity

The results revealed that, between the two genotypes used in this study, significantly higher (14.41 units/min/g FW) SOD activity (*Table 16*) was reported in the QA001 genotype than in the QA002 genotype (13.10 units/min/g FW). Among all the treatments, the ZnO nanopriming treatment resulted in significantly greater (15.62 units/min/g FW) SOD activity followed by SOD activity in the hydropriming treatment (13.53 units/min/g FW), and the significantly lowest (12.13 units/min/g FW) SOD activity was reported in the case of unprimed/control seeds (*Table 16*). The highest SOD activity (15.49 units/min/g FW) was recorded under the 100 mM NaCl salt

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stress condition, followed by that under the 50 mM NaCl salt stress condition (14.03 units/min/g FW), which was 31.83% and 19.83% greater than the SOD activity under the control (no stress) condition (11.75 units/min/g FW), respectively. Considering the interactions of all the treatments tested under all the different stress conditions (C×T), the significantly highest SOD activity (16.67 units/min/g FW) was recorded in the ZnO nanopriming treatment under 100 mM NaCl salt stress conditions. Notably, the unprimed (control) seeds under no stress (control) conditions presented significantly lower (9.94 units/min/g FW) SOD activity. Plants use both enzymatic and nonenzymatic antioxidant mechanisms to protect themselves from the harmful effects of reactive oxygen species (ROS). The key enzymes in this defense system include SOD, which converts superoxide into hydrogen peroxide, and catalase (CAT), which breaks down hydrogen peroxide (Foyer et al., 1994). Our study revealed that salt stress increased both CAT activity and SOD activity in tomato seedlings, suggesting that salt stress activates the antioxidant system. Importantly, ZnO nanopriming further increased the activities of these enzymes under both normal and salt stress conditions. The increase in SOD activity from ZnO nanopriming might be because, among the three isoforms of the SOD enzyme present in plant cells, the Zn/SOD isoform is one of them; thus, Zn acts as a cofactor in the superoxide dismutase (SOD) enzyme. This enhanced ROS scavenging capacity provided by nanopriming likely weakened the negative impacts of salt stress (*Table 16*).

Table 16. *Effects of different treatments on superoxide dismutase (SOD) activity (units/ml/g FW) in tomato seedlings under control and salinity stress conditions*

SOD (units/min/g FW)									
Conditions	**QA001**				**QA002**				**Grand mean**
	Treatments				**Treatments**				
	Control/ unprimed	**Hydro priming**	**NP (ZnO@750 ppm)**	**Mean**	**Control/ unprimed**	**Hydro priming**	**NP (ZnO@750 ppm)**	**Mean**	
Control (no-stress)	10.50	11.65	14.52	12.22	9.37	10.26	14.21	11.28	11.75
50 mM NaCl	13.39	14.85	16.51	14.92	12.29	11.99	15.13	13.14	14.03
100 mM NaCl	13.91	17.10	17.28	16.10	13.32	15.28	16.05	14.88	15.49
Mean	12.60	14.54	16.10	14.41	11.66	12.51	15.13	13.10	13.76
ANOVA									
CD ($p \leq 0.05$)									
Condition (C)	0.582		C×V	NS					
Genotype (V)	0.473		C×T	1.007		C×V×T		NS	
Treatment (T)	0.582		V×T	NS					

PCA biplot analysis

Principal component analysis (PCA) was used to evaluate the effects of salt stress and the effectiveness of different priming methods (untreated, hydroprimed, and nanoprimed) on thirteen measured variables in tomato plants grown under control, 50 mM NaCl, and 100 mM NaCl salt stress conditions. The PCA biplots revealed data points distributed across all four quadrants (*Fig. 3*). Both the first (Dim 1) and second (Dim 2) principal components had eigenvalues greater than one, with cumulative

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variability increasing with increasing salt concentration (*Table 17*). These first two components explained 95.1%, 92.2%, and 94.5% of the total variability observed in the control (*Fig. 3A*), 50 mM NaCl (*Fig. 3B*), and 100 mM NaCl (*Fig. 3C*) treatments, respectively (*Fig. 3* and *Table 17*). Under the control condition, most of the variables belonged to the same quadrant and were thus positively correlated. In the control condition biplot, only the mean germination time (MGT) and MDA had opposite trends from those of the other variables and seemed to be negatively correlated. A similar correlation was observed in the 50 mM NaCl and 100 mM NaCl biplots; however, under 100 mM NaCl stress conditions, both the MDA and MGT contents were in the fourth quadrant. The principal component analysis (PCA) results, which were based on an analysis of thirteen variables under different conditions (control, 50 mM NaCl and 100 mM NaCl), predicted that nanoprimed (@750 ppm) seeds of genotype QA001 performed better under all three conditions (*Table 17*).

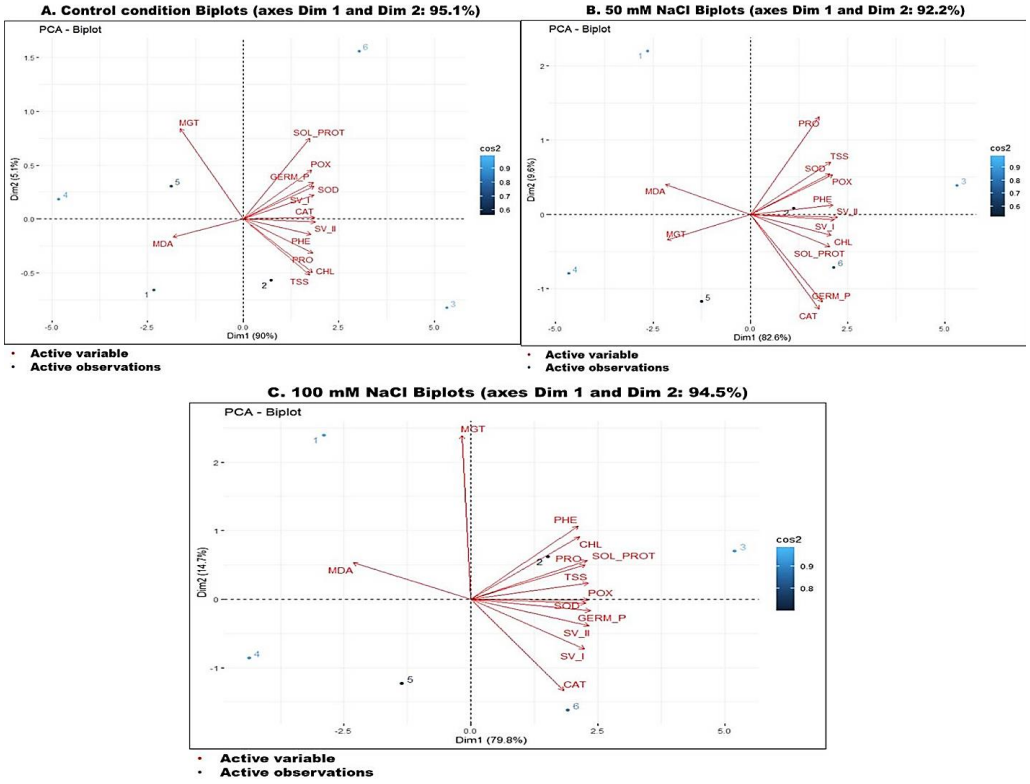

Figure 3. *Principal component analysis (PCA) biplots; (a) control biplot; (b) 50 mM NaCl stress biplot; (c) 100 mM NaCl stress biplot*

Table 17. *Eigenvalues, variability (%), and cumulative (%) of the Dim 1 and Dim 2 axes of the PCA*

	Control		**50 mM NaCl**		**100 mM NaCl**	
	Dim 1	**Dim 2**	**Dim 1**	**Dim 2**	**Dim 1**	**Dim 2**
Eigen value	10.79	1.10	10.74	1.25	10.37	1.91
Variability (%)	83.05	8.52	82.64	9.63	79.83	14.71
Cumulative %	83.05	95.1	82.64	92.27	79.83	94.55

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Conclusion

This study highlights the potential of ZnO nanoparticle (NP) priming as an effective strategy to increase the resilience of two tomato genotypes (QA001 and QA002) to salinity stress. The QA001 genotype performed significantly better than the QA002 genotype under both the control and salinity stress conditions. In this study, nanotechnology was employed in the priming of tomato seeds to determine the efficacy of nanoprimed seeds compared with that of hydropprimed and unprimed controls under different levels of NaCl-mediated salinity stress. Among the various concentrations and soaking durations applied, the performance of tomato seeds primed with ZnO NPs at 750 ppm for 6 h was significantly better than that of those primed with other concentrations. Overall, nanopriming with ZnO NPs at 750 ppm (6 h) significantly increased chlorophyll production, antioxidative mechanisms and osmotic adjustment, which in turn decreased the accumulation of ROS and peroxidation of lipids in the cell membrane; thus, these effects could be used to alleviate the detrimental effects of salinity stress in tomato. Thus, ZnO nanopriming treatment can be used to mitigate the detrimental effects of salt stress on tomato plants through a defensive physicochemical pathway. These findings suggest that ZnO nanopriming could serve as a valuable method for improving seed quality and crop establishment in saline environments. For practical agricultural applications, optimized ZnO nanoparticle (NP) priming treatment (750 ppm for 6 hours) could be integrated into seed enhancement strategies to improve tomato crop establishment, particularly in saline-prone areas. Future research should explore the long-term effects of ZnO nanopriming on plant growth, yield, and soil health. Additionally, investigating the molecular mechanisms underlying ZnO NP-induced stress tolerance and optimizing application strategies for large-scale field conditions would further validate its agricultural significance.

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Data availability statements. The datasets generated during and/or analysed during the current study will be available from the corresponding author upon request.

Conflicts of interest. The authors declare that they have no competing financial interests.

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