Fundamental and Applied Agriculture
Vol. 8(3), pp. 615–626: 2023
doi: 10.5455/faa.160768

SEED SCIENCE | ORIGINAL ARTICLE

Assessing the impact of seed priming by nanomaterials on stevia germination and biochemical attributes under drought stress

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ARTICLE INFORMATION

Article History
Submitted: 11 Jul 2023
Accepted: 04 Aug 2023
First online: 30 Sep 2023

Academic Editor
Md Harun Rashid
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ABSTRACT

Seed priming with nanocompounds can potentially enhance seed germination and tolerance to environmental stress. The study examined the effects of drought stress induced by PEG-6000 at various levels (0, −0.3, −0.6, and −0.9 MPa) and seed priming with different nano-compounds (zinc oxide, titanium oxide, or silicon) on the germination, growth, and biochemical and physiological characteristics of stevia. The results showed that drought stress had a negative impact on most seed germination and growth parameters, while seed priming with zinc oxide nanoparticles had the highest positive impact. Different seed priming treatments produced varying outcomes. Drought stress and seed priming also significantly affected total chlorophyll content, chlorophyll a and b, and antioxidant enzyme activity (catalase and peroxidase). Under severe drought stress, all the three seed priming combinations significantly increased total chlorophyll content. Increasing the concentration of PEG-6000 in the seedling growth medium increased catalase activity. Non-primed seeds and seeds primed with zinc oxide under severe drought stress had the highest peroxidase enzyme activity. The Pearson correlation analysis revealed significant correlations among the measured traits. Lastly, the stepwise regression analysis identified catalase and peroxidase activities as the most influential traits related to stevia seed germination percentage. Seed priming with zinc oxide nanoparticles can enhance stevia seed germination and growth, particularly under drought stress, by adjusting antioxidant enzyme activity and increasing photosynthetic pigment content. Moreover, as a practical outcome, the utilization of priming can serve as an applicable approach in the production of seedlings for this plant.

Keywords: Antioxidant enzymes, chlorophyll content, nanocompound, PEG-6000, zinc oxide, stevia

Cite this article: Safaeipour M, Kalat MN, Shahverdi MA. 2023. Assessing the impact of seed priming by nanomaterials on stevia germination and biochemical attributes under drought stress. Fundamental and Applied Agriculture 8(3): 615–626. doi: 10.5455/faa.160768

1 Introduction

Stevia (*Stevia rebaudiana* Bertoni) is a popular perennial herb known for its sweet-tasting leaves, used as a natural sweetener. Stevia has gained significant attention recently due to its potential as a sugar substitute in various food and beverage industries (Shahverdi et al., 2020; Asmuni and Hakiman, 2020; Buyuk et al., 2022). Due to its self-incompatible flowers, the plant relies on wind and insects for cross-pollination (Shahverdi et al., 2017). Consequently, the plant has a low fertility rate, leading to limited seed germination and growth, ultimately restricting its potential for large-scale cultivation (Gorzi et al., 2018; Afshari et al., 2020).

The successful cultivation of stevia faces challenges due to factors like low seed germination rates and poor seedling establishment, especially in drought stress conditions. Seed priming, a pre-

sowing technique, has shown to improve seed germination and promote plant growth under unfavorable environmental conditions (Arun et al., 2022; Bhatia and Gupta, 2022). Scientists have explored various strategies to counter the negative effects of drought on seed germination and growth, and the use of nanomaterials (NMs) has emerged as a promising approach. Nanomaterials can be applied in small quantities through seed coating, priming, or foliar spray to enhance plant tolerance to environmental stress and improve food quality. Moreover, NMs can stimulate the production of bioactive compounds in plants and activate their defense system against abiotic and biotic stress by triggering key metabolic activities and the accumulation of bioactive compounds (González-García et al., 2022; Nile et al., 2022).

Several research studies have explored how priming with NMs can affect seed germination indices, plant growth, and physiological and biochemical parameters. For example, Behboudi et al. (2018) confirmed that the application of SiO_2 NMs, particularly at concentrations of 30 and 60 ppm, can alleviate the negative impacts of drought stress on wheat plants. Ahmed et al. (2022) reported that the ZnO NMs could be a promising method, side by side with the released osmoprotectants and phytohormones, to relieve salt stress in plants. Another study by Khan et al. (2023) revealed that Ag NMs effectively improved the morphology, physiology, biochemistry, and gene expression pattern under salinity, which could be attributed to positive impacts of Ag NMs on *Pennisetum glaucum* L. These studies demonstrate the potential of NMs priming to enhance germination parameters and plant growth and improve tolerance to environmental stress. There is currently a lack of notable research studies on using NMs for seed priming of stevia plants under drought stress conditions.

Priming with NMs has emerged as a promising technique to improve seed germination and plant growth under adverse environmental conditions. Therefore, this study has been aimed to investigate into the impact of NMs priming (titanium oxide (TiO_2), silicon (Si), and zinc oxide (ZnO)) on stevia seed germination, seedling growth, and biochemical attributes under drought stress conditions.

2 Materials and Methods

2.1 Experimental material

In June 2022, *Stevia rebaudiana* Bertoni seeds were collected from seed production fields in Firozabad city, Fars Province, Iran. The seeds were recently matured and had a mean dry weight of 26.4 ± 0.5 mg per 100 seeds. They were free from impurities and had an 8-9% moisture content.

2.2 Experiment treatments and design

In 2023, a laboratory experiment was conducted at Mashhad University to assess the impact of NMs priming on stevia seed germination, as well as its physiological and biochemical responses under drought stress conditions. The study employed a completely randomized design with three replications, and a factorial experiment was used to evaluate the effects of different levels of drought stress (0, −0.3, −0.6, and −0.9 MPa using PEG-6000) and four treatments (unprimed seed as control, and priming with ZnO, TiO_2, or Si nanoparticles).

For this experiment, Iranian-made TiO_2 (150 mg L^{-1}) with a purity of 99.9% and an average size of 20-30 nm, as well as a specific surface area of 200 m^2 g^{-1}, was used. ZnO nanoparticles at a concentration of 10 mg L^{-1} were obtained from Neutrino Company in Tehran, Iran. Si at a concentration of 20 mg L^{-1} was obtained from a Manvert fertilizer source in Spain. According to (Shahverdi et al., 2017) and Shahverdi et al. (2019), the optimal priming time for stevia seeds is 24 h, and this time frame was used in the experiment.

To prepare for the experiments, stevia seeds were disinfected using a combination of 70% ethanol for one minute and a 20% sodium hypochlorite solution for 15 minutes. They were then rinsed three times with sterile distilled water, as described by Shahverdi et al. (2017). To prepare stevia seeds for germination, they were sterilized and soaked in a solution with the desired concentration before being placed in a germinator in dark conditions for 24 hours. The priming solution was made with distilled water. Next, 50 seeds were placed on Whatman paper in Petri dishes and treated with different levels of salt water or PEG-6000 to create different osmotic potentials. The amount of PEG-6000 was calculated using a formula by Michel and Kaufmann (1973). The Petri dishes were sealed to prevent evaporation.

$$\psi_s = -(1.18 \times 10^{-2}) \times C - (1.18 \times 10^{-4}) \times C_2 + (2.67 \times 10^{-4}) \times CT + (8.39 \times 10^{-7}) \times C_2T \quad (1)$$

where, ψ_s, C, and T represent osmotic potential in bars, the concentration of PEG in g L^{-1} of distilled water, and temperature in °C, respectively. The control treatment, which did not induce drought stress, used distilled water as its baseline with zero potential.

2.3 Seed germination assay

The standard germination test was conducted in a germinator with a temperature of 23 ± 2 °C, 75 ± 5% relative humidity, and a 16-hour light period followed by 8 hours of darkness. Germinated seeds were counted

daily starting from the second day until germination was confirmed for three consecutive days (11 days total) using a criterion of a root length of 2 mm or more. The abnormal seedlings were also recorded based on international seed test criteria. After germination, the length of five normal seedlings from each Petri dish was measured using a ruler. Several characteristics were calculated, including germination percentage, germination rate, mean germination time, mean daily germination, seedling length, and seedling vigor index (Maguire, 1962; Abdul-Baki and Anderson, 1973; A, 2012; Afshari et al., 2020).

$$\text{Germination percentage (GP)} = \frac{N \times 100}{M} \quad (2)$$

$$\text{Germination rate (GR)} = \frac{\sum N_i}{T_i} \quad (3)$$

$$\text{Mean germination time (GT, day)} = \frac{\sum DN}{\sum n} \quad (4)$$

$$\text{Mean daily germination (GD)} = \frac{\sum N_i}{\sum T_i} \quad (5)$$

$$\text{Vigor index } (VI) = \text{GP} \times \text{SL} \quad (6)$$

where, N = sum of germinated seeds at the end of the experiment, M = total planted seeds, T_i = number of days after germination, D_i = the number of day from the start of the test tothe enumeration of nth, SL = Seedling length (mm)

2.4 Measurements of physiological and biochemical traits of seedlings

Once the germination and growth stages were finished, samples were taken from the seedlings in each experimental unit (Petri dish) to measure photosynthetic pigments and the activity of antioxidant enzymes (catalase and peroxidase). The samples were quickly frozen using liquid nitrogen on aluminum sheets and stored at a temperature of −80 °C for later analysis of physiological and biochemical traits.

The method used for analysis included the extraction of 0.25 g of fresh tissue using 5 mL of 80% acetone, followed by centrifugation at 11000 revolutions per minute for 10 minutes. The optical density of the resulting extract was measured at three different wavelengths (646.8 nm and 663.2) using a spectrophotometer (Perkin Elmer, USA, Lambda 25 model), as outlined by Lichtenthaler and Buschmann (2001). Total chlorophyll content was determined by adding the measured amounts of chlorophyll a and chlorophyll b.

$$\text{Chl-a} = (12.25 \times A_{663.2}) - (2.79 \times A_{646.8}) \quad (7)$$

$$\text{Chl-b} = (21.51 \times A_{646.8}) - (5.10 \times A_{663.2}) \quad (8)$$

where, Chl-a and Chl-b represent chlorophyll a and chlorophyll b content in μg g^{-1} FW, respectively.

To measure peroxidase activity (EC 1.11.1.7), 0.2 g of fresh seedlings were ground in a mortar using liquid nitrogen and mixed with 1 mL of Tris-HCl buffer (0.05 M, pH = 7.5). The resulting mixture was centrifuged for 21 minutes at 13,000 rotations per minute and 4 °C, and the supernatant was used to measure enzyme activity. Peroxidase activity was determined by adding a suitable amount of 28 mM guaiacol, 5 mM H_2O_2, 25 mM Na-phosphate buffer (pH 6.8), and the enzyme to the reaction mixture, following the method described by MacAdam et al. (1992).

The catalase activity (EC.1.11.1.6) was measured using the method outlined by Maehly (1954). A reaction mixture consisting of 2.5 mL of 0.05 mM sodium phosphate buffer (pH = 7) and 30 μg of protein was added to cuvettes. At the time of measurement, 30 μL of 30% H_2O_2 was added to the reaction mixture, and the absorbance at 240 nm was recorded spectrophotometrically after 60 seconds at 25 °C. The control group contained 2.5 mL sodium phosphate buffer and 30 μg of protein. Catalase activity was reported as the change in absorption per mg of protein per minute.

2.5 Statistical analysis

After confirming the normal distribution of the data using the Kolmogorov-Smirnov and Shapiro-Wilk tests, statistical analysis of the studied traits was performed using the Statistical Analysis System (SAS) software, version 9.2, by SAS Institute in Cary, North Carolina, USA. Differences among means were determined using the least significant difference (LSD) test at a statistical probability level 0.05. Simple correlation and stepwise regression between germination, physiological, and biochemical traits were performed using Minitab software version 18. Graphs were generated using MS Excel.

3 Results

3.1 Seed germination and seedling growth characteristics

The data's variance analysis revealed that drought stress and seed priming significantly affected various seed germination characteristics and seedling growth

Table 1. ANOVA results for germination and physiological characteristics of Stevia under different levels of drought stress and priming

SOV	Drought (D)	Priming (P)	D × P	Error	CV (%)
df	3	3	9	32	-
GP	1760.9**	484.9**	49.63ns	33.66	13.99
GR	24.69**	10.34**	1.33*	0.5	14.28
GT	0.20*	0.21*	0.04ns	0.05	9.27
GD	3.39**	0.85**	0.11ns	0.071	13.73
SL	73.15**	56.92*	7.51ns	13.87	25.7
SVI	793046.6**	324437.4**	38118.4ns	48832.5	24.95
Tot Chl	19.00**	13.75**	6.10**	1.81	10.13
Chl-a	4.84*	5.88**	1.37ns	1.14	13.16
Chl-b	5.99*	3.17ns	3.52ns	1.72	25.52
CAT	0.34**	0.08**	0.006ns	0.007	11.32
POD	333.1**	5.45*	4.28*	1.73	21.95

SOV = source of variation, GP = germination percentage, GR = germination rate, GT = mean germination time (day), GD = mean daily germination, SL = seedling length (mm), SVI = seedling vigor index, Tot Chl = total chlorophyll (μg g^{-1} FW), Chl-a = chlorophyll a (μg g^{-1} FW), and Chl-b = chlorophyll b (μg g^{-1} FW), CAT = catalase activity (U mg^{-1} protein min^{-1}), POD = peroxidase activity; ns: non-significant;* and **: significant at α = 0.05 and 0.01%

Table 2. Effects of drought stress levels and priming on seed germination and seedling growth characteristics of stevia

Treatments	GP	GT	GD	SL	SVI
Drought stress (MPa)					
0 (as control)	49.5±8.1 a	2.39±0.12 bc	2.27±0.38 a	15.72±3.77 a	787.47±267.86 ab
−0.3	49.3±10.2 a	2.37±0.26 c	2.3±0.48 a	16.67±5.06 a	836.67±356.52 a
−0.6	43.17±8.07 b	2.57±0.17 ab	2.03±0.33 b	14.56±3.57 a	636.23±220.11 b
−0.9	23.83±5.87c	2.63±0.37a	1.17±0.24 c	11.02±3.09 b	268.43±120.11 c
LSD (p≤0.05)	4.82	0.19	0.22	3.09	183.7
Priming					
TiO_2	34.17±11.83 c	2.65±0.33 a	1.63±0.5 c	14.98±4.49 a	543.78±296.18 b
Si	42.33±11.78 b	2.42±0.2 bc	1.96±0.55 b	14.87±3.53 a	646.51±257.33 b
ZnO	49.5±13.57 a	2.35±0.24 c	2.28±0.61 a	16.65±4.05 a	856.79±399.68 a
Control (untreated seed)	39.83±12.49 b	2.55±0.21 ab	1.9±0.55 b	11.46±4.17 b	481.72±275.41 b
LSD (p≤0.05)	4.82	0.19	0.22	3.09	183.7

GP = germination percentage, GR = germination rate, GT = mean germination time (day), GD = mean daily germination, SL = seedling length (mm), SVI = seedling vigor index; Means followed by the same letter in each column are not significantly different according to the LSD test at a 5% level of significance

parameters, such as the percentage and rate of germination, mean germination time, mean daily germination, seedling length, and seedling vigor index. Furthermore, a significant interaction effect between drought stress and priming on germination rate was observed at a probability level of 5% (Table 1).

Drought stress reduced the average values of most seed germination and seedling growth parameters (except for the mean germination time). The comparison of means for the data related to the drought stress treatment revealed that the highest germination percentage (49.5%), mean daily germination (2.27), seedling length (15.72 mm), and seedling vigor index (787.47) were related to free drought stress conditions. Furthermore, the results showed that the partial drought stress (−0.3 MPa) had the highest mean values for the mentioned traits. An increase in osmotic potential due to drought severity significantly reduced the germination percentage, mean daily germination, seedling length, and seedling vigor index.

This reduction was 51.8%, 48.4%, 29.8%, and 65.9%, respectively, at a drought level of −0.9 MPa compared to the control treatment (no drought stress). Drought stress increased in the mean germination time, with the highest value for this trait (2.63 days) observed at the highest level of drought stress (−0.9 MPa) and the lowest mean value recorded for the control treatment and partial drought stress (−0.3 MPa) (Table 2).

Different results were obtained regarding the seed priming treatments, with some combinations leading to an increase and others leading to a decrease in the mean values of the traits compared to the non-primed treatment. Seed priming with ZnO led to the highest mean values for the germination percentage (49.5%), mean daily germination (2.28), seedling length (16.65 mm), and seedling vigor index (856.7), with an increase of 19.5%, 16.6%, 31.1%, and 43.7%, respectively, compared to the control treatment (non-primed). Seed priming with TiO_2 significantly reduced the mean values of germination percentage and mean daily germination compared to the control treatment. In contrast, seed priming with Si did not result in a significant difference in the mean values of germination percentage, mean daily germination, and seedling vigor index compared to the control treatment (Table 2).

In the comparison of the mean interaction effect of drought stress on seed priming, the highest seed germination rate under a drought stress level of −0.3 MPa was observed with seed priming using ZnO, with a mean of 8.32 seeds per day. This result showed a 33.1% increase compared to the control treatment. Conversely, seed priming with TiO_2 under severe drought stress conditions (−0.9 Mpa) resulted in the lowest mean seed germination rate of 2.05 seeds per day. The findings revealed that the application of Si and ZnO under high levels of drought stress (−0.6 and −0.9 MPa) resulted in a significant increase in the mean seed germination rate compared to the non-primed treatment (Fig. 1).

3.2 Biochemical and physiological attributes of seedlings

Based on the analysis of variance (Table 1), it was found that drought stress had a significant effect on the total chlorophyll, chlorophyll a and b, as well as the activity of antioxidant enzymes catalase and peroxidase. Moreover, seed priming significantly affected the total chlorophyll and chlorophyll a, as well as the activity of antioxidant enzymes. The total chlorophyll content and peroxidase enzyme activity significantly interacted with drought stress and seed priming.

The drought stress levels of zero and −0.3 MPa had the highest content of chlorophyll a (8.81 and 8.3 μg g^{-1} FW, respectively) and chlorophyll b (5.38 and 5.97 μg g^{-1} FW, respectively). According to the results, drought stress reduced the mean values of chlorophyll a and b. In particular, the treatment with −0.9 MPa of drought stress decreased 15.48% and 20.4% in chlorophyll a and b, respectively, compared to the control treatment (Table 3).

Priming the seeds with TiO_2, Si, and ZnO NMs led to a rise in chlorophyll a and b content compared to the non-primed treatment. However, there was no notable distinction between the various seed priming combinations in relation to these two characteristics (chlorophyll a and b) (Table 3). As shown in Fig. 2, the highest total chlorophyll content was observed in seed priming with ZnO under non-drought stress conditions (16.87 μg g^{-1} FW), indicating a 27.1% increase compared to the control treatment. Additionally, under a drought stress level of −0.3 MPa, the use of TiO_2 and Si treatments also resulted in the highest mean values of this characteristic. Under severe drought stress conditions (−0.9 MPa), seed priming with all three tested combinations significantly increased total chlorophyll content. The lowest mean value of this characteristic was obtained in the non-primed treatment under severe drought stress conditions (8.54 μg g^{-1} FW).

Increasing the concentration of PEG-6000 in the seedling growth medium resulted in a significant increase in the activity of the antioxidant enzyme catalase. The highest activity was observed under a drought stress level of -0.9 MPa (0.92 U mg^{-1} protein min^{-1}), showing a 42.3% increase compared to the control treatment (Table 3). Furthermore, the comparison between different seed priming treatments showed that the use of ZnO and TiO_2 resulted in an increase and decrease in the catalase enzyme activity, respectively, compared to the non-primed treatment. In other words, the highest activity of the catalase enzyme was observed in the ZnO treatment (0.84 U mg^{-1} protein min^{-1}), while the lowest activity was associated with the TiO2 treatment (0.63 U mg^{-1} protein min^{-1}) (Table 3).

In the comparison of mean values for the interaction between drought stress and seed priming, the highest peroxidase enzyme activity was observed in non-primed seeds and seeds primed with ZnO under the highest drought stress level (15.31 and 15.84 U mg^{-1} protein min^{-1}, respectively). In contrast, the lowest activity of this enzyme was observed in all four seed priming treatments under non-drought stress conditions and at a drought stress level of −0.3 MPa (Fig. 3).

3.3 Correlation coefficients

The results of Pearson correlation analysis between germination, growth, biochemical, and physiological traits revealed significant positive and negative correlations (Table 4). For instance, the germination percentage exhibited a significant negative correlation

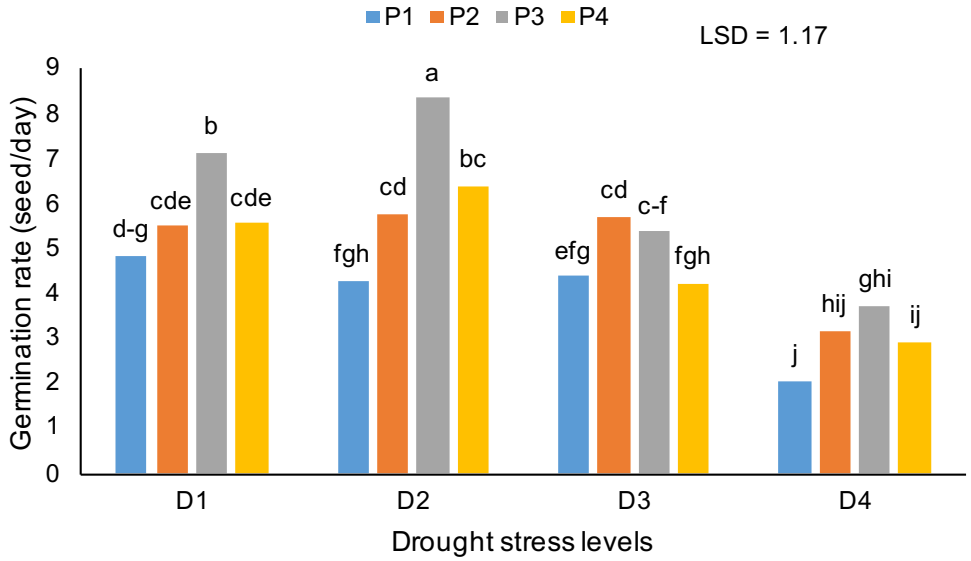

Figure 1. Germination rate of stevia seeds under different drought levels (D1: 0, D2: −0.3, D3: −0.6, and D4: −0.9 MPa) and seed priming treatments (P1: TiO_2, P2: Si, P3: ZnO, P4: non-primed seed as a control) (different letters in each factor indicate significant differences according to LSD test at $p<0.05$)

Table 3. Effects of drought stress levels and priming on seedling biochemical and physiological attributes of stevia

Treatments	Chl-a	Chl-b	CAT
Drought stress (MPa)			
0 (as control)	8.81±1.32 a	5.38±1.87 ab	0.53±0.08 d
−0.3	8.3±0.99 a	5.97±1.52 a	0.69±0.12 c
−0.6	8.17±0.67 ab	4.96±1.08 ab	0.82±0.12 b
−0.9	7.28±1.7 b	4.29±1.35 b	0.92±0.12 a
LSD ($p\leq0.05$)	0.89	1.09	0.06
Priming			
TiO_2	8.46±1.26 a	5.81±1.39 a	0.63±0.15 c
Si	8.76±0.85 a	4.9±1.6 ab	0.76±0.15 b
ZnO	8.2±1.34 a	5.28±1.85 ab	0.84±0.21 a
Control (untreated seed)	7.15±1.3 b	4.62±1.27 b	0.74±0.17 b
LSD ($p\leq0.05$)	0.89	1.09	0.06

Chl-a = chlorophyll a (μg g^{-1} FW), and Chl-b = chlorophyll b (μg g^{-1} FW), CAT = catalase activity (U mg^{-1} protein min^{-1}); Means followed by the same letter in each column are not significantly different according to the LSD test at a 5% level

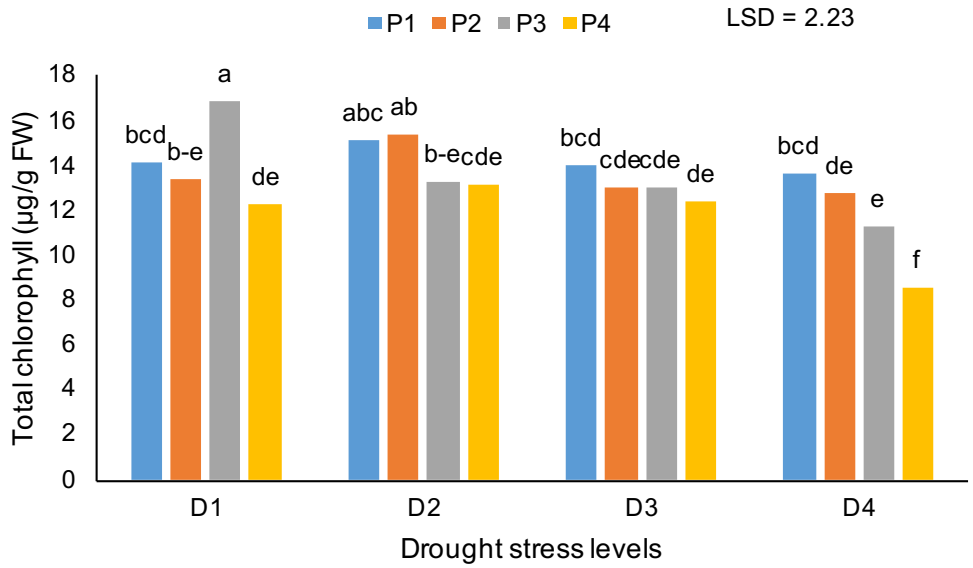

Figure 2. Total chlorophyll content of stevia seeds under different drought levels (D1: 0, D2: −0.3, D3: −0.6, and D4: −0.9 MPa) and seed priming treatments (P1: TiO_2, P2: Si, P3: ZnO, P4: non-primed seed as a control) (different letters in each factor indicate significant differences according to LSD test at $p<0.05$)

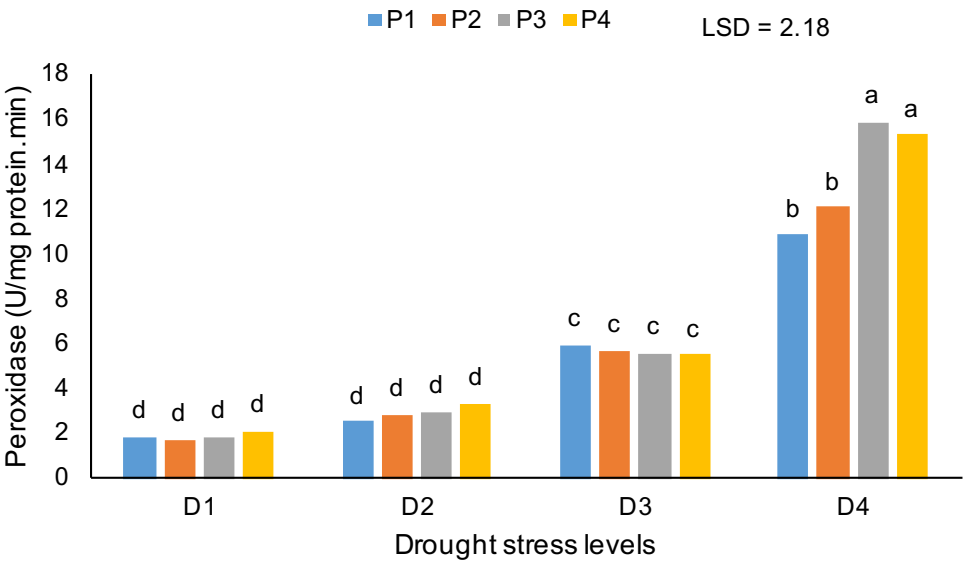

Figure 3. Peroxidase activity of stevia seeds under different drought levels (D1: 0, D2: −0.3, D3: −0.6, and D4: −0.9 MPa) and seed priming treatments (P1: TiO_2, P2: Si, P3: ZnO, P4: non-primed seed as a control) (different letters in each factor indicate significant differences according to LSD test at $p<0.05$)

with the mean germination time (−0.72) and the activity of antioxidant enzymes catalase (−0.43) and peroxidase (−0.80) while showing a significant positive correlation with the other of the measured traits. Conversely, the activity of antioxidant enzymes showed a significant negative correlation with the germination, growth, and biochemical characteristics, unlike the chlorophyll content of the seedlings (Table 4).

3.4 Stepwise regression

To determine the most influential biochemical and physiological attributes related to stevia seed germination percentage, a stepwise regression analysis was performed, and the results are presented in Table 5. The investigation revealed that the regression model included two traits, namely catalase and peroxidase activities, which together explained 71.99% of the variability in seed germination percentage.

4 Discussion

This research aims at assess the performance of various germination and growth indicators, as well as biochemical and physiological parameters of stevia seedlings exposed to drought stress caused by PEG-6000 and seed priming with nano compounds such as ZnO, TiO_2, and Si. According to the findings, elevated levels of drought stress (−0.6 and −0.9 MPa) resulted in a considerable reduction in the germination percentage, germination rate, mean daily germination, and seedling vigor index. Despite this, mild drought stress levels (−0.3 MPa) did not adversely affect the germination and seedling growth parameters. They even caused an improvement in some traits, such as the seedling length and the seedling vigor index. Severe drought stress can have adverse effects on seed germination and seedling growth due to factors such as delayed or reduced germination rates, damage to the seedling structure, reduced root and shoot length, reduced water and nutrient availability, disrupted cellular processes and accumulation of reactive oxygen species (Marthandan et al., 2020; Afshari et al., 2022).

Seed germination is a process that relies heavily on water availability. However, drought stress can impede the absorption of water by seeds, leading to inhibited germination even when other growth conditions are favorable. This can also cause a decrease in seedling vigor and hinder germination by reducing water intake (Gorzi et al., 2018; Wahab et al., 2022). During the early stages of crop development, drought stress can negatively affect the establishment of a stand due to decreased seed germination. In studies conducted on Stevia, exposure to drought stress resulted in poor seedling germination (Gorzi et al., 2018; Afshari et al., 2022). According to Jain et al. (2019), drought stress has reduced the germination rate. This is because PEG molecules impede oxygen flow through the cell membranes, disrupting the oxygen supply to the radicals.

The findings indicated that treating seeds with NMs like ZnO and Si through priming enhanced seed germination parameters and seedling growth in contrast to the untreated control. Studies have demonstrated that treating wheat (Rai-Kalal and Jajoo, 2021), rice (Anwar et al., 2021), and stevia (Shahverdi et al., 2017; Afshari et al., 2022) with nutrient solutions during priming can enhance seed germination and seedling vigor.

In general, ZnO priming was found to be the most effective. These results align with several other studies that reported increased and quicker seed germination and synchronized emergence of primed seeds in crop species (Rai-Kalal and Jajoo, 2021; Afshari et al., 2022). Among metal oxide nanoparticles, ZnO has garnered the attention of numerous researchers due to their exceptional photocatalytic and photo-oxidizing capabilities against chemical and biological species (Salem and Awwad, 2022). ZnO and zinc sulphates ($ZnSO_4.H_2O$ or $ZnSO_4.7H_2O$) are commonly utilized as Zn fertilizers to address Zn deficiency in plants. However, their use as fertilizers is limited because of their low solubility in soil, which results in limited Zn availability to plants Lateef et al. (2016). Due to their higher reactivity, ZnO nanoparticles can overcome this limitation by providing a more soluble and available form of Zn to plants. Studies have shown that priming seeds with ZnO nanoparticles can increase the zinc content in primed seeds, thus contributing to better seed germination, seedling growth, and yield (Rameshraddy et al., 2017; Rai-Kalal and Jajoo, 2021). The increase in the seed germination parameters observed in NMs seeds can be explained by the influence of Zn, which triggers various biochemical changes in the seeds that are essential for initiating the germination process. These changes include breaking dormancy, hydrolyzing or metabolizing inhibitors, facilitating imbibition, and activating enzymes (Samad et al., 2014; Rai-Kalal and Jajoo, 2021). Previous studies have shown that Zn is essential for metabolizing carbohydrates and proteins, resulting in improved and synchronized germination of seeds treated with ZnO NMs. Adding Zn to the priming solution enhances emergence and growth of seedlings, which may be attributed to its involvement in the early stages of coleoptile and radicle development (Ozturk et al., 2006).

The presence of ZnO NMs can increase the seedling length due to the role of Zn in promoting the biosynthesis of hormones like auxins and gibberellins, which are essential for seed growth (Mishra et al., 2023). Zn also plays a significant role in carbohydrate and protein metabolism, resulting in better and synchronized germination of seeds primed with ZnO NMs (Rai-Kalal and Jajoo, 2021). The addition

Table 4. Correlation coefficients among seed germination, seedling growth, biochemical and physiological characteristics of Stevia under drought stress and priming treatments

	GP	GR	GT	GD	SL	SVI	Tot Chl	Chl−a	Chl−b	CAT
GR	0.97**									
MGT	−0.72**	−0.81**								
MDG	0.99**	0.97**	−0.71**							
SL	0.71**	0.68**	−0.57**	0.69**						
SVI	0.93**	0.92**	−0.73**	0.92**	0.89**					
TChl	0.46*	0.42*	−0.29ns	0.46*	0.72**	0.59**				
Chl-a	0.47*	0.43*	−0.36**	0.45*	0.66**	0.58**	0.84**			
Chl-b	0.34*	0.31*	−0.16ns	0.34*	0.58**	0.44*	0.88**	0.48*		
CAT	−0.43**	−0.35*	0.17ns	−0.43*	−0.41*	−0.42*	−0.64**	−0.54**	−0.56**	
POD	−0.80**	−0.72**	0.48*	−0.80**	−0.67**	−0.76**	−0.70**	−0.70**	−0.53**	0.80**

ns: non-significant, * and **: significant at $\alpha = 0.05$ and 0.01%, GP: germination percentage, GR: germination rate, GT: mean germination time, GD: mean daily germination, SL: seedling length, SVI: seedling vigor index, Tot Chl: total chlorophyll, Chl-a: chlorophyll a, Chl-b: chlorophyll b, CAT: catalase activity, and POD: peroxidase activity

Table 5. Stepwise regression for stevia seed germination percentage as a dependent variable and other seedling biochemical and physiological attributes as independent variables

Term	Coef	SE Coeff	T-value	P-value
Constant	30.39	9.86	3.08	0.009
CAT (X_1)	41.3	16.6	2.49	0.027
POD (X_2)	−3.262	0.5903.08	−5.52	0

R^2(adj) = 71.99%

$Y = 30.39 + 41.3(X_1) - 3.262(X_2)$

CAT = catalase activity, POD = peroxidase activity

of Zn to the priming solution promotes emergence and growth of seedlings by aiding in the early development of coleoptile and radicle (Rehman et al., 2021).

The current research findings indicate a significant reduction in photosynthetic pigments, such as chlorophyll a, b, and total, when seedlings are subjected to drought stress. Previous studies conducted by other researchers have reported a decline in the level of photosynthetic pigments in stevia seedlings under drought stress conditions (Gorzi et al., 2018; Afshari et al., 2022). Parveen et al. (2019) reported that *Zea mays* L. exposed to drought stress experienced a decrease in their photosynthetic pigments. This was attributed to excessive production of ROS, inefficient nutrient uptake by the plants, and disruptions in enzyme activities at the cellular level.

Furthermore, the research results indicated that seed priming, specifically under severe drought stress conditions (−0.9 MPa), increased the average total chlorophyll content. Seed priming with Si had an increasingly positive impact on the total chlorophyll content, both under stress and non-stress conditions (Fig. 2). In accordance with the results of this research, previous studies have reported an increase in the photosynthetic pigment content in seed priming of various plants under different stress conditions. For instance, seed priming of *Lathyrus odoratus* L. under salinity stress (El-Serafy et al., 2021), *Triticum aestivum* L. under salinity stress (Mushtaq et al., 2017), *Medicago sativa* L. under alkaline stress (Liu et al., 2018), and *Zea mays* L. under drought stress (Parveen et al., 2019) have all shown positive effects on the photosynthetic pigment content. Similar to the results of the present study, the application of Si treatments improved seed germination in *Lathyrus* (El-Serafy et al., 2021). Related research discovered that Si played a vital role in the physiological process of seed germination in *Glycyrrhiza uralensis* when subjected to saline conditions (Zhang et al., 2015).

Under stress conditions, Si can improve photosynthetic activity by reducing ion toxicity and reactive oxygen species (ROS) content (Manzoor et al., 2022). Si also helps maintain various photosynthesis aspects, including stomatal conductance, transpiration, membrane permeability, net photosynthesis, and chlorophyll levels (El-Serafy et al., 2021). Furthermore, Si can decrease leaf curvature angle and

increase leaf flatness, which allows for greater light interception and more photosynthetic pigments. As a result, seedlings can accumulate more carbohydrates and dry matter (Ning et al., 2020).

The findings indicated that the activity of antioxidant enzymes, specifically catalase and peroxidase, increased due to drought stress. The results of the current study were consistent with similar findings previously reported (Parveen et al., 2019). A complex antioxidant defense system consisting of enzymatic (peroxidase and catalase) and non-enzymatic antioxidants significantly removes ROS (Dumanović et al., 2021).

The observed results could be explained by the improvement of seedling vigor and the strengthening of the antioxidant defense system induced by seed priming. Additionally, seed priming with ZnO may have reduced oxidative damage in the primed seedlings. Based on stepwise regression results, the effect of enzymatic activity, specifically catalase, and peroxidase, on the seed germination percentage was more significant than that of photosynthetic pigment content. Severe drought stress conditions caused certain NMs to reduce the activity of antioxidant enzymes, while others caused an increase in activity. ZnO was identified as the most effective priming compound based on its level of antioxidant enzyme activity. An increase in superoxide dismutase, catalase, and peroxidase activities was observed in rice plants derived from ZnO NMs-primed seeds (Mazhar et al., 2022).

5 Conclusion

The study found that different types of nanocompounds resulted in either an increase or decrease in certain traits compared to the control treatment (unprimed seed). Overall, priming seeds with ZnO nanoparticles yielded more favorable outcomes regarding seed germination, seedling growth, and biochemical and physiological characteristics than other treatments. This was attributed to the adjustment of antioxidant enzyme activity levels and increased photosynthetic pigment content, particularly under severe drought stress conditions, which helped mitigate the adverse effects of increased PEG levels. For obtaining more reliable results and exploring additional physiological and biochemical effects (including the effects of these compounds on the levels of medicinal metabolites in this plant), conducting this study under diverse environmental conditions is suggested.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

A LT. 2012. Effect of salicylic acid (SA) and gibberellic acid (GA3) pre-soaking on seed germination of stevia (stevia rebaudiana) under salt stress. Journal of Medicinal Plants Research 6. doi: 10.5897/jmpr11.1106.

Abdul-Baki AA, Anderson JD. 1973. Vigor determination in soybean seed by multiple criteria. Crop Science 13:630–633. doi: 10.2135/cropsci1973.0011183x001300060013x.

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

Afshari F, Nakhaei F, Mosavi S, Seghatoleslami M. 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. doi: 10.1016/j.indcrop.2021.114399.

Ahmed M, Decsi K, Tóth Z. 2022. Different tactics of synthesized zinc oxide nanoparticles, homeostasis ions, and phytohormones as regulators and adaptatively parameters to alleviate the adverse effects of salinity stress on plants. Life 13:73. doi: 10.3390/life13010073.

Anwar MP, Jahan R, Rahman MR, Islam AKMM, Uddin FMJ. 2021. Seed priming for increased seed germination and enhanced seedling vigor of winter rice. IOP Conference Series: Earth and Environmental Science 756:012047. doi: 10.1088/1755-1315/756/1/012047.

Arun MN, Hebbar SS, Senthivel T, Nair AK, Padmavathi G, Pandey P, Singh A. 2022. Seed priming: The way forward to mitigate abiotic stress in crops. volume 11. IntechOpen London, UK.

Asmuni NA, Hakiman M. 2020. *In vitro* responses of *Stevia rebaudiana* Bert. to MS basal medium supplemented with 6-benzylaminopurine and indole-3-butyric acid. Fundamental and Applied Agriculture 5:243–247. doi: 10.5455/faa.103572.

Behboudi F, Tahmasebi Sarvestani Z, Kassaee MZ, Modares Sanavi SAM, Sorooshzadeh A. 2018. Improving growth and yield of wheat under drought stress via application of SiO_2 nanoparticles. Journal of Agricultural Science and Technology 20:1479–1492.

Bhatia P, Gupta M. 2022. Micronutrient seed priming: new insights in ameliorating heavy metal stress. Environmental Science and Pollution Research 29:58590–58606. doi: 10.1007/s11356-022-21795-6.

Buyuk G, Bayram CA, Kırpık M, İnan M. 2022. Agronomic performance and nutrient content of stevia (*Stevia rebaudiana* Bertoni) in different semi-arid locations. Communications in Soil Science and Plant Analysis 53:848–861. doi: 10.1080/00103624.2022.2028819.

Dumanović J, Nepovimova E, Natić M, Kuča K, Jaćević V. 2021. The significance of reactive oxygen species and antioxidant defense system in plants: A concise overview. Frontiers in Plant Science 11:552969. doi: 10.3389/fpls.2020.552969.

El-Serafy RS, El-Sheshtawy ANA, Atteya AK, Al-Hashimi A, Abbasi AM, Al-Ashkar I. 2021. Seed priming with silicon as a potential to increase salt stress tolerance in *Lathyrus odoratus*. Plants 10:2140. doi: 10.3390/plants10102140.

González-García Y, López-Vargas ER, Pérez-Álvarez M, Cadenas-Pliego G, Benavides-Mendoza A, Valdés-Reyna J, Pérez-Labrada F, Juárez-Maldonado A. 2022. Seed priming with carbon nanomaterials improves the bioactive compounds of tomato plants under saline stress. Plants 11:1984. doi: 10.3390/plants11151984.

Gorzi A, Omidi H, Bostani AB. 2018. Morpho-physiological responses of Stevia (*Stevia rebaudiana* Bertoni) to various priming treatments under drought stress. Applied Ecology and Environmental Research 16:4753–4771. doi: 10.15666/aeer/1604_47534771.

Jain M, Kataria S, Hirve M, Prajapati R. 2019. Water deficit stress effects and responses in maize. In: Plant Abiotic Stress Tolerance: Agronomic, Molecular and Biotechnological Approaches. Springer. doi: 10.1007/978-3-030-06118-0_5.

Khan I, Awan SA, Rizwan M, Akram MA, ur Rehman MZ, Wang X, Zhang X, Huang L. 2023. Physiological and transcriptome analyses demonstrate the silver nanoparticles mediated alleviation of salt stress in pearl millet (*Pennisetum glaucum* L). Environmental Pollution 318:120863. doi: 10.1016/j.envpol.2022.120863.

Lateef A, Nazir R, Jamil N, Alam S, Shah R, Khan MN, Saleem M. 2016. Synthesis and characterization of zeolite based nano–composite: An environment friendly slow release fertilizer. Microporous and Mesoporous Materials 232:174–183. doi: 10.1016/j.micromeso.2016.06.020.

Lichtenthaler HK, Buschmann C. 2001. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. Current Protocols in Food Analytical Chemistry 1:F4.3.1–F4.3.8. doi: 10.1002/0471142913.faf0403s01.

Liu D, Liu M, Liu XL, Cheng XG, Liang ZW. 2018. Silicon priming created an enhanced tolerance in alfalfa (*Medicago sativa* L.) seedlings in response to high alkaline stress. Frontiers in Plant Science 9:716. doi: 10.3389/fpls.2018.00716.

MacAdam JW, Nelson CJ, Sharp RE. 1992. Peroxidase activity in the leaf elongation zone of tall fescue. Plant Physiology 99:872–878. doi: 10.1104/pp.99.3.872.

Maehly AC. 1954. The Assay of Catalases and Peroxidases. Wiley. doi: 10.1002/9780470110171.ch14.

Maguire JD. 1962. Speed of germination—aid in selection and evaluation for seedling emergence and vigor. Crop Science 2:176–177. doi: 10.2135/cropsci1962.0011183x000200020033x.

Manzoor N, Ali L, Ahmed T, Rizwan M, Ali S, Shahid MS, Schulin R, Liu Y, Wang G. 2022. Silicon oxide nanoparticles alleviate chromium toxicity in wheat (*Triticum aestivum* L.). Environmental Pollution 315:120391. doi: 10.1016/j.envpol.2022.120391.

Marthandan V, Geetha R, Kumutha K, Renganathan VG, Karthikeyan A, Ramalingam J. 2020. Seed priming: A feasible strategy to enhance drought tolerance in crop plants. International Journal of Molecular Sciences 21:8258. doi: 10.3390/ijms21218258.

Mazhar MW, Ishtiaq M, Hussain I, Parveen A, Bhatti KH, Azeem M, Thind S, Ajaib M, Maqbool M, Sardar T, Muzammil K, Nasir N. 2022. Seed nano-priming with zinc oxide nanoparticles in rice mitigates drought and enhances agronomic profile. PLOS ONE 17:e0264967. doi: 10.1371/journal.pone.0264967.

Michel BE, Kaufmann MR. 1973. The osmotic potential of polyethylene glycol 6000. Plant Physiology 51:914–916. doi: 10.1104/pp.51.5.914.

Mishra D, Chitara MK, Negi S, singh JP, Kumar R, Chaturvedi P. 2023. Biosynthesis of zinc oxide nanoparticles via leaf extracts of *Catharanthus roseus* (L.) G. Don and their application in improving seed germination potential and seedling vigor of *Eleusine coracana* (L.) Gaertn. Advances in Agriculture 2023:1–11. doi: 10.1155/2023/7412714.

Mushtaq A, Jamil N, Riaz M, Hornyak G, Ahmed N, Ahmed SS, Shahwani MN, Malghani MNK. 2017. Synthesis of silica nanoparticles and their effect on priming of wheat (*Triticum aestivum* L.) under salinity stress. In: Biol. Forum. volume 9. p. 150–157.

Nile SH, Thiruvengadam M, Wang Y, Samynathan R, Shariati MA, Rebezov M, Nile A, Sun M, Venkidasamy B, Xiao J, Kai G. 2022. Nano-priming as emerging seed priming technology for sustainable agriculture—recent developments and future perspectives. Journal of Nanobiotechnology 20:1–31. doi: 10.1186/s12951-022-01423-8.

Ning D, Qin A, Liu Z, Duan A, Xiao J, Zhang J, Liu Z, Zhao B, Liu Z. 2020. Silicon-mediated physiological and agronomic responses of maize to drought stress imposed at the vegetative and reproductive stages. Agronomy 10:1136. doi: 10.3390/agronomy10081136.

Ozturk L, Yazici MA, Yucel C, Torun A, Cekic C, Bagci A, Ozkan H, Braun HJ, Sayers Z, Cakmak I. 2006. Concentration and localization of zinc during seed development and germination in wheat. Physiologia plantarum 128:144–152. doi: 10.1111/j.1399-3054.2006.00737.x.

Parveen A, Liu W, Hussain S, Asghar J, Perveen S, Xiong Y. 2019. Silicon priming regulates morpho-physiological growth and oxidative metabolism in maize under drought stress. Plants 8:431. doi: 10.3390/plants8100431.

Rai-Kalal P, Jajoo A. 2021. Priming with zinc oxide nanoparticles improve germination and photosynthetic performance in wheat. Plant Physiology and Biochemistry 160:341–351. doi: 10.1016/j.plaphy.2021.01.032.

Rameshraddy, Pavithra GJ, Reddy BHR, Salimath M, Geetha KN, Shankar AG. 2017. Zinc oxide nano particles increases Zn uptake, translocation in rice with positive effect on growth, yield and moisture stress tolerance. Indian Journal of Plant Physiology 22:287–294. doi: 10.1007/s40502-017-0303-2.

Rehman A, Nadeem F, Farooq M. 2021. Role of Seed Priming in Root Development and Crop Production. In: In The root systems in sustainable agricultural intensification. Wiley. doi: 10.1002/9781119525417.ch8.

Salem NM, Awwad AM. 2022. Green synthesis and characterization of ZnO nanoparticles using solanum rantonnetii leaves aqueous extract and antifungal activity evaluation. Chem. Int 8:12–17.

Samad A, Khan MJ, Shah Z, Tariq Jan M. 2014. Determination of optimal duration and concentration of zinc and phosphorus for priming wheat seed. Sarhad Journal of Agriculture 30.

Shahverdi MA, Omidi H, Damalas CA. 2020. Foliar fertilization with micronutrients improves stevia rebaudiana tolerance to salinity stress by improving root characteristics. Brazilian Journal of Botany 43:55–65. doi: 10.1007/s40415-020-00588-6.

Shahverdi MA, Omidi H, Mosanaiey H, Pessarakli M, Mousavi SE, Ghasemzadeh M. 2019. Effects of light and temperature treatments on germination and physiological traits of stevia seedling (*Stevia rebuadiana* Bertoni). Journal of Plant Nutrition 42:1125–1132. doi: 10.1080/01904167.2019.1567781.

Shahverdi MA, Omidi H, Tabatabaei SJ. 2017. Effect of nutri-priming on germination indices and physiological characteristics of stevia seedling under salinity stress. Journal of Seed Science 39:353–362. doi: 10.1590/2317-1545v39n4172539.

Wahab A, Abdi G, Saleem MH, Ali B, Ullah S, Shah W, Mumtaz S, Yasin G, Muresan CC, Marc RA. 2022. Plants' physio-biochemical and phytohormonal responses to alleviate the adverse effects of drought stress: A comprehensive review. Plants 11:1620. doi: 10.3390/plants11131620.

Zhang XH, Zhou D, Cui JJ, Ma HL, Lang DY, Wu XL, Wang ZS, Qiu HY, Li M. 2015. Effect of silicon on seed germination and the physiological characteristics of *Glycyrrhiza uralensis* under different levels of salinity. The Journal of Horticultural Science and Biotechnology 90:439–443. doi: 10.1080/14620316.2015.11513207.

The Official Journal of the **Farm to Fork Foundation**
ISSN: 2518–2021 (print)
ISSN: 2415–4474 (electronic)
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