Growth and morpho-physiological response of stevia seedlings to nano-chemical pretreatments and salt stress

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Abstract

The purpose of this study was to evaluate the effects of salinity stress on germination, growth, and physiological characteristics of Stevia and to investigate whether pre-treatment with nano-compounds, specifically zinc oxide (ZnO), titanium oxide (TiO_2), and silicon (Si), could enhance these traits. The study revealed a significant improvement in the germination, growth, and physiological properties of Stevia seeds under varying levels of salinity stress (0, 2.5, 5, and 10 dS/m) using eight different seed priming treatments. The results showed that the effects of salinity stress and seed priming, as well as the interaction between these two factors, were significant on seed germination parameters and physiological characteristics. The most considerable germination percentage (54%) and rate (11.89 seeds/day), germination power (0.018), and seedling vigor index (609.6) were observed in seed priming with a combination of TiO_2 and Si under normal conditions. Under non-stress conditions, the combination of Si and ZnO used for seed pre-treatment resulted in the highest mean photosynthetic pigments, including chlorophyll a, b, and total (17.49, 8.72, and 26.22 µg/g FW, respectively) as well as the lowest mean proline content (0.3µmol/g FW) and enzymatic activities of antioxidants such as catalase and peroxidase (8.82 and 16.72 U/mg protein . min). The integrated application of Si + TiO_2 or Si + ZnO was particularly effective in enhancing germination and growth indices, as well as improving physiological traits under salt stress conditions. These results suggest that nano-fertilization can be a promising approach to enhance seedling growth and development, particularly under stress conditions.

Keywords: antioxidant activity, chlorophyll content, NaCl, proline content, seed priming

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Introduction

Stevia (*Stevia rebaudiana* Bertoni) is a plant species belonging to the Asteraceae and native to South America. It is highly valued for its sweet-tasting steviol glycosides, which have zero calories and do not raise blood sugar levels ((Afshari et al., 2020; Aghighi Shahverdi et al., 2020). The plant's value as a natural sweetener has made it a popular subject of study in recent years. Stevia cultivation faces several challenges, including its sensitivity to

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environmental stressors such as salinity (Aghighi Shahverdi et al., 2020; Shahverdi et al., 2017).

Salinity stress is a significant issue affecting crop yields globally. It causes damage to plant growth and development, including reduced seed germination and seedling growth. In this context, evaluating Stevia seed germination, growth, and physiological characteristics under salinity stress can provide valuable information for improving Stevia cultivation under saline conditions. One of the most sensitive stages of plant growth under salinity stress is the germination stage (Sivakumar et al., 2020). This is because this stage is the basis for the plant's initial establishment and salinity significantly impacts the final yield. Stress in this stage can have irreparable consequences for the plant. Salinity stress leads to disruption of plant growth and development through reduction of water potential (osmotic stress), accumulation of sodium and chlorine ions (ionic toxicity), damage by active oxygen groups, and disruption of the balance of nutrient ions in the root environment (Arif et al., 2020; Shahverdi et al., 2017; Yadav et al., 2020).

Seed priming is an advanced seed treatment technique that involves pre-sowing seed hydration to enhance the its germination under various environmental stress (Nejati et al., 2020). Seeds require precise moisture levels to begin the germination process, and stress factors such as drought, salinity, temperature, and other environmental factors can limit their germination and growth potential (Shahverdi et al., 2017). Seed priming effectively counters these stressors by allowing the seeds to imbibe water and activate the germination process during the treatment period. This process enhances enzyme activity, metabolic activity, oxygen uptake, and nutrient mobilization within the seeds, resulting in faster and more uniform germination of seeds ((Johnson and Puthur, 2021; Mansouri et al., 2022; Shaikh-Abol-hasani and Roshandel, 2019). Seed priming has been found to improve crop yields and promote crop resilience to environmental stress (Afshari et al., 2022; Shahverdi et al., 2017).

Seed priming is a seed treatment technique that involves hydration and partial germination of the seeds before planting. This method can enhance the seed's performance under stressful conditions by promoting enzymatic and metabolic activities within the seed. Studies have shown that different seed priming solutions can improve Stevia seed germination under salinity stress, including solutions of plant hormones, antioxidants, and osmoprotectants. For instance, pretreatment with paclobutrazol (Afshari et al., 2020) and selenium (Shahverdi et al., 2017) have been found to enhance Stevia seed germination under salt and drought stresses significantly. Such research results can greatly improve Stevia cultivation practices under saline environments, leading to improved yield and quality of the plant's sweet-tasting steviol glycosides (Rai and Han, 2022).

It has been confirmed that priming using nanoparticles (nano-priming) is more promising than traditional priming methods to achieve feasible agricultural yields (Nile et al., 2022). Nano-priming uses nanoparticles with a size of less than 100 nm, and "priming" refers to the development of stress tolerance under moderate and repeated stress (Chandrasekaran et al., 2020). It has been reported that seed germination and seedling establishment are potentially induced in various crops by nano-priming (Chandrasekaran et al., 2020; Zhu et al., 2019). Furthermore, it may be one of the best methods to overcome dormancy problems and increase seed germination in medicinal and forest species (Chandrasekaran et al., 2020). However, many studies have shown that high amounts of nanoparticles can have toxicological effects on a number of crops, including lettuce, tomato, wheat, and cucumber (Hatami et al., 2016). Several reports have been reported on applying nano-compounds for seed priming and their positive effects on germination, growth, and changes in physiological processes. For example, the application of nano-scale ZnO on *Zea mays* L. (Tondey et al., 2021), nano-Si solutions on *Helianthus annuus* L. (Janmohammadi and Sabaghnia, 2015), and TiO_2 nanoparticle seed priming on *Zea mays* L. under salinity stress (Shah et al., 2021).

Furthermore, studying the germination parameters of medicinal plants under stress conditions can identify the best conditions for optimized seed germination rates, leading to an increased yield of active agents used in various

herbal products. This information can be used to develop improved seed treatments, irrigation, and other agronomic practices that increase seedling establishment and plant growth, ultimately improving plant productivity and economic profits. This article explores the potential of seed priming techniques to improve seed germination and development, as well as to enhance seedling physiological characteristics under stressful conditions. The study investigates the use of various priming solutions, including nano-chemical fertilizers, to develop new approaches that can increase crop productivity in challenging environments. Addressing the need for sustainable agricultural systems, these methods have the potential to be highly effective. Specifically, this experiment aimed to evaluate the impact of nano compounds, either alone or in combination, on the growth, germination, and physiological attributes of Stevia, a medicinal plant, under salt stress induced by sodium chloride.

Materials and methods

Plant materials and experiment conditions

In 2023, a factorial experiment was conducted in the Laboratory of Seed Science and Technology at Islamic Azad University, Mashhad Branch. The investigation, which involved three replications and was based on a completely randomized design, aimed to assess the impact of nano-compound pretreatment on the growth, germination, and physiological responses of Stevia under conditions of salt stress. In this experiment, the first factor was different salinity levels of 0, 2.5, 5, and 10 dS/m using sodium chloride. Eight treatments (control, zinc oxide (ZnO), titanium oxide (TiO_2), silicon (Si), ZnO + TiO_2, ZnO + Si, TiO_2 + Si, and ZnO + TiO_2 + Si) were considered as the second factor.

TiO_2 (150 mg/L) was obtained from Neonano Co, with average dimensions of 20-30 nm, purity of 99.9%, and specific surface area of 200 square meters per gram (Sayedena et al., 2019).

ZnO nanoparticles were obtained from Neutrino Co., Tehran, Iran, and used at a concentration of 10 mg/L (Esparham et al., 2017).

Nano-Si was used from Manvert fertilizer source in Spain with a concentration of 20 mg/L (Almutairi, 2016). The literature suggests the best time for the pretreatment of stevia seeds as 24 hours (Aghighi Shahverdi et al., 2019), and this was followed in the present experiment.

Stevia seeds *var.* Bretoni were prepared from the fields of Stevia seed production in Firozabad, Fars province, Iran, in 2021-2022. The prepared seeds with a moisture content of 8.64% and a 1000-seed weight of 27.7 ± 0.5 mg were stored in paper envelopes at a temperature of 4 ± 1 °C until the start of the experiment.

Before the experiments, stevia seeds were disinfected with 70% ethanol for one minute and 20% sodium hypochlorite solution for 15 minutes and then washed three times with sterile distilled water (Shahverdi et al., 2017). All Petri dishes, glassware, and forceps were first disinfected with 70% ethanol and then sterilized in an oven at 160 °C for 2 hours

For pretreatment, sterilized Stevia seeds were immersed in priming solutions at the desired concentrations and transferred to a germinator under dark conditions. The seeds were treated under optimal priming conditions at 15 ± 1 °C for 24 hours (Shahverdi et al., 2017). Priming solutions were prepared using distilled water.

In the next step, 50 seeds were placed on Whatman paper in each Petri dish. According to the treatment, 7 ml of salt water (with different electrical conductivity of 2.5, 5, and 10 dS/m) was added to each Petri dish. Distilled water was added for the control treatment, and to reduce the amount of evaporation, the Petri dishes were sealed with paraffin. The preparation of saline water with different electrical conductivity was conducted using sodium chloride salt. The following formula was used to ensure the solution's electrical conductivity (EC) checked by an EC meter (Aghighi Shahverdi et al., 2019)

$$NaCl\ (mg.L^{-1}) = EC\ (dS.m^{-1}) \times 640$$

The standard germination test was performed at 23 ± 2 °C, a relative humidity of 75 ± 5%, and a light period of 16 hours and 8 hours of darkness in the

germinator (Aghighi Shahverdi et al., 2019). The daily counting of the germinated seeds from the second day until the time of germination was confirmed for three consecutive days (11 days), conducted daily at a certain time. During the counting process, seeds were considered germinated when the radicle reached a length of 2.

The number of abnormal seedlings (plants without sufficient growth or with abnormal growth) was also determined based on international seed test criteria by determining the cumulative number of germinated seeds in each experiment. Furthermore, at the end of germination, five normal seedlings were randomly selected from each petri dish, and the seedlings length was determined using a ruler (Afshari et al., 2020). Then, the characteristics of germination percentage (Liopa-Tsakalidi et al., 2012), germination rate (Maguire, 1962), mean germination time (Salehzade et al., 2009), germination power (Czabator, 1962), and seedling length vigor index (Abdul-Baki and Anderson, 1973) were calculated.

Measurement of biochemical and physiological characteristics

After the completion of germination and growth stages, sampling from the seedlings of each experimental unit (Petri dish) was performed to measure photosynthetic pigments, activity of antioxidant enzymes (catalase and peroxidase), and free proline content. The samples were immediately frozen in aluminum sheets by liquid nitrogen before they were transferred to a -80 °C freezer to measure biochemical traits.

Measurement of chlorophyll a, b, total, and carotenoid

To measure the chlorophyll a, b, and total contents, the method of Lichtenthaler and Buschmann (2001)was used. According to this method, 0.1 g of fresh seedling material was poured into a Chinese mortar and crushed with liquid nitrogen. Then, 5 ml of 80% acetone was added to the sample which was placed in a centrifuge (5000 rpm) for 10 min. Finally, the absorbance was read at wavelengths of 663.2 and 646.8 nm for chlorophyll a and b of the extract using a spectrophotometer (Perkin Elmer, USA, Lambda 25 model). The concentration of photosynthetic pigments was calculated using the following formulas, and the results of measuring the amount of these pigments were presented in terms of microgram per gram fresh weight. Total chlorophyll was calculated from the sum of chlorophyll a and b.

$$\text{Chl a} = (12.25 \times A_{663.2}) - (2.79 \times A_{646.8})$$

$$\text{Chl b} = (21.51 \times A_{646.8}) - (5.1 \times A_{663.2})$$

Determination of proline content of seedlings

Bates et al.'s (1973)method was used to measure free proline content in Stevia seedlings. Based on this method, 0.5 g of seedling sample from each petri dish was placed in 10 ml of 3% sulfosalicylic acid, and the resulting mixture was homogenized entirely in a Chinese mortar and centrifuged at 4000 rpm for 10 minutes. Then, 2 ml of a filtered extract with 2 ml of ninhydrin reagent and 2 ml of glacial acetic acid were poured into a test tube, and then the tubes were placed in a water bath at a temperature of 100 °C for one hour. In the next step, 4 ml of toluene was added to each test tube, and the samples were vortexed for 30 seconds to make them completely uniform. To determine the concentration of proline according to the standard curve of proline, the spectrophotometer was used at a wavelength of 520 nm.

Catalase enzyme activity assay

To measure the activity of catalase enzyme (EC.1.11.1.6), the method of Chance and Maehly (1995)was used. For this purpose, the reaction mixture containing 0.75 mL of 100 mM potassium phosphate buffer with pH = 7, 20 µL of soluble protein, and 1500 µL of double distilled water was added to the quartz cuvette, and 750 µL of peroxide was added during enzyme measurement. Seventy (70) mM H_2O_2 was added to the reaction mixture. Catalase enzyme activity was assayed by measuring the removal of hydrogen peroxide for 60 seconds at 240 nm at 25 °C using the spectrophotometer.

Peroxidase enzyme activity assay

Table 1
Effect of different levels of salinity stress (0, 2.5, 5, and 10 dS/m) and pre-treatments on germination and growth parameters of Stevia

Treatments	Germination percentage (%)	Mean germination time (day)	Germination rate (seed/day)	Germination power	Seedling length (cm)	Seedling vigor index
Salinity stress (dS/m)						
0 as control	41.83±7.34a	2.88±0.55d	9.32±2.58a	0.014±0.002a	10.11±1.98a	427.51±128.82a
2.5	44.08±5.22a	3.95±0.47c	6.54±1.2b	0.015±0.002a	8.03±2.08b	360.71±122.55b
5	38.5±5.28b	5.06±0.52b	4.26±0.8c	0.013±0.002b	5.19±1.09c	201.53±57.2c
10	35.83±9.45c	5.7±0.36a	3.38±0.99d	0.012±0.003c	2.99±0.7d	109±42.62d
LSD (p≤0.05)	2.39	0.26	0.53	0.0008	0.62	33.7
Pre-treatments						
Control (untreated seed)	33.83±7.93e	4.34±1.32	5.18±2.85d	0.011±0.003e	5.27±2.66d	192.18±124.7e
Titanium Oxide (TiO_2)	43.33±6.51ab	4.42±1.31	5.98±2.03bc	0.014±0.002ab	7.25±3.15ab	313.88±149.14ab
Silicon (Si)	39.17±7.46cd	4.62±0.89	5.05±1.81d	0.013±0.002cd	6.55±3.06bc	262.85±134.73cd
Zinc Oxide (ZnO)	42±6.38abc	4.35±1.42	6.35±3.23ab	0.014±0.002abc	7.87±4.32a	338.77±207.38a
TiO_2+ Si	44.83±9.04a	4.28±1.12	6.74±3.33a	0.015±0.003a	7.67±3.27a	350.03±189.22a
TiO_2+ ZnO	40.83±5.69bc	4.54±1.27	6±3abc	0.014±0.002bc	5.38±2.21d	228.07±110.61de
Si + ZnO	36.5±4.36de	4.44±1.24	5.36±2.42cd	0.012±0.001de	6.03±2.63cd	225.15±111.45de
TiO_2+ Si + ZnO	40±8.27bc	4.22±1.11	6.36±3.41ab	0.013±0.003bc	6.64±3.18bc	286.57±175.69bc
LSD (p≤0.05)	3.38	0.46(NS)	0.75	0.0011	0.88	47.7
Salinity × Pre-treatments						
	**	NS	**	**	**	**

Means followed by the same letter in each column are not significantly different according to LSD test at 5%
NS: non-significant; * and **: significant at α=0.05 and 0.01%, respectively.

To measure the activity of peroxidase enzyme (EC 1.11.1.7), the method of MacAdam et al. (1992)was used. In this method, 50 µL of the enzyme extract is mixed with 3 mL of 0.1 M potassium phosphate buffer solution (pH = 6) and 50 µL of pure guaiacol liquid, as an electron donor, ($C_7H_8O_2$). Then, 50 µL of hydrogen peroxide 3% was added as an electron acceptor. The changes in optical absorption at the wavelength of 436 nm were recorded immediately using the spectrophotometer at intervals of 15 seconds for 3 minutes.

Statistical Analysis

According to the levels of each test factor (4 levels of salinity stress and eight levels of priming) in three repetitions, the statistical population was 96 Petri dishes. The sampling method of experimental units (each Petri dish) was also done randomly. After collecting the germination, growth, and physiological data, the normality test of the experimental error was done using the Kolmogorov-Smirnov method. Further, the data were analyzed as a factorial experiment based on a completely randomized design using SAS statistical software version 9.2. The mean data comparison was conducted using the least significant difference (LSD) test at 5% probability level. Afterward, a simple correlation between germination traits, seedling growth, and some physiological characteristics was conducted using Minitab version 18 software.

Results

Germination and growth parameters

Results showed that the effects of salinity stress and seed priming, as well as the interaction between these two factors were significant on seed germination parameters such as germination percentage and rate, germination power, seedling length, and seed vigor index (Table 1).

According to the findings, the most significant germination percentage was observed in seed

Table 2
Interaction effect of different levels of salinity stress (0, 2.5, 5, and 10 dS/m) and pre-treatments on germination and growth parameters of Stevia

Salinity (dS/m)	Pre-treatment	Germination percentage (%)	Germination rate (seed/day)	Germination power	Seedling length (cm)	Seedling vigor index
0 control	Control (untreated seed)	36±5.29h-k	8.53±2.54b	0.012±0.002h-k	8.0±1.65f-i	287.2±65.34i-m
	Titanium Oxide (TiO_2)	35.33±1.15i-l	7.01±1.07cde	0.012±0i-l	9.6±1.77b-f	338.13±54.16f-j
	Silicon (Si)	33.33±2.31jkl	4.87±0.5g-k	0.011±0.001jkl	9.37±0.81c-f	313.47±49.76h-l
	Zinc Oxide (ZnO)	43.33±4.16b-g	11.33±1.14a	0.014±0.001b-g	13.33±1.5a	578.67±94.98ab
	TiO_2+ Si	54±2a	11.89±1.31a	0.018±0.001a	11.3±0.75b	609.6±32.23a
	TiO_2+ ZnO	46±3.46b-e	10.55±0.61a	0.015±0.001b-e	8.3±1.39e-h	378.6±32.22e-i
	Si + ZnO	38.67±2.31f-j	8.92±1.14b	0.013±0.001f-j	10±1.11b-e	386.4±47.04e-h
	TiO_2+ Si + ZnO	48±0a-d	11.48±0.15a	0.016±0a-d	11±0.82bc	528±39.29abc
2.5	Control (untreated seed)	42±6d-i	6.38±0.85def	0.014±0.002d-i	6.97±2.03h-k	300.4±122.88h-m
	Titanium Oxide (TiO_2)	50±3.46ab	8.39±1.03bc	0.017±0.001ab	10.13±0.83bcd	508±71.21bcd
	Silicon (Si)	47.33±5.03a-d	7.68±1.22bcd	0.016±0.002a-d	9±0.72d-g	424.13±29.29def
	Zinc Oxide (ZnO)	44.67±8.08b-f	6.06±0.88e-h	0.015±0.003b-f	9.77±2.17b-f	446.13±159.34cde
	TiO_2+ Si	42.67±3.06c-h	6.25±0.57d-g	0.014±0.001c-h	9.57±1.44b-f	411.07±92.16efg
	TiO_2+ ZnO	42±2d-i	5.84±0.94e-i	0.014±0.001d-i	5.67±0.76j-m	237.87±32.41k-n
	Si + ZnO	39.33±1.15e-j	5.33±0.61f-j	0.013±0e-j	5.83±1.26j-m	230.33±55.14lmn
	TiO_2+ Si + ZnO	44.67±6.43b-f	6.39±0.69def	0.015±0.002b-f	7.33±1.22g-j	327.73±71.96g-k
5	Control (untreated seed)	34±2jkl	3.75±0.74k-n	0.011±0.001jkl	3.7±0.36n-r	125.93±16.34o-s
	Titanium Oxide (TiO_2)	45.33±3.06b-f	4.72±0.75h-k	0.015±0.001b-f	6.1±1.49j-m	276.47±71.73j-m
	Silicon (Si)	42.67±4.62c-h	4.44±0.19i-m	0.014±0.002c-h	5.5±0.53klm	234.13±26.61k-n
	Zinc Oxide (ZnO)	34.67±5.03jkl	3.84±0.96j-n	0.012±0.002jkl	4.97±1.33l-o	176.13±72.82n-q
	TiO_2+ Si	33.33±2.31jkl	3.92±0.93j-n	0.011±0.001jkl	6.47±0.68i-l	216.27±34.84mno
	TiO_2+ ZnO	42.67±3.06c-h	4.65±0.81h-l	0.014±0.001c-h	4.83±0.45l-p	207.13±34.24m-p
	Si + ZnO	36.67±4.62g-k	4.1±0.56j-n	0.012±0.002g-k	4.57±0.4m-q	168.07±31.38n-r
	TiO_2+ Si + ZnO	38.67±2.31f-j	4.71±1.28h-k	0.013±0.001f-j	5.37±0.64k-n	208.13±33.99m-p
10	Control (untreated seed)	23.33±2.31m	2.05±0.19o	0.008±0.001m	2.4±0.52r	55.2±7.27s
	Titanium Oxide (TiO_2)	42.67±6.43c-h	3.81±0.41k-n	0.014±0.002c-h	3.17±0.81pqr	132.93±23.97o-s
	Silicon (Si)	33.33±5.77jkl	3.2±0.47l-o	0.011±0.002jkl	2.33±0.81r	79.67±37.02rs
	Zinc Oxide (ZnO)	45.33±2.31b-f	4.16±0.65j-n	0.015±0.001b-f	3.4±1.1o-r	154.13±49.03n-r
	TiO_2+ Si	49.33±8.33abc	4.91±1f-k	0.016±0.003abc	3.33±0.23o-r	163.2±17.82n-r
	TiO_2+ ZnO	32.67±2.31jkl	2.97±0.35mno	0.011±0.001jkl	2.73±0.45r	88.67±9.3qrs
	Si + ZnO	31.33±4.16kl	3.09±0.99mno	0.01±0.001kl	3.7±0.17n-r	115.8±14.44p-s
	TiO_2+ Si + ZnO	28.67±2.31lm	2.86±0.16no	0.01±0.001lm	2.87±0.29qr	82.4±12.96qrs

Means followed by the same letter in each column are not significantly different according to LSD test at 5 % level.

priming with a combination of TiO_2 and Si under normal conditions, with an average of 54%. Furthermore, the highest average of this trait was obtained through the combined use of three elements, including TiO_2, Si, and ZnO, under non-stressful conditions, as well as the separate application of Si and TiO_2 under salinity stress of 2.5 dS/m. The germination percentage was at its lowest when the level of salinity stress was the highest (10 dS/m) and the seeds were not subject to any priming treatment, with an average of 23.33% (Table 2).

Under non-stressful conditions, seed priming with ZnO, a combination of Si and TiO_2, ZnO and TiO_2, as well as the combination of all three elements led to the highest average germination rate, which was observed to be 11.33, 11.89, 10.55, and 11.48 seeds per day, respectively. Similar to the germination percentage, the lowest germination rate was observed at the highest level of salinity stress and absence of seed priming, with an average of 2.05 seeds per day (Table 2).

Under non-stressful conditions, combining TiO_2 and Si resulted in the highest germination power (0.018). However, salinity stress decreased the germination power, with the lowest average observed at the highest level of salinity stress and without any seed priming (0.008). Nevertheless, seed priming with a combination of three elements also showed the lowest average of this trait at the same level of salinity stress (Table 2).

According to the findings, the average length of seedlings ranged from 2.33 to 13.33 cm. The highest seedling length was observed after seed priming with ZnO under non-stressful salinity conditions. In contrast, the shortest seedlings were associated with the absence of seed priming

Table 3
Effect of different levels of salinity stress (0, 2.5, 5, and 10 dS/m) and pre-treatments on physiological characteristics of Stevia seedling

Treatments	Total chlorophyll content (µg/g FW)	Chlorophyll a content (µg/g FW)	Chlorophyll b content (µg/g FW)	Proline content (µmol/g FW)	Catalase activity (U/mg protein.min)	Peroxidase activity (U/mg protein.min)
Salinity stress (dS/m)						
0 as control	16.97±5.33a	12.23±3.42a	4.74±2.14a	0.26±0.06d	8.25±1.01d	13.71±5.03b
2.5	8.87±2.93b	6.71±2.82b	2.16±1.23b	0.49±0.09c	11.9±3.27c	14.94±4.86b
5	4.32±1.28c	3.27±1c	1.06±0.52c	0.78±0.15b	14.24±2.3b	15.08±3.6b
10	4.34±1.64c	3.12±1.16c	1.22±0.58c	0.93±0.17a	15.34±3.66a	40.0±11.27a
LSD (p≤0.05)	5.21	4.53	1.49	0.08	0.89	3.17
Pre-treatments						
Control (untreated seed)	5.49±2.29d	4.18±1.77e	1.31±0.85d	0.71±0.30a	14.37±5.1a	21.8±21.5ab
Titanium Oxide (TiO_2)	7.31±5.13c	5.17±3.65d	2.14±1.55c	0.56±0.27d	14.44±5.32a	21.5±14.4ab
Silicon (Si)	8.64±6.09b	6.43±4.35c	2.22±1.86bc	0.62±0.27c	13.24±4.13a	22.1±11.6a
Zinc Oxide (ZnO)	9.45±5.97b	7.39±4.65b	2.06±1.47c	0.62±0.30c	11.29±2.34b	20.5±13.2abc
TiO_2+ Si	9.66±6.6b	6.69±4.99bc	2.97±2.03a	0.66±0.27bc	11.23±2.7b	18.7±12.7bc
TiO_2+ ZnO	9.16±6.67b	6.34±4.66c	2.82±2.12ab	0.70±0.30ab	11.79±2.85b	22.8±9.4a
Si + ZnO	12.49±8.97a	9.36±5.92a	3.14±3.45a	0.70±0.30ab	11.82±3.3b	21.9±13.8a
TiO_2+ Si + ZnO	6.79±3.34c	5.09±2.63d	1.7±0.8cd	0.35±0.14e	11.28±3.22b	17.9±3.6c
LSD (p≤0.05)	1.16	0.85	0.60	0.041	1.26	3.07
Salinity × Pre-treatments						
	**	**	**	**	**	**

Means followed by the same letter in each column are not significantly different according to LSD test at 5 % level.
NS: non-significant; * and **: significant at α=0.05 and 0.01%.

and seed priming with Si under severe salinity stress (10 dS/m), with an average of 2.4 and 2.33 cm, respectively.

The seedling vigor index, influenced by the germination percentage and seedling length, had the highest average of 609.6 in seed priming with a combination of Si and TiO_2 under non-stressful salinity conditions. Additionally, applying ZnO alone or combining all three elements under non-stressful conditions resulted in the highest average of this trait. Salinity led to a significant decrease in the average seedling vigor index, with the lowest average observed at the highest level of salinity stress and absence of seed priming, with an average of 55.2 (Table 2).

Photosynthetic pigments

The study results revealed that salinity stress, seed priming, and their interaction significantly impacted the content of photosynthetic pigments such as total chlorophyll, chlorophyll a, and chlorophyll b (p<0.01). Overall, salinity stress caused a significant decrease, while seed priming significantly increased the average content of photosynthetic pigments (Table 3). Under non-saline conditions, seed priming with a combination of Si and ZnO led to the highest average content of total chlorophyll, chlorophyll a, and chlorophyll b, with averages of 26.22, 17.49, and 8.72 µg/g FW, respectively. Under the highest level of salinity stress and absence of seed priming, the total chlorophyll and chlorophyll b content was observed to be the lowest, with averages of 2.91 and 0.75 µg/g FW, respectively. On the other hand, the lowest average of chlorophyll a was obtained when TiO_2 was applied at the salinity level of 5 dS/m (Table 4).

Proline content

The study results indicated a significant impact of salinity stress, seed priming, and the interaction between salinity and priming on the proline content. Salinity stress caused an increase in proline content, with the greatest proline content observed in Stevia seedlings at the highest stress

Table 4
Interaction effect of different levels of salinity stress (0, 2.5, 5, and 10 dS/m) and pre-treatments on physiological characteristics of Stevia seedlings

Salinity (dS/m)	Pre-treatment	Total chlorophyll content (µg/g FW)	Chlorophyll a content (µg/g FW)	Chlorophyll b content (µg/g FW)	Proline content (µmol/g FW)	Catalase activity (U/mg protein.min)	Peroxidase activity (U/mg protein.min)
0 (control)	Control (untreated seed)	8.67±0.94gh	6.37±0.24f	2.31±1.18def	0.33±0.02lm	7.86±1.41o	6.66±1.83k
	Titanium Oxide (TiO_2)	15.48±0.64d	10.83±0.04d	4.65±0.61bc	0.23±0.07no	8.39±1.55o	17.25±2.19d-h
	Silicon (Si)	18.11±1.94bc	13.12±0.94bc	4.99±1.61bc	0.26±0.02mn	8.01±0.14o	14.03±1.58e-j
	Zinc Oxide (ZnO)	17.14±1.35cd	12.93±0.78bc	4.21±0.57c	0.25±0.04mn	8.65±1.43o	12.51±2.86g-k
	TiO_2+ Si	19.21±3.13bc	14.52±1.81b	4.68±1.46bc	0.31±0.07mn	8.69±0.69o	9.17±0.81ijk
	TiO_2+ ZnO	19.61±1.77b	13.76±0.93b	5.86±1.53b	0.3±0.09mn	8.82±1.01o	16.72±2.67d-h
	Si + ZnO	26.22±1.32a	17.49±0.6a	8.72±0.85a	0.3±0.03mn	7.77±0.87o	12.25±5.29g-k
	TiO_2+ Si + ZnO	11.3±1.32ef	8.78±0.99e	2.52±0.68de	0.16±0.01o	7.79±1.04o	21.12±3.26d
2.5	Control (untreated seed)	5.4±1j-n	4.34±1.63g-m	1.06±0.66gh	0.56±0.07h	17.48±2.63bcd	8.48±3.03jk
	Titanium Oxide (TiO_2)	6.43±1.14h-l	4.96±1.09f-j	1.47±0.28e-h	0.41±0.04kl	12.38±4.27i-m	12.21±1.08g-k
	Silicon (Si)	7.98±0.85ghi	6.24±0.76f	1.74±0.28d-h	0.5±0.05hij	11.55±1.07j-n	20.16±8de
	Zinc Oxide (ZnO)	12.38±2.31ef	10.22±2.5de	2.16±0.52d-g	0.44±0.06ijk	10.02±0.35l-o	11.61±4.42h-k
	TiO_2+ Si	10.27±2.2fg	5.67±0.87fg	4.6±1.43c	0.55±0.03h	9.27±0.74no	14.94±1.42e-i
	TiO_2+ ZnO	8.05±1.7ghi	5.23±1.1f-i	2.81±0.62d	0.57±0.04h	15.08±1.7d-h	18.1±2.67d-g
	Si + ZnO	12.95±0.89e	11.66±0.84cd	1.29±0.57fgh	0.56±0.05h	10.14±0.53k-o	17.49±2.22d-h
	TiO_2+ Si + ZnO	7.54±1.99hij	5.39±1.53fgh	2.15±0.56d-g	0.33±0.01lm	9.28±0.4no	16.52±1.84d-h
5	Control (untreated seed)	4.99±1.06k-o	3.85±0.91h-o	1.13±0.21fgh	0.89±0.06de	12.18±0.92i-m	15.51±1.96d-h
	Titanium Oxide (TiO_2)	2.99±0.87o	1.84±0.7p	1.15±0.28fgh	0.76±0.07g	16.32±2.84cde	11.7±0.65h-k
	Silicon (Si)	4.07±1.32mno	3.1±0.9k-p	0.97±0.44gh	0.8±0.02fg	18.05±2.08bc	14.66±3.5e-i
	Zinc Oxide (ZnO)	4.2±0.76l-o	3.49±0.38j-p	0.7±0.38h	0.81±0.04efg	12.98±0.57g-j	17.08±2.64d-h
	TiO_2+ Si	6.16±1.29i-m	4.35±0.39g-l	1.81±0.91d-h	0.81±0.03efg	14.29±1.07e-i	12.04±3.28g-k
	TiO_2+ ZnO	3.11±0.73no	2.39±0.61nop	0.72±0.18h	0.9±0.02cd	13.37±1.28f-j	18.71±3.23def
	Si + ZnO	4.25±0.81k-o	3.54±1.2i-o	0.71±0.44h	0.9±0.03d	14.23±0.56e-i	13.82±2.22f-j
	TiO_2+ Si + ZnO	4.82±0.86k-o	3.56±0.69i-o	1.25±0.25fgh	0.42±0.08jk	12.5±1.27i-l	17.16±5.8d-h
10	Control (untreated seed)	2.91±0.55o	2.17±0.52op	0.75±0.06h	1.07±0.07a	20.69±1.34a	56.54±7.55a
	Titanium Oxide (TiO_2)	4.36±0.23k-o	3.06±0.3k-p	1.3±0.28fgh	0.86±0.06def	19.97±1.05ab	44.98±2.53b
	Silicon (Si)	4.42±2.17k-o	3.24±1.58k-p	1.18±0.59fgh	0.94±0.04cd	15.35±1.4d-g	39.69±3.6bc
	Zinc Oxide (ZnO)	4.08±1.44mno	2.93±1.13l-p	1.15±0.34fgh	0.99±0.05abc	13.5±1.77f-j	41.01±7.78bc
	TiO_2+ Si	2.99±1.14o	2.21±0.96op	0.78±0.19h	0.98±0.02bc	12.66±2.32h-k	38.96±6.83bc
	TiO_2+ ZnO	5.87±1.03i-m	3.99±0.99g-n	1.88±0.45d-h	1.04±0.03ab	9.88±0.8mno	38.02±1.28c
	Si + ZnO	6.56±1.36h-k	4.74±0.34f-k	1.82±1.02d-h	1.04±0.08ab	15.13±2.13d-h	44.27±1.52b
	TiO_2+ Si + ZnO	3.51±1.28no	2.64±1.09m-p	0.87±0.2h	0.53±0.07hi	15.57±0.18c-f	17.16±2.62d-h

Means followed by the same letter in each column are not significantly different according to the LSD test at the 5 % level.

level (10 dS/m) in the absence of seed priming, with an average of 1.07 µmol/g FW. Furthermore, at the highest stress level, the use of ZiO, a combination of ZnO and TiO_2, and a combination of Si and ZnO also led to the highest average proline content. The lowest average proline content was achieved through seed priming with various elements - ZnO, TiO_2, and Si - under non-stressful conditions, with an average of 0.16 µmol/g FW (Table 4).

Table 5
Simple correlation between seed germination traits and physiological characteristics of Stevia under the influence of salinity stress and seed priming.

	1	2	3	4	5	6	7	8	9	10	11	12
1	1											
2	-0.41*	1										
3	0.65**	-0.92**	1									
4	0.99**	-0.41*	0.65**	1								
5	0.51**	-0.91**	0.87**	0.51**	1							
6	0.68**	-0.86**	0.91**	0.68**	0.96**	1						
7	0.22ns	-0.78**	0.71**	0.22ns	0.74**	0.66**	1					
8	0.24ns	-0.78**	0.72**	0.24ns	0.75**	0.67**	0.99**	1				
9	0.16ns	-0.70**	0.62**	0.16ns	0.66**	0.56**	0.93**	0.86**	1			
10	-0.37*	0.91**	-0.80**	-0.37*	-0.85**	-0.80**	-0.72**	-0.73**	-0.63**	1		
11	-0.27ns	0.72**	-0.65**	-0.27ns	-0.68**	-0.62**	-0.72**	-0.72**	-0.64**	0.68**	1	
12	-0.32ns	0.65**	-0.51**	-0.32ns	-0.65**	-0.59**	-0.39*	-0.41*	-0.32ns	0.67**	0.47*	1

ns: non-significant; * and **: significant at α=0.05 and 0.01%.

Antioxidant enzyme activities

The activity levels of antioxidant enzymes, catalase and peroxidase, were significantly influenced by salinity stress, seed priming, and their interaction, with a statistically significant difference observed at a probability level of 1% (Table 3). The study results revealed that the highest activity levels of catalase and peroxidase enzymes were observed without seed priming under severe salinity stress conditions, with enzyme unit levels of 20.69 and 56.54 U/mg protein.min, respectively. Salinity stress increased the activity levels of antioxidant enzymes while non-saline conditions showed the lowest activity levels of catalase and peroxidase enzymes. The lowest activity level of catalase enzyme was observed in all eight levels of seed priming under non-stress conditions. On the other hand, the lowest activity of peroxidase enzyme was related to the non-seed priming treatment under non-stress conditions, with an average of 6.66 U/mg protein.min (Table 4).

Correlation analysis

Table 5 presents the results of the simple correlation between seed germination traits and physiological characteristics, indicating significant positive and negative correlations between certain features. The findings revealed that the percentage of Stevia seed germination positively correlated with germination rate, seedling vigor index, and seedling length. In contrast, it negatively correlated with mean germination time and proline content. Additionally, the seedling length had a positive correlation with seedling vigor index and shoot length but a negative correlation with mean germination time. The seedling vigor index also negatively correlated with mean germination time and proline content. There was a significant positive correlation between the total chlorophyll content and seed germination rate, seedling length, and seedling vigor index. On the other hand, a negative correlation was observed between the total chlorophyll content and proline content, as well as the activity of catalase and peroxidase enzymes.

Discussion

This study aimed to assess Stevia seedlings' germination and physiological responses to salt stress and priming with various nano-fertilizer compounds. The results demonstrated that salt stress caused a reduction in germination parameters such as germination percentage and rate, germination power, seedling length, and seedling vigor index. However, priming with different nano-fertilizer compounds mitigated the adverse effects of salt stress and improved germination and seedling growth. It should be noted that the impact of these compounds varied depending on their types, and there were differences among various germination and growth parameters. Other investigators have

reported comparable findings (Ahmadi et al., 2020; Torabzadeh et al., 2019).

Overall, this study can enhance our understanding of plant responses to salt stress and provide insights into enhancing them. Depending on their species and sensitivity level, different plants use complex and diverse mechanisms to counter the effects of osmotic and ionic toxicity in response to salt stress. For example, in salt-sensitive plants, an increase in the concentration of sodium ions in the cytoplasm of cells can lead to severe cellular damage, whereas in salt-tolerant plants, the harmful ions are redirected and accumulated in vacuoles or inactive plant organs or are expelled from the cell wall and plant tissues, resulting in reduced damage caused by stress through the management of ion accumulation. Generally, plants employ various mechanisms to cope with salt stress, enabling them to survive in dry and semi-arid regions.

Additionally, the production of phytohormones and other compounds, such as polyamines and polysaccharides, can also improve a plant's response to salt stress (Arif et al., 2020; Shahverdi et al., 2017). Numerous studies have confirmed that salinity (NaCl) has a negative impact on the germination and growth of various crops. Other researchers have also reported reduced germination percentages, which can be attributed to a decrease in primary water uptake and the harmful effects of osmotic potential and ion toxicity on the physiological processes of seed germination (Nejati et al., 2020). Salt stress has an inhibitory effect on seed germination, which occurs due to an increase in osmotic pressure and a subsequent reduction in water uptake by the seed, as well as the toxic effects of sodium and chloride ions (Manaa et al., 2019). The reduced germination percentage under salt stress is caused by the disruption of ion balance due to the osmotic pressure of the solution, which affects the biological processes and reactions in the seed. This, in turn, leads to the cessation of enzyme activity in the seed or those produced for growth, resulting in a lack of energy required for germination and other growth activities (Joshi, 2018). These findings suggest that salt stress can have a detrimental impact on seed germination, which is caused by a combination of osmotic and ionic factors that disrupt the normal physiological processes of the seed (Nejati et al., 2020; Shahverdi et al., 2017).

Recent studies have shown that applying nano-fertilizers can improve seed germination under various stress conditions, including salt stress. Nano-fertilizers are particles that are smaller than 100 nanometers and can be designed to release nutrients slowly over a prolonged period, providing a sustained source of nutrition to the seed. When seeds are primed with nano-fertilizers before sowing, they can absorb the nutrients more efficiently and utilize them for growth and development (El-Sharkawy et al., 2022). In particular, studies have shown that priming with various types of nano-fertilizers, such as ZnO nanoparticles, silver nanoparticles, and nano-chitosan, can improve seed germination under salt-stress conditions. This improvement in germination can be attributed to the ability of these nanoparticles to enhance the uptake and utilization of water and nutrients by the seed, as well as to mitigate the toxic effects of salt ions (El-Sharkawy et al., 2022; Esparham et al., 2017). The study results indicated that pre-treating seeds with a combination of Si, TiO_2, and ZnO nanoparticles can lead to a higher germination percentage and rate under high salt-stress conditions. Additionally, seed priming increased the content of photosynthetic pigments under both saline and non-saline conditions. These findings suggest that the use of these nano-compounds for seed priming can be an effective strategy to enhance seedling growth and development under salt-stress conditions, potentially leading to increased crop productivity in salt-affected areas. The improved rate of germination observed in the study was linked to the infiltration of nanoparticles either into the seed coat or onto its surface, followed by their transportation inside through the texture parenchymatous. The nanoparticles' small size and large surface area facilitated their entry into the cell. This phenomenon has been reported in a recent study by El-Badri et al. (2021).

Seed priming with nutrients has been shown to increase seed vigor indexes, resulting in a significant improvement in seedling length and weight, as well as seed germination. These two

parameters are involved in the calculation of seed vigor, and the increase in their values may be attributed to the role of these nutrients in promoting cell division, cell elongation, and growth of meristematic cells. This suggests that nutrient priming can effectively enhance seedling growth and development, potentially leading to increased crop productivity (Shahverdi et al., 2017).

A study conducted by Shahverdi et al. (2017) found that proline can act as a signaling and regulatory molecule that enhances the ability of plants to resist salinity, particularly under salt-stress conditions. Afshari et al. (2022) also reported a positive correlation between proline accumulation and the level of antioxidants in plants. They suggested that stress-induced free radicals cause plants' free proline accumulation. Nutrient elements were found to increase the activity of proline 5-carboxylase synthase, which is a key enzyme in the synthesis of proline, leading to enhanced synthesis of these secondary metabolites under conditions of high salinity (Aghighi Shahverdi et al., 2020; Ahmadi et al., 2020). These findings suggest that proline can protect plants against salt stress by enhancing antioxidant defense mechanisms.

Plants have developed defense mechanisms that include both enzymatic and non-enzymatic antioxidants to protect against cellular damage caused by ROS while also regulating ROS levels through scavenging. Previous research by Shahverdi et al. (2017)has highlighted the importance of these mechanisms in maintaining plant health under stress conditions. In a recent investigation, the application of nanoparticles was found to enhance enzymatic activity and improve antioxidative mechanisms, suggesting that the use of ZnO, TiO_2, and Si nanoparticles may elevate the expression level of stress-responsive genes and ROS accumulation. This, in turn, leads to improved development under stress conditions (Almutairi, 2016; El-Badri et al., 2021). These findings suggest that the use of nanoparticles can be a practical approach to enhance the antioxidant defense system of plants, thereby improving their ability to cope with stress and maintain cellular health. Consistent with the results of this study, it has been reported that priming seeds with ZnO nanoparticles can regulate the activity of antioxidant enzymes, such as catalase and superoxide dismutase, in *Brassica napus* L. seedlings under salt stress caused by sodium chloride (El-Badri et al., 2021).

The application of ZnO nanoparticles has been found to positively impact the germination and morpho-physiological parameters of plants subjected to NaCl stress. Zn is crucial in various physiological processes, such as protein synthesis, indole acetic acid production, and chlorophyll synthesis. Furthermore, Zn helps maintain the structural integrity of biological membranes and reduces the uptake of excess Na^+ and Cl^- ions, which are harmful to plants under salt-stress conditions (Faizan et al., 2021). The increase in chlorophyll content resulting from the application of Si can be attributed to the potential of Si to preserve water and enhance nutrient contents in plants (Almutairi, 2016). Additionally, Si application has been found to enhance seedling photosynthesis by regulating nutrient transport via the opening of xylem vessels and the thickening of cell walls (Janmohammadi and Sabaghnia, 2015).

Conclusion

The findings of this study indicate that seed priming with nano-fertilizers, including Si, TiO_2, and ZnO, can enhance germination and growth parameters, promote the synthesis of photosynthetic pigments, and improve osmoregulation, ultimately alleviating salt stress in stevia seeds. Furthermore, applying nano-fertilizers was found to mediate the alleviation of salt stress by modulating various biochemical pathways and the antioxidant system. The integrated application of Si + TiO_2 or Si + ZnO was particularly effective in enhancing germination and growth indices, as well as improving physiological traits under salt stress conditions. These results suggest that nano-fertilization can be a promising approach to improve plant growth and development, particularly under stress conditions.

References

Abdul-Baki, A. A. and J. D. Anderson. 1973. Vigor determination in soybean seed by multiple criteria 1. *Crop science,* 13, (6) 630-633.

Afshari, F., F. Nakhaei, S. Mosavi and M. Seghatoleslami. 2020. Evaluating the role of nutri-priming in improving PEG-induced drought stress tolerance of stevia (*Stevia rebuadiana* Bertoni). *Iranian Journal of Plant Physiology,* 11, (1) 3509-3522.

Afshari, F., F. Nakhaei, S. Mosavi and M. Seghatoleslami. 2022. Physiological and biochemical responses of *Stevia rebaudiana* Bertoni to nutri-priming and foliar nutrition under water supply restrictions. *Industrial Crops and Products,* 176, 114399.

Aghighi Shahverdi, M., H. Omidi and C. A. Damalas. 2020. Foliar fertilization with micronutrients improves *Stevia rebaudiana* tolerance to salinity stress by improving root characteristics. *Brazilian Journal of Botany,* 43, (1) 55-65.

Aghighi Shahverdi, M., H. Omidi, H. Mosanaiey, M. Pessarakli, S. Mousavi and M. Ghasemzadeh. 2019. Effects of light and temperature treatments on germination and physiological traits of stevia seedling (*Stevia rebuadiana* Bertoni). *Journal of Plant Nutrition,* 42, (10) 1125-1132.

Ahmadi, N., H. Hassanpour, M. Hekmati and M. Ghanbarzadeh. 2020. Effect of SiO_2 nanoparticles on phytochemical and anatomical alterations in *Anthemis gilanica*. *Iranian Journal of Plant Physiology,* 10, (3) 3223-3231.

Almutairi, Z. M. 2016. Effect of nano-silicon application on the expression of salt tolerance genes in germinating tomato ('S*olanum lycopersicum*'L.) seedlings under salt stress. *Plant Omics,* 9, (1) 106-114.

Arif, Y., P. Singh, H. Siddiqui, A. Bajguz and S. Hayat. 2020. Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. *Plant Physiology and Biochemistry,* 156, 64-77.

Bates, L. S., R. Waldren and I. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant and soil,* 39, 205-207.

Chance, J. and S. Machely. 1995. Assay of Catalase and peroxidase. Meth. Enzymo

Chandrasekaran, U., X. Luo, Q. Wang and K. Shu. 2020. Are there unidentified factors involved in the germination of nanoprimed seeds? *Frontiers in Plant Science,* 11, 832.

Czabator, F. J. 1962. Germination value: an index combining speed and completeness of Pine seed gerrmination.

El-Badri, A. M., M. Batool, C. Wang, A. M. Hashem, K. M. Tabl, E. Nishawy, J. Kuai, G. Zhou and B. Wang. 2021. Selenium and zinc oxide nanoparticles modulate the molecular and morpho-physiological processes during seed germination of *Brassica napus* under salt stress. *Ecotoxicology and Environmental Safety,* 225, 112695.

El-Sharkawy, M., E. Mahmoud, M. Abd El-Aziz and T. Khalifa. 2022. Effect of zinc oxide nanoparticles and soil amendments on wheat yield, physiological attributes and soil properties grown in the saline–sodic soil. *Communications in Soil Science and Plant Analysis,* 53, (17) 2170-2186.

Esparham, E., S. Saeidisar, H. Mahmoodzadeh and M. Hadi. 2017. The effects of zinc oxide (ZnO) nanoparticles on the germination, biochemical and ultrastructural cell characteristics of Ricinus communis. *Cell and Tissue Journal,* 8, (2) 151-164.

Faizan, M., J. A. Bhat, C. Chen, M. N. Alyemeni, L. Wijaya, P. Ahmad and F. Yu. 2021. Zinc oxide nanoparticles (ZnO-NPs) induce salt tolerance by improving the antioxidant system and photosynthetic machinery in tomato. *Plant Physiology and Biochemistry,* 161, 122-130.

Hatami, M., K. Kariman and M. Ghorbanpour. 2016. Engineered nanomaterial-mediated changes in the metabolism of terrestrial plants. *Science of the total environment,* 571, 275-291.

Janmohammadi, M. and N. Sabaghnia. 2015. Effect of pre-sowing seed treatments with silicon nanoparticles on germinability of sunflower (*Helianthus annuus*). *Bot Lith,* 21, (1) 13-21.

Johnson, R. and J. T. Puthur. 2021. Seed priming as a cost effective technique for developing

plants with cross tolerance to salinity stress. *Plant physiology and biochemistry,* 162, 247-257.

Joshi, R. 2018. Role of enzymes in seed germination. *International Journal of Creative Research Thoughts,* 6, (2) 1481-1485.

Lichtenthaler, H. K. and C. Buschmann. 2001. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. *Current protocols in food analytical chemistry,* 1, (1) F4. 3.1-F4. 3.8.

Liopa-Tsakalidi, A., G. Kaspiris, G. Salahas and P. Barouchas. 2012. Effect of salicylic acid (SA) and gibberellic acid (GA3) pre-soaking on seed germination of stevia (*Stevia rebaudiana*) under salt stress.

Macadam, J. W., C. J. Nelson and R. E. Sharp. 1992. Peroxidase activity in the leaf elongation zone of tall fescue: I. Spatial distribution of ionically bound peroxidase activity in genotypes differing in length of the elongation zone. *Plant Physiology,* 99, (3) 872-878.

Maguire, J. D. 1962. Speed of germination-aid in selection and evaluation for seedling emergence and vigor.

Manaa, A., R. Goussi, W. Derbali, S. Cantamessa, C. Abdelly and R. Barbato. 2019. Salinity tolerance of quinoa (*Chenopodium quinoa* Willd) as assessed by chloroplast ultrastructure and photosynthetic performance. *Environmental and Experimental Botany,* 162, 103-114.

Mansouri, A., H. Omidi and A. Bostani. 2022. Effects of boron priming on the chemicals and antioxidant of quinoa seedling. *Iranian Journal of Plant Physiology,* 5, (4) 4289.

Nejati, M., A. Alipour, M. Yousefirad, Y. Sharghi and H. Zahedi. 2020. Effects of seed germination with gibberellin on safflower germination under salinity stress. *Iranian Journal of Plant Physiology,* 5, (1) 3443.

Nile, S. H., M. Thiruvengadam, Y. Wang, R. Samynathan, M. A. Shariati, M. Rebezov, A. Nile, M. Sun, B. Venkidasamy and J. Xiao. 2022. Nano-priming as emerging seed priming technology for sustainable agriculture—recent developments and future perspectives. *Journal of nanobiotechnology,* 20, (1) 254.

Rai, A. and S.-S. Han. 2022. Critical review on key approaches to enhance synthesis and production of steviol glycosides: a blueprint for zero-calorie sweetener. *Applied Sciences,* 12, (17) 8640.

Salehzade, H., M. I. Shishvan, M. Ghiyasi, F. Forouzin and A. A. Siyahjani. 2009. Effect of seed priming on germination and seedling growth of wheat (*Triticum aestivum* L.). *Res J Biol Sci,* 4, (5) 629-631.

Sayedena, V., B. Pilehvar, K. Abrari-Vajari, M. Zarafshar and H. R. Eisvand. 2019. Effects of TiO2 nanoparticles on germination and primary growth of mountain ash (*Sorbus luristanica*). *Iranian Journal of Seed Research,* 6, (1) 173-184.

Shah, T., S. Latif, F. Saeed, I. Ali, S. Ullah, A. A. Alsahli, S. Jan and P. Ahmad. 2021. Seed priming with titanium dioxide nanoparticles enhances seed vigor, leaf water status, and antioxidant enzyme activities in maize (*Zea mays* L.) under salinity stress. *Journal of King Saud University-Science,* 33, (1) 101207.

Shahverdi, M. A., H. Omidi and S. J. Tabatabaei. 2017. Effect of nutri-priming on germination indices and physiological characteristics of stevia seedling under salinity stress. *Journal of Seed Science,* 39, (04) 353-362.

Shaikh-Abol-Hasani, F. and P. Roshandel. 2019. Effects of priming with salicylic acid on germination traits of *Dracocephalum moldavica* L. under salinity stress. *Iranian Journal of Plant Physiology,* 10, (1) 3035-3045.

Sivakumar, J., J. E. P. Prashanth, N. Rajesh, S. M. Reddy and O. B. Pinjari. 2020. Principal component analysis approach for comprehensive screening of salt stress-tolerant tomato germplasm at the seedling stage. *Journal of biosciences,* 45, 1-11.

Tondey, M., A. Kalia, A. Singh, G. S. Dheri, M. S. Taggar, E. Nepovimova, O. Krejcar and K. Kuca. 2021. Seed priming and coating by nano-scale zinc oxide particles improved vegetative growth, yield and quality of fodder maize (*Zea mays*). *Agronomy,* 11, (4) 729.

Torabzadeh, D., H. Hassanpour, J. Asgarpanah and M. Rezayian. 2019. Nanoparticles induced antioxidative compounds in Matricaria chamomilla. *Iranian Journal of Plant Physiology,* 9, (4) 2955-2961.

Yadav, S., P. Modi, A. Dave, A. Vijapura, D. Patel and M. Patel. 2020. Effect of abiotic stress on

crops. *Sustainable crop production,* 3, (17) 5-16.

Zhu, J., Z. Zou, Y. Shen, J. Li, S. Shi, S. Han and X. Zhan. 2019. Increased ZnO nanoparticle toxicity to wheat upon co-exposure to phenanthrene. *Environmental Pollution,* 247, 108-117.