

## Impact of Zinc Oxide Nanoparticles on Seed Germination Characteristics in Rice (*Oryza sativa* L.) Under Salinity Stress

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### ABSTRACT

An exciting new window of opportunity has opened up for environmentally responsible farming with the advent of the nanotechnology era: the role of nanoparticles (NPs) to mitigate abiotic stresses. NPs have unique physiochemical characteristics that make them an attractive study subject. Rice growth and yield are severely inhibited by salinity, a major detrimental abiotic factor. However, the impact of NPs on rice seeds germination characteristics and physio-biochemical phenomena under salt stress conditions remains poorly understood. Accordingly, we intended to look at how zinc oxide nanoparticles (ZnO-NPs) affected germination processes and the early seedling stage while the rice plants (Kargi and CSR 30 rice genotypes) were put under salinity stress. Different germination characteristics parameters were considered, e.g., germination percentage (GP) relative seed germination rate (RGR), and seed vigour index (SVI) determined after eight days of treatment with ZnO-NPs at a concentration of 50 mg/L on rice seed. After passing the germination test, the seeds were placed in Hoagland hydroponic solution and given another week of ZnO-NPs treatment to evaluate the seedling growth and phyto-biochemical characteristics, such as shoot height and root length, inhibition percentage of shoot height and root length, chlorophyll and carotenoid stability index, chlorophyll and carotenoid inhibition percentage, malondialdehyde (MAD) content and antioxidant enzymatic activities (SOD, APX). This investigation demonstrated that 50 mg/L ZnO-NPs have the potential to alleviate the effect of salt stress on rice genotypes during the germination stage.

**Keywords:** Nanoparticle, salinity, Kargi, CSR 30, germination, rice, ZnO-NPs,

### INTRODUCTION

Soil salinity reduced crop production in several countries worldwide including USA, Australia, India, China, Egypt, Iran, Iraq, Thailand and Pakistan, where more than 3.85 million ha of land is affected by salinity and that is half of the arable land (Kumar and Sharma, 2020). According to a prediction, by 2050, 16.2 million

ha of agricultural lands will be affected by salinity, which can cause serious problems related to agricultural productivity and endanger the availability of food (Van Zelm et al., 2020). In the southeast Asia, approximate 5.8 million ha of agricultural land is recognized as territory damaged by salt and 6.727 million sodic-saline lands in India (Kumar and Sharma, 2020). More than fifty percent of the global population consume

rice every day, making it the most edible grain in the world (Liu et al., 2019). 90% of the rice consumed worldwide is grown in Asia (Khush, 2005; Tian et al., 2014).

Seed is a crucial input in agriculture because it directly affects crop yield and productivity (Sarkhosh et al., 2022). In all stages of plant development, salt stress negatively impacted growth and yield (García-López et al., 2018). Reduced crop yield is a result of poor seed germination, slowed plant growth, and increased duration of the dormant period (Itrotwar et al., 2020a). Salt tolerance during germination is essential for the stand establishment of plants and for growth in saline soils (Shu et al., 2017). Seeds of most species germinate best in D/W, and they are very susceptible to saline throughout the process of germination and seedlings phases of growth, as shown by numerous studies of seed germination under salinity stress (Shu et al., 2017). Most halophyte seeds germinate best when exposed to low NaCl levels and extremely susceptible to high salt levels throughout the process of germination and initial growth phases (Soltabayeva et al., 2021).

Rice's growth and development are influenced by salinity in a variety of ways, including the germination of seeds, seedling growth, length of roots, shoot height, dry fresh weight of the root and shoot, the number of cultivars per plant, plant height, flag leaf length, blooming stage, spikelet number, panicle length, etc (Ashraf and Akram, 2009; Gupta and Huang, 2014; Horie et al., 2012; Läuchli and Grattan, 2007; Moradi and Ismail, 2007; Zeng and Shannon, 2000). Osmotic and ionic stressors caused by salinity stress combine with oxidative damage to impede plant development. Oxidative stress is a secondary kind of stress brought on by the loss of oxygen-carrying electrons during the processes of photosynthesis, respiration, and transpiration. It further leads to the generation of reactive oxygen species (ROS) such as singlet oxygen ( $^1O_2$ ), hydroxyl radical ( $OH^\bullet$ ), superoxide radical ( $O_2^{\bullet-}$ ), hydrogen peroxide ( $H_2O_2$ ) produced in different subcellular components, e.g., cell walls, peroxisomes, chloroplasts, endoplasmic reticulum, mitochondria, plasma membranes, and apoplasts region (Das and Roychoudhury, 2014; Massange-Sánchez et al., 2021; Mittler, 2002; Sharma et al., 2012).

Salinity stress also enhances the production of ROS in these subcellular compartments that inhibits the biological process occurring inside them e.g. photosynthesis, respiration, protein

synthesis, lipid biosynthesis, membrane transport system, and nucleic acids biosynthesis that ultimately cause program cell death of plant cell (Gill and Tuteja, 2011, 2010). The detoxification process requires some detoxifying machinery that is antioxidant enzymes ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), and glutathione reductase (GR). Decreasing the activity of antioxidative enzymes increases the risk of oxidative damage due to increasing MDA (Malondialdehyde) content, which is a product of lipid peroxidation. Thus, increasing the activity of the antioxidative enzyme and decreasing the MDA level show tolerance activity of plants against salinity (Adhikary et al., 2022; Demiral and Türkan, 2005). Zinc is an important micronutrient for all living things. Zn acts as a co-factor for many plant enzymes that help required them to function efficiently (Tripathi et al., 2015). It plays a vital role in the biosynthesis of tryptophan and auxins and also essential metal element for enzymes like aldose, pectinase, cysteine desulphhydrase, histidine deaminase, carbonic anhydrase, dehydropeptidase, glycyl-glycine dipeptidase (Natasha et al., 2022). Zinc in the soil is easily absorbed by plants when it is in the water-soluble, exchangeable, and complex form (Wang et al., 2020). The availability of soil Zn is impacted by salinity stress-induced elevation of soil pH (Alenezi et al., 2022). A zinc deficiency in plants slows photosynthesis rate and decreases nitrogen metabolism, resulting in diminished flowering, fruit development, and crop yield (Alloway, 2008). Nanotechnology is a developing scientific discipline that helps mitigate abiotic stress (Zafar et al., 2021). The use of NPs during salt stress condition could protect the plants (Elhaj Baddar and Unrine, 2018; Zafar et al., 2021), as it may be absorbed rapidly by plants through plant organs e.g., root, shoot, epidermis, cuticle, hydathodes, stomata, root tips, root junctions, stigma, wounds, or other pore areas of the plant (Dietz and Herth, 2011; Lai et al., 2020). In order to combat zinc deficiency, zinc oxide is potential NPs that incorporated into agricultural fertilizers or apply through priming of seeds, poured over roots, or foliar spraying to plants (Rossi et al., 2019). Plants can easily absorb and store ZnO-NPs due to their high solubility in water. It has been demonstrated that these particles can be utilized to create zinc nanofertilizers that have superior solubility and distribution characteristics, which is a significant advancement in the agricultural sector (Awasthi

et al., 2017). Recent various studies on Arabidopsis (Syu et al., 2014), *Brassica pekinensis* (Xiang et al., 2015), *Sorghum bicolor* (Rakgotho et al., 2022), maize (Tondey et al., 2022), barley (Ali et al., 2022), rice (Prakash et al., 2022), tomato (Faizan et al., 2021) demonstrated that dose-dependent application of ZnO-NPs can enhance germination under stress conditions. Similarly, we hypothesized that ZnO-NPs would enhance germination characteristics such as germination percentage (GP), relative seed germination rate (RGR), and seed vigour index (SVI), as well as phyto-biochemical characteristics such as shoot height and root length, inhibition percentage of shoot height and root length, chlorophyll and carotenoid stability index, chlorophyll content, and carotenoid content. Although the effect of NPs on molecular and biochemical processes, during germination, is poorly understood. This study, therefore aims to determine the impact ZnO-NPs have on various seed characters and phyto-biochemical changes that occur in *Oryza sativa* L. seeds when the plant is stressed by salt, during absorption and the early seedlings stages.

## MATERIALS AND METHODS

### Characterization of nanoparticle

ZnO-NPs (particle size < 50 nm) were purchased from Sisco Research Laboratories (SRL) Pvt. Ltd, Maharashtra, India, and characterized by UV-visible spectrophotometer (SYSTRON-ICS AU 2701, Gujarat India) from the range between 100–900 nm wavelength and the absorption of ZnO-NPs range between 350–385 nm (Amendola and Meneghetti, 2009; Pérez-Hernández et al., 2012). The morphology surface properties and size level-based characterization were analyzed by transmission electron microscopy (JEOL, JEM1011, USA).

### Preparation of ZnO-NPs solution

The Rajput et al. approach was used to create a 50 mg/L solution containing ZnO-NPs (Rajput et al., 2021) with the help of a sonicator.

The treatments of NaCl and ZnO-NPs are mentioned below:

- T0: Control
- T1: 60 mM NaCl
- T2: 80 mM NaCl

- T3: 100 mM NaCl
- T4: ZnO NPs (50 mg/L) + 60 mM NaCl
- T5: ZnO NPs (50 mg/L) + 80 mM NaCl
- T6: ZnO NPs (50 mg/L) + 100 mM NaCl

### Seed germination assay evaluation

The seeds were carefully washed a total of three times in D/W to sterilise the surface, twice in 70% ethanol for 2 mins, and once in 1.5% sodium hypochlorite solution for 5 mins. Using D/W for four rounds of washing, the surface disinfection procedure was completed. ZnO-NPs solutions with a 50 mg/L concentration was used to study the impact of these NPs. These surface-disinfected 25 seedlings were added to each petri dish, which held 10 mL of NPs formulations, in the initial phase of the experiment. The investigation was set up in a set of three separate replications for each treatment, with each Petri dish containing 25 seeds. In first phase of this experiment germination rates were evaluated at intervals of 24 hours throughout the experiment. The seeds were permitted to sprout for eight days at a temperature of 25 °C in a dark environment. At this stage, following seed germination assay parameters were evaluated:

#### Germination%

In germination, percentage (Germination %) observed the germination rate of the average number of seeds of Kargi and CSR 30 (25 of each genotypes) germinated over the 8-days periods under saline and ZnO-NPs treatments (Singh et al., 2016).

$$GP = \left( \frac{\text{Number of normally germinated seeds}}{\text{Total number of seeds sown}} \right) \times 100 \quad (1)$$

#### Relative seed germination Rate (RGR)

RGR is described as a relative seed germination rate of control plant seeds and treated seeds. The germination of 25 seeds of CSR 30 and Kargi rice genotypes in control and ZnO-NPs treatment under the saline condition with the following formula were calculated:

$$RGR = \left( \frac{SC}{SS} \right) \times 100 \quad (2)$$

where: SC is the number of seeds germinated in control and SS is the number of seeds germinated in treatment (Itrotwar et al., 2020; Mahender et al., 2015).

### Seed vigour index (SVI)

SVI (seedling vigour index) is the sum of a seed's properties that define its degree of activity and efficiency throughout germination and the development of seedlings. This approach was used to determine the seed germination rates and seedling lengths (root and shoot lengths) of the rice genotypes CSR 30 and Kargi. The SVI is calculated as described by Mahender et al. (2015).

$$\text{SVI} = \text{mean germination percentage} \times \text{mean seedling length} \quad (3)$$

### Evaluating Seedlings Growth and Phyto-Biochemical parameters

Rice seeds were shifted from petri plates to half-strength modified hydroponic Hoagland's solution (without Zn micronutrient) with and without NaCl and ZnO-NPs in the second phase of our experiment to assess the effects of ZnO-NPs on seeds growth parameters and phyto-biochemical evaluation. The rice seeds were subsequently permitted to germinate for seven days in growth chamber at 28 °C and 14 h/10 h photoperiod.

#### Seedlings growth parameters

##### Shoot height and root length

The CSR 30 and Kargi seedlings' root and shoot lengths were calculated on a meter scale and expressed in centimeters.

##### Inhibition percentage of shoot height and root length

According to Ali et al. (2014), the inhibitory impact of shoot height and root length of the CSR 30 and Kargi rice genotypes was assessed with some modifications.

$$\begin{aligned} \text{shoot height inhibition percentage} &= \\ &= 100 - (\text{Response of treatments} \times \\ &\quad \times 100 / \text{Response of control}) \end{aligned} \quad (4)$$

$$\begin{aligned} \text{root length inhibition percentage} &= \\ &= 100 - (\text{Response of treatments} \times \\ &\quad \times 100 / \text{Response of control}) \end{aligned} \quad (5)$$

#### Phyto-Biochemical parameters

##### Carotenoid and Chlorophyll stability index

The assessment of the chlorophyll and carotenoid stability index in seedlings leaf tissue depends on pigmentation variations caused by

heating and evaluated using a spectrophotometer (Takagi and Yamada, 2013). Fresh leaf samples were taken from seedlings that were 8 days old and put in two clean glass tubes with 10 ml of D/W. These two glass tubes were then heated on a water bath for approximately 30 minutes at  $56 \pm 1$  °C before the water was discarded. Both tubes were placed in an incubator in the dark for a whole night after being added 10 ml of acetone (80%). Finally, using acetone (80%) as a blank, absorbance (A) was measured at 645, 663, and 470 nm after mixing 1 ml of sample with 2 ml of acetone (80%) in a 1:2 ratio. The following formula is used to calculate the total chlorophyll, carotenoid, and chlorophyll stability index:

$$\begin{aligned} \text{Total chlorophyll content} &= 20.2 \times \\ &\quad \times (A_{645}) + 8.02 \times (A_{663}) \times \\ &\quad \times V / (1000 \times W \times a) \text{ (mg/g fr. Wt.)} \end{aligned}$$

$$\begin{aligned} \text{Carotenoid (mg/g):} \\ 46.95 \times (A_{470} - 0.268 \times \text{Chl a} + \text{b}) \end{aligned}$$

where: V is the final volume (ml);

W is the fresh weight of the sample (g);

A is the absorbance for chlorophyll-a (chl-a) at 663 nm, chlorophyll-b (chl-b) at 645 nm, and carotenoid at 470 nm is the path length of light (3 cm).

$$\text{Chlorophyll stability index} = \text{Cs} / \text{Cc} \times 100 \quad (6)$$

where: Cs is the Chlorophyll content of plants under stress (mg/g) and Cc is the Chlorophyll content of the control plant (mg/g).

$$\text{Carotenoid stability index} = \text{Cs} / \text{Cc} \times 100 \quad (7)$$

where: Cs is the Carotenoid content of the stressed plant (mg/g) and Cc is the Carotenoid content of control plant mg/g).

#### Chlorophyll and Carotenoid Inhibition Percentage

The inhibition effect on chlorophyll and carotenoid of Kargi and CSR 30 rice genotypes was calculated according to Ali and Ashraf, (2011) with modification.

$$\begin{aligned} \text{Chlorophyll inhibition percentage} &= \\ &= 100 - (\text{Response of treatments} \times \\ &\quad \times 100 / \text{Response of control}) \end{aligned} \quad (8)$$

$$\begin{aligned} \text{Carotenoid inhibition percentage} &= \\ &= 100 - (\text{Response of treatments} \times \\ &\quad \times 100 / \text{Response of control}) \end{aligned} \quad (9)$$

## MAD

The Assaha et al. (2017) approach was used to calculate the MDA level. Using a spectrophotometer, the absorbance was determined at 532 and 600 nm. Fresh leaves from the Kargi and CSR 30 plants totaled 0.1 g each, and they were blended in an extraction solution (10 mM HEPES, pH 7.0, 15% tricarboxylic acid, 0.375% thiobarbituric acid, 0.25 N HCl, 0.04% butylated hydroxyl toluene, and 2% ethanol). By measuring the supernatant's absorbance between 532 and 600 nm and using the extinction coefficient ( $155 \text{ mM}^{-1} \text{ cm}^{-1}$ ), the MDA concentration was identified.

## SOD

Using Takagi and Yamada (2013) methodology, the SOD concentration was calculated. A spectrophotometer was used to measure the absorbance at 590 nm. Applying 500 mL of 10 mM K-P buffer (pH 7.8) and 1 mL of crude enzyme extracts through a 12-hour dialysis process, changing the buffer per three hours. The SOD activity of the enzyme was then determined at 560 nm after the enzyme had been dialyzed to remove extra ions.

## APX

Takagi and Yamada (2013) analysis was used to evaluate the APX concentration. A spectrophotometer was used to quantify the absorbance at 290 nm. The activity of APX was assessed in 1 mL of an experiment solution consisting 100 mM K-P Buffer (pH 7.8), 0.5 mM L-ascorbic acid, and

2% (v/v) crude enzyme extracts. The quantity of oxidised L-ascorbic acid per minute was then measured at a specified 290 nm absorbance.

## RESULTS

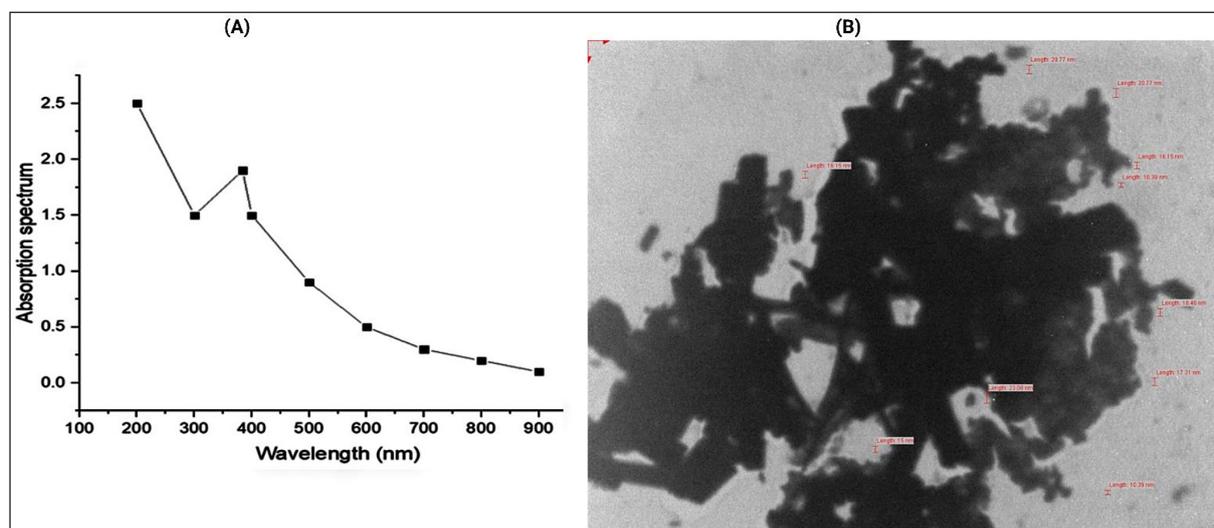
### Characterization of ZnO-NPs

ZnO-NPs were first confirmed at the base detection level using UV-Vis spectroscopy. Figure 1a depicts the highest absorption of NPs at a wavelength that ranges between 350 and 385 nm, and similarly ZnO-NPs also absorbed the wavelength between 350–385 nm. The TEM image (Figure B) (Supplementary file S 1 TEM characterization image) revealed the morphological surface properties of ZnO-NPs that were spherical with regular diameters < 50 nm.

### Seed germination assay evaluation

#### Germination percentage (GP)

Kargi and CSR 30 GP were tested under salt stress circumstances and with ZnO-NPs. The GP of Kargi (30.82–48%) and CSR 30 (24.18–44.20%) under NaCl treatments is reduced as compared with their controls (Table 1). Combined application of ZnO-NPs and NaCl treatments suggests a reduced impact on GP of Kargi (21.70–30.88%) and CSR 30 (15.68–30.88%) respectively their control (Table 1). Higher GP was shown in untreated Kargi rice plants compared to CSR 30 rice genotypes.



**Figure 1.** Characterization of ZnO-NPs. Fig. 1a show the UV-vis spectroscopy analysis and Fig. 1b characterization of ZnO-NPs

**Table 1.** Evaluation of GP, RGR, and SVI of Kargi and CSR 30 seeds after 8 days of being treated with ZnO-NPs and NaCl under control and saline conditions. Values are three replicated means. The least significant value (LSD) for each treatment at  $p$  0.01 is displayed in error bars

Genotypes	Treatments	GP	RGR	SVI
Kargi	T0	85 ± 3.15	1.003 ± 0.015	803.73 ± 11.60
	T1	60 ± 0.49	0.680 ± 0.023	509.50 ± 18.24
	T2	52 ± 1.26	0.590 ± 0.023	402.23 ± 14.40
	T3	44 ± 1.22	0.500 ± 0.006	304.27 ± 2.46
	T4	68 ± 0.98	0.680 ± 0.017	611.47 ± 14.83
	T5	60 ± 2.15	0.773 ± 0.020	513.57 ± 14.23
	T6	56 ± 0.81	0.637 ± 0.015	447.47 ± 10.85
CSR 30	T0	84 ± 2.22	1.000 ± 0.006	748.87 ± 26.81
	T1	62 ± 0.90	0.700 ± 0.017	508.70 ± 4.11
	T2	54 ± 1.93	0.610 ± 0.017	400.17 ± 9.70
	T3	46 ± 1.65	0.520 ± 0.006	308.20 ± 8.54
	T4	70 ± 0.57	0.790 ± 0.029	597.03 ± 8.62
	T5	63 ± 1.53	0.700 ± 0.023	523.33 ± 18.73
	T6	58 ± 0.47	0.660 ± 0.012	447.07 ± 12.39

#### Relative seed germination rate (RGR)

Salt stress reduces the germination rate of seeds. In saline medium, RGR was reduced 1.70–2.01 fold in the Kargi and 1.42–1.92 fold in the CSR 30 rice genotypes (T1, T2, T3). Rates of RGR in Kargi (1.30–1.57 folds) and CSR 30 (1.27–1.52 folds) were less affected by ZnO-NPs treatment (T4, T5, T6). The application of ZnO-NPs at dosage of 50 mg/L has a protective effect, as evidenced by the reduced effect of salinity stress on RGR parameters in the Kargi and CSR 30 genotypes.

#### Seed vigour index (SVI)

Compared to the control group, the SVI of Kargi (36.61–62.14%) and CSR 30 (32.07–58.84%) showed significantly more variation after NaCl treatment (Table 1). However, after ZnO-NPs (50 mg/L) treatments were applied in Kargi (23.92–44.33%) CSR 30 (20.28–40.30%), the level of SVI decreased significantly compared to stressed plants (Table 1)..

### Evaluating seedlings growth and phyto-biochemical parameters

#### Seedlings growth parameters

##### Length of the root and the shoot

The lengths of both the shoot and the root were decreased when exposed to NaCl stress. The shoot reduction show under saline conditions

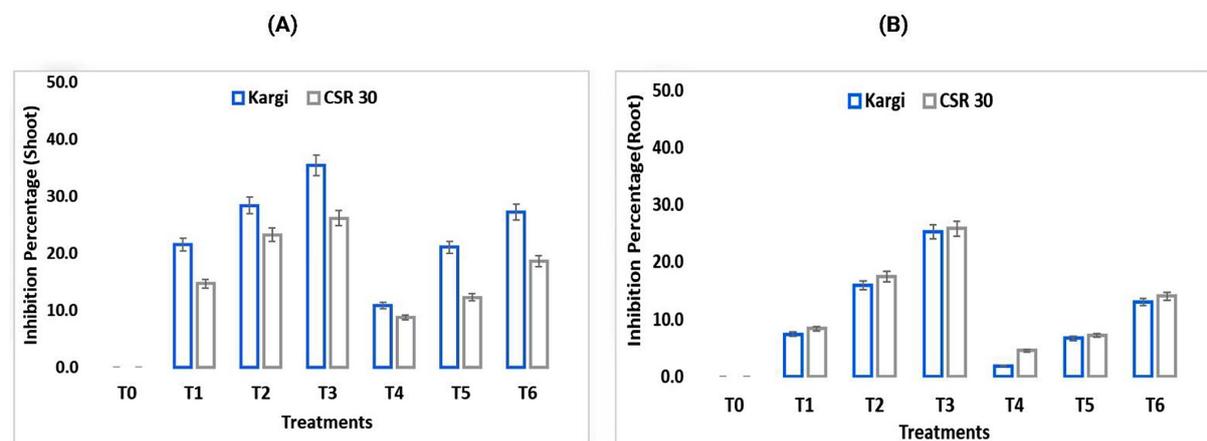
is 21.59–35.45% for the Kargi rice genotype and 14.73–26.22% for the CSR 30 genotype (Table 2). Kargi (10.89–27.25%) and CSR 30 (8.82–18.71%) shoot growth can be stimulated by exposure to ZnO-NPs at dosage of 50 mg/L. Kargi (7.33–25.28%) and CSR 30 (8.18–25.86%) root growth were inhibited during saline circumstances, but ZnO-NPs administration resulted in a lower decrease in root growth in Kargi (6.86–12.97%) and CSR 30 (5.38–14.08%) compared to control (Table 2). The findings of this study showed that a ZnO-NPs dosage of 50 mg/L was sufficient to promote the development of rice shoots and roots from the Kargi and CSR 30 genetic variants.

#### Inhibition Percentage of shoot and root

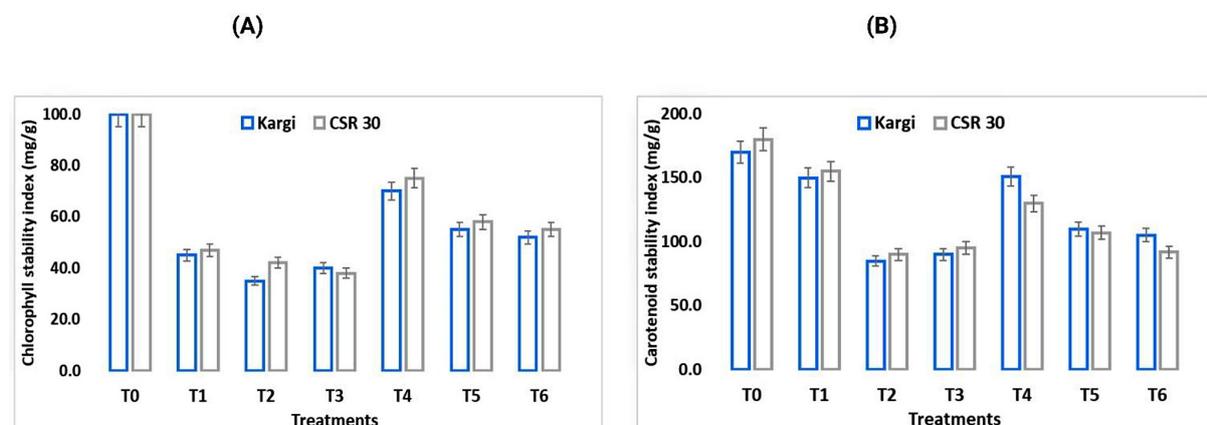
Root and shoot development were both hindered by NaCl salt stress. NaCl treatments reduce shoot growth in the Kargi (21.59–35.45%) and CSR 30 (14.71–26.20%) rice genotypes (Figure 2 a). Kargi (10.9–27.27%) and CSR 30 (8.82–18.69%) benefited from ZnO-NPs (50 mg/L), as their shoot growth was stimulated (Figure 2 a). Root growth inhibition was greater in Kargi (7.33–25.28%) and CSR 30 (8.37–25.86%) under saline conditions compared to controls, but this effect was mitigated by the incorporation of ZnO-NPs at a concentration of 50 mg/L in Kargi (5.86–13.01%) and CSR 30 (4.56–14.07%), respectively (Figure 2 b).

**Table 2.** After 8 days of treatment, the seedlings’ RL (Root Length) and SL (Shoot Length) of Kargi and CSR 30 variables have been affected by ZnO-NPs, NaCl in the control, and saline environment. Values are three replicated means. The least significant value (LSD) for each treatment at p 0.01 is displayed in error bars

Genotypes	Treatments	RL (cm)	SL (cm)
Kargi	T0	8.97 ± 0.32	14.67 ± 0.12
	T1	8.30 ± 0.07	11.50 ± 0.28
	T2	7.53 ± 0.18	10.50 ± 0.29
	T3	6.70 ± 0.19	9.47 ± 0.14
	T4	8.80 ± 0.13	13.07 ± 0.47
	T5	8.37 ± 0.30	11.57 ± 0.10
	T6	7.80 ± 0.11	10.67 ± 0.38
CSR 30	T0	8.77 ± 0.21	12.47 ± 0.35
	T1	8.03 ± 0.22	10.63 ± 0.15
	T2	7.23 ± 0.10	9.57 ± 0.34
	T3	6.50 ± 0.23	9.20 ± 0.33
	T4	8.37 ± 0.30	11.37 ± 0.09
	T5	8.13 ± 0.07	10.93 ± 0.27
	T6	7.53 ± 0.27	10.14 ± 0.08



**Figure 2.** After 8 days of treatment, the effects of ZnO-NPs, NaCl in the control and saline environment on the seedlings inhibitory % parameters of (a) the shoots and (b) the roots of Kargi and CSR 30. Values are three replicated means. The least significant value (LSD) for each treatment at p 0.01 is displayed in error bars.3.4.2 Phyto-Biochemical parameters



**Figure 3.** After 8 days of treatment, the effects of ZnO-NPs, NaCl in the control and saline environments on the (a) carotenoid stability index and (b) chlorophyll stability index of Kargi and CSR 30. Values are three replicated means. The least significant value (LSD) for each treatment at p 0.01 is displayed in error bars

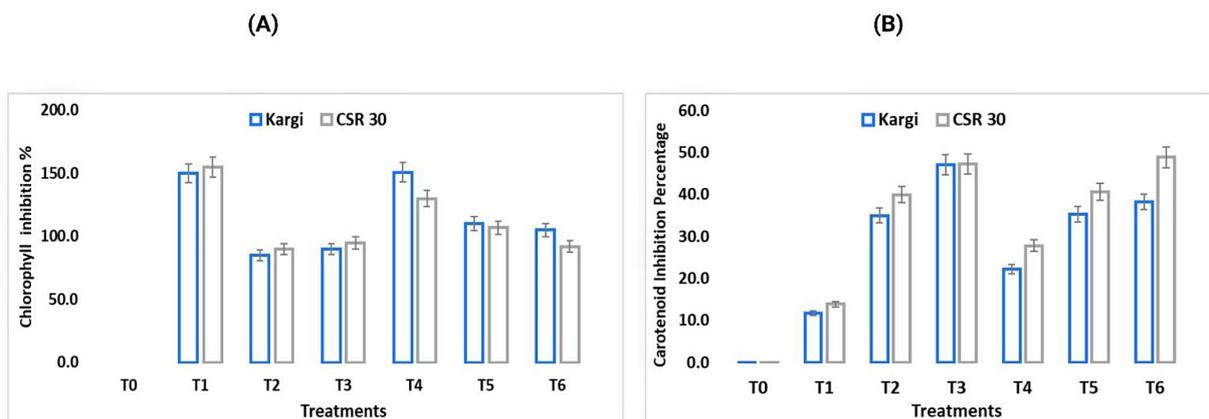
### Chlorophyll and carotenoid stability index

The stability index of carotenoids and chlorophyll was measured and compared with the standard value to analyze the effects of ZnO-NPs on photosynthetic pigments in the presence of NaCl stress. Chlorophyll stability index values were found to drop by 55–60% in Kargi and 53–62% in CSR 30 after NaCl treatments (Figure 3 a, b). Similar decreases were seen in the carotenoid stability index from Kargi (11.76%–47.06%) and CSR 30 (13.89–47.22%) (Figure 3 a, b). Comparatively, the increased chlorophyll stability index in Kargi (30–48%) and CSR 30 (25–45%) after introducing 50 mg/L ZnO-NPs demonstrates its protective role during salinity stress. The carotenoid stability index significantly increased from 11.18 to 38.24% in the Kargi, and from 27.78 to 48.89% in the CSR 30 rice genotype, both of which showed a protective effect of 50 mg/L of ZnO-NPs (Figure 3a, b).

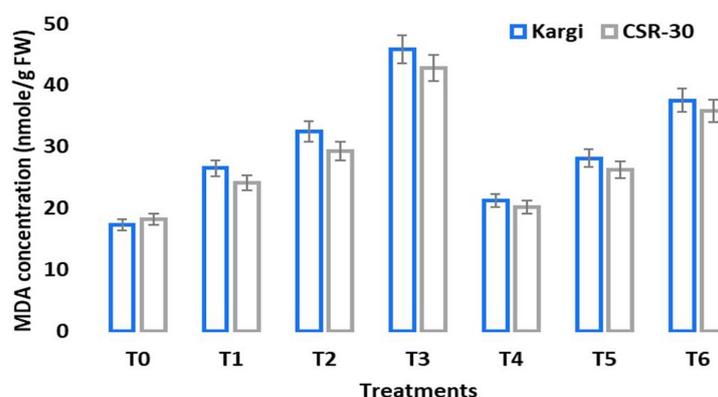
A high stability index for chlorophyll and carotenoids predicts a high tolerance for salt after the use of 50 mg/L of ZnO-NPs.

### Chlorophyll and carotenoid inhibition percentage

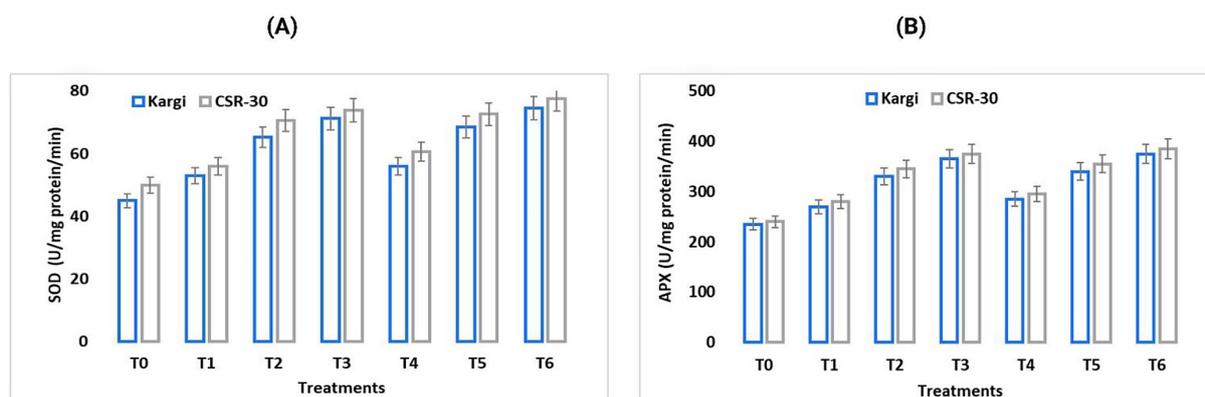
Inhibition of photosynthetic pigments by salinity stress can be measured through analyses of chlorophyll and carotenoid percentages. Kargi (55–65%) and CSR 30 (53–62%) rice genotypes show greater chlorophyll and carotenoid inhibition in a salty environment. Inhibition by carotenoids was higher in Kargi (11.76–47.06%) and CSR 30 (13.89–47.22%). (Figure 4 a, b). The rice genotypes Kargi (30–48%) and CSR 30 (25–45%) treated with ZnO-NPs show less inhibition of chlorophyll percentage under saline conditions. ZnO-NPs increase carotenoids in Kargi (11.18–38.24%) and CSR 30 (27.78–48.89%) rice compared to untreated control plants (Figure 4a, b).



**Figure 4.** The effects of ZnO-NPs, NaCl under control and a saline environment on the (a) chlorophyll and (b) carotenoid inhibition percentage of Kargi and CSR 30, seedlings after 8 days of treatment. Values are three replicated means. The least significant value (LSD) for each treatment at p 0.01 is displayed in error bars



**Figure 5.** The effects of ZnO-NPs, NaCl in the control, and salty conditions on the MDA levels of Kargi and CSR 30, seedlings after 8 days of treatment. Values are three replicated means. The least significant value (LSD) for each treatment at p 0.01 is displayed in error bars



**Figure 6.** After 8 days of treatments, the effects of ZnO-NPs, NaCl in the control and saline conditions on the expression of antioxidative enzymes (a) SOD and (b) APX level of Kargi and CSR 30, seeds. Values are three replicated means. The least significant value (LSD) for each treatment at p 0.01 is displayed in error bars

### Malondialdehyde (MDA) content

The amount of MDA produced by rice plants under saline conditions increased dramatically (Figure 5). Under the saline condition, higher peroxidation was seen in both rice genotypes, leading to higher accumulation of MDA levels in Kargi (1.53–2.65 folds) and CSR 30 (1.33–2.35 folds) (Figure 5). However, 50 mg/L ZnO-NPs application reduced MDA levels by 1.23–2.17 folds in the Kargi and 1.11–1.97 folds in the CSR 30 rice genotypes, respectively.

### Superoxide dismutase (SOD) activity

Plants respond to salinity stress by producing more of an antioxidant enzyme. Increases in SOD activity of 1.18–1.57 folds for Kargi and 1.12–1.48 folds for CSR 30 relative to controls were observed following NaCl treatment of the rice genotypes (Figure 6 a). However, when ZnO-NPs are applied at a concentration of 50 mg/L, the expression of SOD is increased by 1.24–1.66 folds in the Kargi and by 1.21–1.55 folds in the CSR 30 rice genetic variants were compared to the standard (Figure 6a).

### Activity of ascorbate peroxidase (APX)

Compared to controls, the expression of APX was increased by 1.15–1.55 folds in the Kargi and 1.17–1.56 folds in the CSR 30 rice genotypes after exposure to salinity (Figure 6 b). However, 50 mg/L ZnO-NPs exhibited to increase APX level the most in Kargi (1.21–1.60 folds) and CSR 30. (1.23–1.65 folds).

## DISCUSSION

The germination of a seed is an important stage in the growth of a plant, and it is particularly

vulnerable to biotic and abiotic stressors like salt (Singh et al., 2020). Salt stress has an effect on a multitude of biological and physiological functions at all development stages, including germination (El-Badri et al., 2021). In terms of germination of seeds features, salinity stress has a substantial impact on the proliferation and maturation of plants. To lessen the detrimental effects of salinity stress on rice crops, researchers are working on salt-tolerance techniques, such as the use of external shields to decrease salt-induced oxidative damage in plants (Spielman-Sun et al., 2019). The external usage of ZnO-NPs has enough potential to combat against climate change-induced salinity and other critical abiotic stressors that impacted plant development and growth thus ZnO-NPs act as exogenous protectants that minimized the impact of salt on early germination stage of plants. In this investigation, the use of NPs has been postulated to have a big impact in alleviating ionic toxicity and osmotic stress. Under salt stress, seedling growth slowed, this may have been brought on by toxic effects of ions, osmotic stress, nutritional imbalance, and oxidative stress that results from an excess of Na<sup>+</sup> and Cl<sup>-</sup> absorption in plants.

In contrast to some studies that have found higher doses of ZnO-NPs to be harmful that show phytotoxicity effect on crops (Lee et al., 2010; Liu et al., 2016; Saleh, 2020). Other research suggests that low and controlled dose-dependent applications of ZnO-NPs can have a positive effect on plants experiencing salinity stress. For example, Torfeh et al. (2020) study recommended concentration of 20 mg/L ZnO-NPs can alleviate the impact of salinity stress on *Brassica napus*, while Mogazy and Hanafy, (2022) found that a combination of 50 mg/L ZnO-NPs and NaCl improved

growth and antioxidant activity in a under salinity stress. Some study also suggested that dose-dependent application of ZnO-NPs can improved the yield parameters of rice (Zhang et al., 2021). In our research, we utilized a small quantity of ZnO-NPs (50 mg/L) and observed no adverse effects on the rice genotypes.

Our research revealed that the usage of ZnO-NPs improved the assessment of the germination of seeds under salt stress. Present research data obtained through analysis of germination traits such as GP, RGR, SVI, under saline conditions. At the dose 50 mg/L ZnO-NPs seed germination traits GP, RGR, SVI of rice plants under salinity stress were efficiently to improved (Table 1). There have been reports of similar findings by Sarkhosh et al. (2022) on *Brassica* plants. The reason for increasing GP of rice seed after being treated with ZnO-NPs under salinity stress is absorption of water, light, and activity of the Rubisco enzyme that also upregulated the phytohormone gibberellic acid activity that enhanced the starch hydrolysis which provided energy for germination of seed (Assaha et al., 2015; H, 2017; Hoshmand, 1994; Oh et al., 2006; Sarkhosh et al., 2022; Sharma et al., 2021; Yang et al., 2006).

Phytochemical parameters, chlorophyll and carotenoid stability index, chlorophyll and carotenoid inhibition percentage, and MAD content evaluation of current study, are affected under salinity stress that reduced the growth of plants. Lipid peroxidation in membranes is an indicator of membrane breakdown in saline stress regimes (El-Shintinawy and El-Shourbagy, 2001). According to the present research's findings, it seems that salinity stress can damage different cell components such lipids and protein molecules as well as the structural stability of the cell membrane. The application of ZnO-NPs effectively decreased MDA content, protecting cells from the damage that is typically caused by salinity stress (Yasmin et al., 2021). MDA primarily affected the architecture of chloroplast membrane that altered the pigment-protein complex and reduced the photosynthetic pigments resulting photosynthesis process decreased. Our examination of data reveals that the rice plants' pigment chlorophyll and carotenoids concentrations were decreased by salt stress. Similar outcomes were made by Zafar et al. (Zafar et al., 2020) in wheat crop and showed at seedling growth stage, the amount of chlorophyll and carotenoids was decreased under salinity stress conditions.

The reduction in photosynthetic pigment in salty circumstances is directly linked to the oxidation of photosynthetic pigments via free radicals that affect the protein-pigment complexes of photosynthetic pigments that hydrolyze chlorophyllase, an essential enzyme involved in the production of chlorophyll (Parida et al., 2004). In our findings, the use of 50 mg/L ZnO-NPs improves the content of photosynthetic pigments-chlorophyll and carotenoid under stress caused by salt in rice cultivars. A related finding was observed by Rahnesan et al. (2018) and Alabdallah and Alzahrani, (2020) that application of ZnO-NPs improves chlorophyll and carotenoid levels in Pistachio vera and *Abelmoschus esculentus* plants. Enhancement of photosynthetic pigment content also related to promotion of carboxylation and enzymatic process of  $C_3$  plants after the application NPs (Lowry et al., 2019).

Seedlings parameters, shoot length and root length, inhibition percentage of root and shoot are directly affected due to situation like osmotic stress that caused by salinity stress which highly affected the intake of nutritional components and water that reduced the metabolic activity of plants (Zafar et al., 2022). The finding of Xu et al. (2016) suggested that the length of the plant's shoots and roots decreased under salt stress circumstances. However, an external use of ZnO-NPs enhanced the development of rice plants as shown in Table 2. A similar finding have been revealed by Zafar et al. (2020) that the use of external ZnO-NPs can upregulate the growth of *Abelmoschus esculentus* plants.

Numerous studies have demonstrated that being subjected to salinity stress causes the production of ROS, which in turn raises the expression of antioxidant enzymes as a protective mechanism (Abdel Latef, 2010; Khan and Upadhyaya, 2019; Singh et al., 2022, 2021). In the current study, we examined the enhancement of antioxidative catalysts (APX and SOD) under salinity stress. The upregulation of antioxidant enzymes for combating salinity stress is a part of the internal defense system of plants (Ali and Ashraf, 2011). Application of exogenous ZnO-NPs with concentration of 50 mg/L upregulate the antioxidant enzymes SOD and APX maximum activity level than normal saline conditions. These findings are in line with a prior work that shown how applying 50 mg/L biogenic ZnO-NPs to rice might enhance the efficiency of antioxidant enzymes (Itrotwar et al., 2019).

The content of MDA was also recorded highest under saline in rice but the exogenous application of ZnO-NPs decreased the MDA level production may be due to NPs mediated cell membrane recovery resulting improve plant vigor and reduced the risk of injury caused by salinity stress at germination to seedling stage. Our study is supported by Burman et al. (2013) with findings in chickpea seedlings suggesting that ZnO-NPs application in salinity stress can reduce the MDA by upregulation of the antioxidant enzyme activity that reduced the risk of ROS to alternations of membrane permeability.

## CONCLUSIONS

This study's findings hold great promise for the widespread implementation of ZnO-NPs, as we presented a cost-effective scientific approach to enhancing seed germination under varying environmental stresses without resorting to potentially harmful chemicals. Our data suggest that ZnO-NPs ameliorated salt stress in two rice varieties, Kargi and CSR 30, by promoting seed germination and promoting antioxidant enzyme activity. Due to their capacity to reduce the negative impact of salinity on crops, ZnO-NPs have shown great promise for boosting yields. In this study, we examined the properties of rice across multiple stages, from the germination of seeds to the development of seedlings, by measuring a variety of seed germination and phyto-biochemical parameters. Despite having the presence of stress caused by NaCl, application of 50 mg/L ZnO-NPs improved both the germination of seeds and rice plant development. It also lowers the MDA level, which subsequently boosts the amount of photosynthetic components (such as carotenoids and chlorophyll) and the degree of germination. Also, our results indicated that the landrace Kargi responded favourably to salinity and ZnO-NPs, albeit to a lesser extent than the salinity-tolerant genotype CSR 30. However, under normal conditions, Kargi has a stronger germination rate than the CSR 30 rice genotypes. Altogether, the results point to ZnO-NPs as a potentially useful and environmentally friendly application for increasing plant tolerance to salinity stress and reducing the damage it causes through antioxidant activity. ZnO-NPs applied to plants might provide new agricultural insights.

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