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Effects of Nano-Silica and Multi-Walled Carbon Nanotubes on Grape Seedlings under Salt Stress

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Abstract: To improve the salt tolerance of grape seeds and seedlings under salt stress, this study was conducted including two control groups (CK, S) and five experimental groups (S + Si40, S + Si60, S + Si80, S + C90, S + Si40 + C90), and the physiological characteristics of grape seed germination and seedlings were studied using 40, 60, and 80 µg/mL of nano-silica treatments and by mixing 40 µg/mL of nano-silica with 90 µg/mL of multi-walled carbon nanotubes (MWCNTs), respectively. The combined treatment of 40 µg/mL nano-silica and 90 µg/mL MWCNTs resulted in the best rate of growth in grape seeds and root length and an increased germination rate when compared with the other concentrations. The combined treatment reduced the MDA content in the grape seedling leaves and increased the activities of superoxide (SOD), peroxidase (POD), catalase (CAT), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), ascorbate peroxidase (APX), glutathione-s-transferase (GT), and glutathione reductase (GR). In addition, the scavenging activity of DPPH· was also maintained by the combined treatment. In conclusion, a combined treatment with 40 µg/mL nano-silica and 90 µg/mL MWCNTs significantly increased the reduction capacity through the direct and indirect antioxidant systems (AsA-GSH cycle) and maintained a high antioxidant capacity of grape seedlings under salt stress.

Keywords: nano-silica; combined treatment; active oxygen; photosynthetic properties; antioxidase activity

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1. Introduction

As a deciduous vine of the genus Vitis of the Vitaceae, grape features a relatively high economic value and has, thus, become one of the most widely distributed fruit trees across the world. Soil salinization is a worldwide problem impairing agricultural production, with salt stress leading to the substantial inhibition of plant growth and a significant reduction in fruit yield and quality. Although grapes exhibit a certain level of salt tolerance, severe salinity still limits the healthy and sustainable development of the grape industry.

Plants have direct and indirect antioxidant systems to remove excessive reactive oxygen species and maintain the balance of reactive oxygen species [1,2]. The ascorbic acid–glutathione (AsA-GSH) cycle is a representative indirect antioxidant system catalyzing the conversion of hydrogen peroxide to water, which is mainly composed of three interdependent redox pairs and the following enzymes: dehydroascorbate reductase (DHAR); glutathione reductase (GR); monodehydroascorbate reductase (MDHAR); and ascorbate peroxidase (APX) [1,3].

As a member of the carbonaceous nanomaterials family, multi-walled carbon nanotubes (MWCNTs) are well known for their nanoscale size below 100 nm and unique physical/chemical properties, including a high-surface area-to-volume ratio and high reactivity. Notably, MWCNTs can act as a plant growth regulator [4,5].

Silicon is one of the most abundant elements on Earth, but no specific research has shown that silicon is an essential element for plant growth and development. In recent years, due to the particularity of nano-silica materials, scholars have carried out in-depth research on their application in agricultural production, obtaining some positive results. For example, Yuvakkumar et al. [6] mixed nanosilicon (30 nm) in the soil phase and found that 100 and 200 mg/kg nanosilicon treatments significantly increased the germination rate of maize seeds, the number of seedling leaves, and the height and roughness of corn stalks. Siddiqui et al. [7] found that nanosilicon (12 nm) effectively improved the tomato seed vitality and seed germination rate.

However, there are few studies on the salt tolerance of grapes treated with nano-silica and MWCNTs together. Based on previous studies from the same research group (School of Agricultural Science and Technology, Jinan, China), it was found that 90 μg/mL of MWCNTs can enhance the reducing ability of the antioxidant system in grape seedlings and can alleviate salinity [2]. Building on previous research, in this study, grape seedlings were used as test materials to study the effects of co-treatment with nano-silica and MWCNTs on the photosynthetic characteristics of grape seedling leaves and plant growth. The optimal treatment conditions, including material compositions and concentrations, were screened to alleviate salt stress in grape seedlings to achieve the maximum effect. This study provides a theoretical basis for further research on the possible mechanism of grapevine tolerance to salt stress and the regulatory role of nano-silica and multi-walled carbon nanotubes in this process.

2. Materials and Methods

2.1. Experiment Material

From the grape seedlings of the American red grape variety, the seedlings with relatively robust and approximate growth were selected as the test materials, and pot experiments were carried out in a greenhouse. In this experiment, sodium chloride (analytical pure NaCl) was used as the salt source of salt stress with a concentration of 4 g/L, and MWCNTs and nanosilicon were purchased from Chengdu Organic Chemical Co., Ltd., Chinese Academy of Sciences (Sichuan, China). The outer diameter of MWCNTs was 4–6 nm; the purity was over 98%; the length was 10–20 microns, and the specific surface area was 380–550 m^2/g. The average particle size of nano-silica was 30 nm, with a purity of over 99%, a specific surface area of 42.4 m^2/g, and a bulk density of 0.19 g/cm^3.

2.2. Methods

2.2.1. Determination of Germination Indexes of Grape Seeds

Grape seeds were soaked in a 4 g/L GA solution for 48 h to release the dormancy. Subsequently, they were transferred to a sterile workbench, disinfected with 70% ethanol for 15 s, treated with 0.2% mercuric chloride for 5 min, washed with distilled water five times, and finally immersed in deionized water and a suspension of MWCNTs for 5 h each. Seeds were transferred into Petri dishes, each containing 10 seeds. Each treatment was repeated with 10 Petri dishes. A filter paper (100 mm × 100 mm) was placed into each culture dish, and 5 mL of deionized water and 5 mL of NaCl solution were added, respectively. The next day, multi-walled carbon nanotubes (MWCNTs) and nano-silica suspensions with varying concentrations were utilized for treatment. Then, 5 mL of multi-walled carbon nanotubes (MWCNTs) and 5 mL nano-silica were added, respectively, according to treatment groups for the first three days and 5 mL of deionized water for the following days. Dark treatment was performed in an incubator (with foil paper). When 65% of the control roots were longer than 5 mm, the germination rate of seeds was measured. After 7 days, the seedlings were washed, and the dry weight, fresh weight, and root length were measured.

The experiment consists of 7 treatment groups:

(1) CK, deionized water;
(2) S, 4 g/L NaCl solution;
(3) S + Si40, 4 g/L NaCl solution + 40 μg/mL nano-silica suspension;
(4) S + Si60, 4 g/L NaCl solution + 60 μg/mL nano-silica suspension;
(5) S + Si80, 4 g/L NaCl solution + 80 μg/mL nano-silica suspension;
(6) S + C90, 4 g/L NaCl solution + 90 μg/mL multi-walled carbon nanotube suspension;
(7) S + Si40 + C90, 4 g/L NaCl solution + 40 μg/mL nano-silica + 90 μg/mL MWCNTs suspension.

2.2.2. Determination of Photosynthetic Indexes

In this experiment, the soil culture pot was used, and the river sand was washed with water before loading the basin to wash away the salt ions. After washing, the basin was filled, the Hoagland culture medium was used, and the culture medium was changed every 4 days. Strong grape seedlings (cuttings) with consistent growth potential and a well-developed root system were selected for planting in April 2023 and watered after planting. After about 70 days of cultivation, the solid and consistent seedlings (seedlings were in the perennial growth stage) were selected for each treatment group, which is identical to that described in Section 2.2.1. Three replications were set up for each treatment.

Seven days after treatment, 3–4 functional leaves from top to bottom were selected from each plant, and some physiological indexes of the grape were measured [1] in the daytime from 10:00 to 12:00, including stomatal conductance (Gs), intercellular CO_2 concentration (Ci), net photosynthetic rate (Pn), and water efficiency rate (We), with an LI-6400XT portable photosynthesis system (LI-COR company, Lincoln, NE, USA). The built-in chamber temperature was set at (25 ± 2) °C, the CO_2 flux was 540 μmol/mol, and the optical quantum density was 900 μmol/(m^2·s).

2.2.3. Determination of Soluble Protein Content, Enzymatic Antioxidant Enzyme (SOD, POD, CAT) Activity, MDA Content, and DPPH· Free Radical Scavenging Activity

Samples were taken from the 3rd–4th functional leaves of each plant in pots counting down from the top in the first, second, and third weeks after salt treatment, respectively. After picking seedling leaves, they were immediately flash-frozen with liquid nitrogen and then stored at −80 °C in an ultra-cold refrigerator to measure the activity of various antioxidant enzymes.

Soluble protein content was determined via the Coomassie brilliant blue colorimetric method [8]. The MDA content was determined according to the thiobarbituric acid method proposed by Velikova et al. [9]. The measurement of SOD activity refers to the method of Prochazkova et al. [10], and the measurement of CAT activity and POD activity refers to the method of Cakmak et al. [11].

The scavenging activity of the DPPH· radical was assayed using the method described by Brand-Williams et al. [12].

2.2.4. Determination of Related Enzyme Activities in the AsA-GSH Cycle

The sampling method is identical to that described in Section 2.2.3. The ascorbate peroxidase (APX) activity was measured according to Nakano's method [13]. The MDHAR activity was determined using the method of Hodges and Forney [14]. The determination of DHAR activity was based on the method of Shan et al. [15]. The GR activity was determined according to the method of Hodges and Forney [14]. The GT activity was determined according to the method of Rogiers et al. [16].

2.3. Data Analysis

The processing and drawing of the experimental data were conducted using the Excel 2016 software package, and the analysis of significance was performed using the SPSS 26.0 software package.

3. Results

3.1. Effects of Two Nanomaterial Treatments on Grape Germination and Seedling Growth under Salt Stress

In order to investigate the potential impact of MWCNTs on grape root elongation and seed germination, sterile seeds were incubated in a combined treatment suspension with various concentrations and compositions (S + Si40, S + Si60, S + Si80, S + C90, and S + Si40 + C90). In a Petri dish, water was used as a control in the experiment. Compared with salt stress (S), the relative root length of seeds treated with S + Si40 + C90 increased significantly by 1.61 times. Compared with S + Si40 and S + C90, the combined treatment of S + Si40 + C90 resulted in root elongation by 15% and 21%, respectively (Table 1), while achieving a peak seed germination rate of 95%. No significant difference was observed between S + Si40 and S + C90. These results indicate that S + Si40 + C90 promoted root growth and enhanced seed germination rate following brief exposure to grape seeds in experimental settings without causing any significant impact on dry and fresh weight compared to the control group.

Table 1. Effect of MWCNTs of different concentrations on grape seed germination and varying root lengths under salt stress. CK indicates blank control; S indicates salt treatment but no namo-silica and MWCNTs. No. indicates seed numbers and root numbers. GR indicates seed germination rate. FW indicates average value of seed fresh weight. DW indicates average value of seeds dry weight. Different small letters after data indicate the significance at $p < 0.05$ level.

	Seedling				Root	
Treatment (T)	**No.**	**GR**	**FW (g)**	**DW (g)**	**No.**	**Length (cm)**
CK	86	86%	0.2162 ± 0.0044 b	0.0422 ± 0.0036 a	86	2.54 ± 0.14 d
S	57	57%	0.1477 ± 0.0049 e	0.0211 ± 0.0031 e	57	1.27 ± 0.18 f
S + Si40	90	90%	0.2052 ± 0.0105 c	0.0313 ± 0.0003 bc	90	2.89 ± 0.27 b
S + Si60	80	80%	0.1633 ± 0.0037 d	0.0284 ± 0.0014 d	80	2.41 ± 0.19 e
S + Si80	83	83%	0.1393 ± 0.0032 f	0.0280 ± 0.0009 d	83	2.69 ± 0.16 c
S + C90	88	88%	0.2059 ± 0.0035 c	0.0311 ± 0.0013 bc	88	2.84 ± 0.15 b
S + Si40 + C90	96	96%	0.2455 ± 0.0233 a	0.0316 ± 0.0014 b	96	3.32 ± 0.33 a

3.2. Effects of Two Nanomaterial Treatments on Photosynthetic Characteristics of Grape Seedling Leaves under Salt Stress

3.2.1. Effects of Two Nanomaterial Treatments on Leaf Stomatal Conductance (Gs) of Grape Seedlings under Salt Stress

As shown in Figure 1A, compared with the blank control (CK), the stomatal conductance of leaves in the salt stress treatment (S) decreased by 41.2%. Under salt stress, the indicator of nano-silica-treated samples was significantly lower than that of CK-treated leaves, regardless of nano-silica concentrations. After the combined treatment of S + Si40 + C90, there was an evident increase of 13.3% compared to CK. The results showed that salt stress would decrease the stomatal conductance of grape seedling leaves, while the combined treatment of nano-silica and MWCNTs significantly improved the stomatal conductance of grape seedling leaves under salt stress.

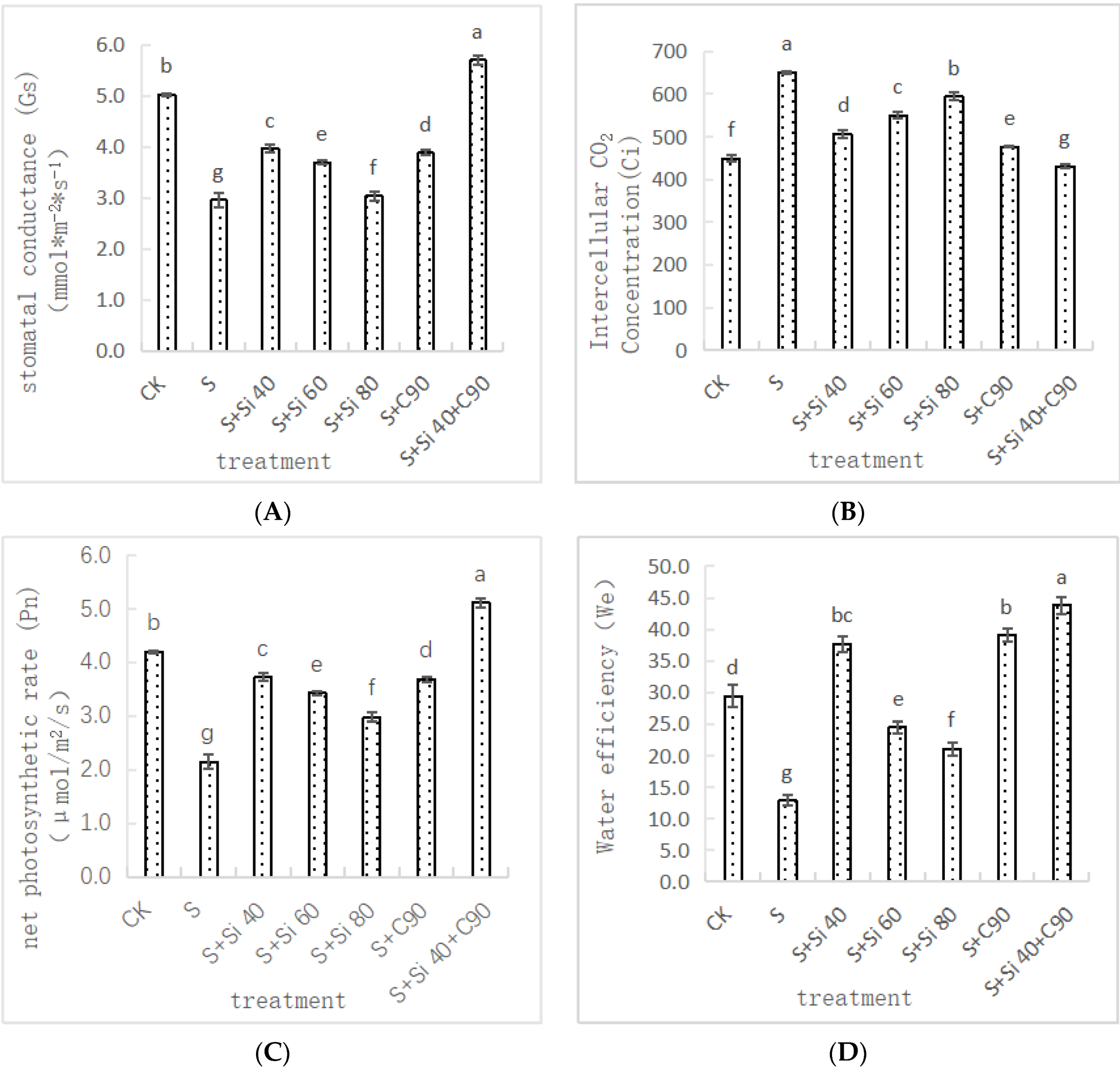


Figure 1. Effect of different treatments on grape photosynthetic parameters stomatal conductance (**A**), intercellular CO_2 concentration (**B**), net photosynthetic rate (**C**), and water efficiency (**D**) under salt stress. Different small letters after data indicate the significance at $p < 0.05$ level.

3.2.2. Effects of Two Nanomaterial Treatments on Intercellular CO_2 Concentration (Ci) in Grape Leaves under Salt Stress

As can be seen from Figure 1B, compared with the blank control (CK), the intercellular CO_2 concentration of the leaves treated with salt stress (S) increased by 51.7%. Under salt stress, the 40 μg/mL, 60 μg/mL, and 80 μg/mL nano-silica treatments (S + Si40, S + Si60, and S + Si80) resulted in reductions of 22.1%, 15.6%, and 8.7%, respectively. However, these values remained higher than the intercellular CO_2 concentration in CK leaves. After the combined treatment of S + Si40 + C90, the growth was suppressed to 15.0% lower than that of CK. The results indicated that salt stress increased the intercellular CO_2 concentration in grape seedling leaves. The combined treatment of nano-silica and MWCNTs was found to be the most effective in enhancing the intercellular CO_2 concentration in grape seedling leaves under salt stress, consequently boosting the photosynthetic rate of grape seedling leaves.

3.2.3. Effects of Two Nanomaterial Treatments on Net Photosynthetic Rate (Pn) of Grape Seedlings under Salt Stress

As shown in Figure 1C, compared with the blank control (CK), the net photosynthetic rate of leaves under salt stress (S) was significantly constrained, decreasing by 49% in the first week after treatment. Under the condition of salt stress, the net photosynthetic rates of nano-silica treatments at concentrations of 40 μg/mL, 60 μg/mL, and 80 μg/mL were 73.5%, 59.5%, and 38.6% higher than those of the salt stress treatments, respectively, but all were lower than those of the control (CK) leaves. The net photosynthetic rate of grape leaves treated with 40 μg/mL nano-silica was the highest. After the S + Si40 + C90 combined treatment, the net photosynthetic rate was significantly boosted by 21.8% compared to that of CK leaves. It was indicated that salt stress could significantly reduce the net photosynthetic rate of grape seedling leaves, a situation that can be reversed by nano-silica treatment during salt stress. The combined treatment of nano-silica and MWCNTs had the greatest impact on increasing the net photosynthetic rate of grape seedling leaves under salt stress.

3.2.4. Effects of Two Nanomaterial Treatments on Water Efficiency (We) of Grape Leaves under Salt Stress

As shown in Figure 1D, compared with the blank control (CK), the water efficiency of leaves exposed to salt stress treatment (S) decreased by 56.0%. The water efficiency of leaves treated with S + Si40 was 1.91 times higher than that under salt stress. After a combined treatment of S + Si40 + C90, the growth was significantly increased by 2.39 times compared to that under salt stress. The results indicated that salt stress would decrease the water efficiency of grape leaves, while the combined treatment of nano-silica and MWCNTs significantly improved the water efficiency of grape leaves under salt stress.

3.3. Effects of Two Nanomaterial Treatments on Soluble Protein Content and Antioxidant Enzyme Activities of Grape Seedlings under Salt Stress

3.3.1. Effects of Two Nanomaterial Treatments on Soluble Protein Content of Grape Seedlings under Salt Stress

It can be seen from Figure 2A that under salt stress, among the nano-silica treatments, the 40 μg/mL nano-silica treatment (S + Si40) induced the most significant enhancement in the soluble protein content. It increased by 82.9%, 85.4%, and 61.6%, respectively, compared to the samples undergoing the same period of salt stress. The combined treatment of S + Si40 + C90 resulted in an increase of 88.7%, 121.1%, and 89.0% over the salt stress control (S). The combined treatment also led to a 3.1%, 14.4%, and 10.2% increase compared to the S + Si40 treatment group and a 10.0%, 15.9%, and 9.8% increase compared to the S + C90 treatment group during the same period. The experiment demonstrated that the combined treatment of nano-silica and MWCNTs was the most effective in increasing the soluble protein content of grape seedling leaves under salt stress.

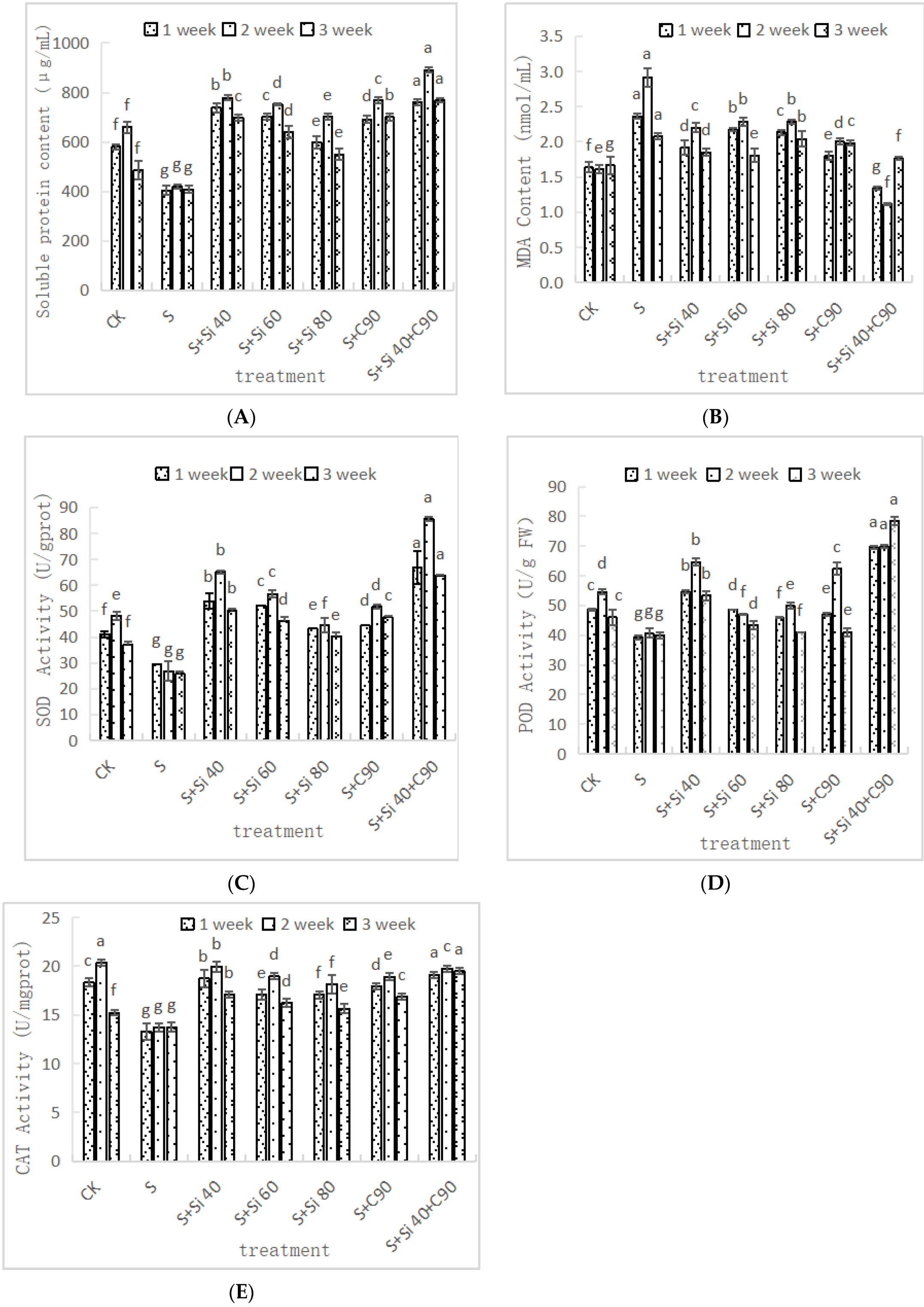


Figure 2. (**A**) Effects of different treatments on soluble protein content of grape seedlings; (**B**) effects of different treatments on malondialdehyde (MDA) content in leaves of grape seedlings; (**C**) effects of different treatments on the vitality of total superoxide dismutase (SOD) in leaves from grape seedlings; (**D**) effects of different treatments on leaf peroxidase (POD) vitality of grape seedlings; (**E**) effects of different treatments on CAT vitality of leaves from grape seedlings. Different small letters after data indicate the significance at $p < 0.05$ level.

3.3.2. Effects of Two Nanomaterial Treatments on Malondialdehyde (MDA) Content in Grape Seedlings under Salt Stress

It can be seen from Figure 2B that under salt stress control (S), the MDA content in the leaves of grape seedlings increased by 43.4%, 81.2%, and 17.6% after 1 week, 2 weeks, and 3 weeks, respectively, compared with CK in the same period. Under salt stress, among the nano-silica treatments, 40 μg/mL was the optimal concentration for reducing the MDA content, inducing a decrease of 18.8%, 24.4%, and 10.8% compared with that of the salt stress control in the same period. Meanwhile, after the combined treatment of S + Si40 + C90, the reductions were 43.1%, 61.9%, and 15.0%, respectively, compared with the salt stress control in the same period. Compared with the S + Si40 treatment in the same period, the reductions were 29.9%, 49.6%, and 4.7%, respectively. In comparison with the S + C90 treatment during the same period, the reductions were 25.3%, 44.5%, and 11%, respectively. The experiment showed that the combined treatment of nano-silica and MWCNTs was more beneficial in reducing the content of MDA in the leaves of grape seedlings under salt stress.

3.3.3. Effects of Two Nanomaterial Treatments on Superoxide Dismutase (SOD) Activity of Grape Seedlings under Salt Stress

According to Figure 2C, under salt stress, the 40 μg/mL nano-silica treatment (Si40) resulted in a rapid increase of 82.9%, 146.1%, and 98.7% over that under salt stress control (S) in the same period, respectively. In comparison, the combined treatment of S + Si40 + C90 increased the values by 1.26 times, 2.23 times, and 1.51 times, respectively, compared to the salt stress control in the same period. The increase rates were 23.8%, 31.3%, and 26.7% compared with the S + Si40 treatment and 50.2%, 65.5%, and 33.9%, respectively, compared with the S + C90 treatment group in the same period, suggesting that the combined treatment of nano-silica and MWCNTs (S + Si40 + C90) was the most effective in enhancing the SOD activity of grape seedling leaves under salt stress.

3.3.4. Effects of Two Nanomaterial Treatments on Peroxidase (POD) Activity of Grape Seedlings under Salt Stress

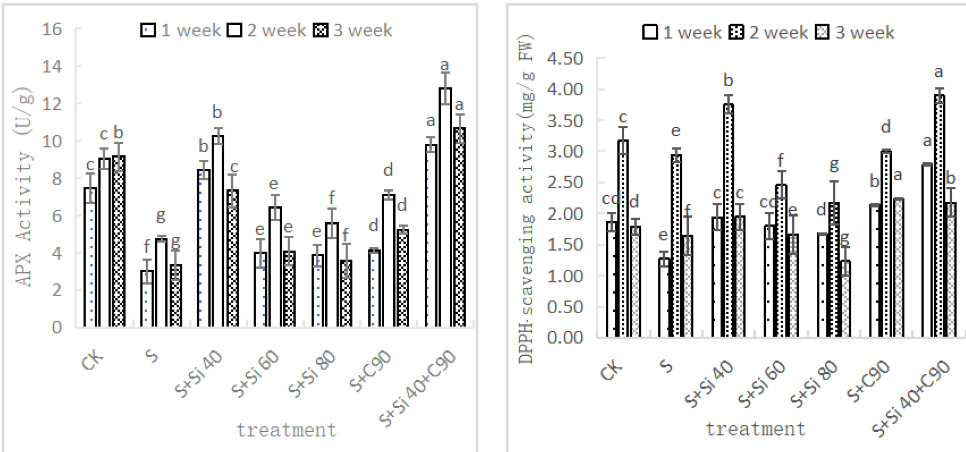
As shown in Figure 2D, under salt stress, among the nano-silica treatments, 40 μg/mL (S + Si40) was the most efficient concentration for increasing POD activity. It led to enhancements of 1.46 times, 2.41 times, and 1.16 times compared to the salt stress control during the same period, respectively. By comparison, the combined treatment of S + Si40 + C90 exhibited an even greater effect and increased POD activity by 2.97 times, 2.93 times, and 2.63 times, respectively, over the salt stress control in the same period. The increases were 27.4%, 8.2%, and 47.1% compared with the S + Si40 group and 47.8%, 12.1%, and 91.0% compared with the S + C90 group in the same period. The experiment demonstrated that the combined treatment of nano-silica and MWCNTs was the most effective in enhancing the POD activity of grape seedling leaves under salt stress.

3.3.5. Effects of Two Nanomaterial Treatments on Catalase (CAT) Activity in Leaves of Grape Seedlings under Salt Stress

It can be seen from Figure 2E that under stress from salt, among the nano-silica treatments, the maximum level of CAT activity was reached at a concentration of 40 μg/mL (S + Si40), which was 41.0%, 45.7%, and 24.1% higher than that observed over the same duration under salt stress control (S), respectively. Comparing the S + Si40 + C90 treatment to the salt stress control (S), the increases were 43.9%, 44.3%, and 41.6%, respectively. The rate of increase in the S + Si40 + C90 treatment was 6.7%, 4.5%, and 15.4%, respectively, in contrast to S + C90 therapy, and these rate increases were only marginally greater than the S + Si40 treatment throughout the same time period. These findings demonstrated that in grape seedlings under salt stress, the application of S + Si40 + C90 could considerably boost the CAT activity of the leaves.

3.4. Effects of Two Nanomaterial Treatments on APX Activity and DPPH· Scavenging Activity of Grape Seedling Leaves under Salt Stress

It can be seen from Figure 3A that salt stress treatment (S) greatly suppressed the APX activity in the leaves of grape seedlings. This resulted in a decrease of 59.81%, 47.51%, and 63.31% compared with the CK group in the first, second, and third weeks, respectively. After the treatment with 40 μg/mL nano-silica (S + Si40), the APX activity increased by 181%, 116%, and 118%, respectively, compared to the activity under salt stress treatment during the same period. Meanwhile, the combined treatment of S + Si40 + C90 showed a remarkable increase of 2.26 times, 1.69 times, and 2.18 times, respectively, compared to the salt stress treatment during the same period. The increases were 16.1%, 24.9%, and 45.7% compared with the S + Si40 group and 137.1%, 79.7%, and 103.4%, respectively, compared with the S + C90 group.



(**A**) (**B**)

Figure 3. (**A**) Effects of different treatments on APX vitality of leaves from grape seedlings; (**B**) effect of different treatments on free radical scavenging activity of DPPH· leaves of grape seedlings. Different small letters after data indicate the significance at $p < 0.05$ level.

As shown in Figure 3B, under the salt stress treatment (S), the DPPH· free radical scavenging activity in the leaves of grape seedlings decreased significantly. In the first three weeks, it decreased by 31.72%, 7.32%, and 8.55%, respectively, compared with that of the CK group in the same period. The 40 μg/mL nano-silica treatment (S + Si40) increased by 52.92%, 27.56%, and 18.77%, respectively, compared with the salt stress treatment (S) in the same period. The combined treatment of S + Si40 + C90 outperformed the treatments of single-component nano-silica in this regard, showing an increase of 120%, 32.73%, and 33.19%, respectively, compared with the salt stress treatment at the same period. The increases were 43.8%, 4.1%, and 12.1%, respectively, compared with the S + Si40 group in the same period. Compared with the S + C90 treatment in the same period, it increased by 30.7% and 30.1%, respectively, in the first and second weeks but decreased slightly in the third week.

The results showed that under salt stress, the combined treatment of S + Si40 + C90 was the most effective in increasing the APX activity and DPPH· free radical scavenging activity of grape seedling leaves under salt stress.

3.5. Effects of Two Nanomaterial Treatments on the Activities of Related Enzymes in the AsA-GSH Cycle of Grape Seedling Leaves under Salt Stress

It can be seen from Figure 4A that under the salt stress treatment (S), the activity of GR in the leaves of grape seedlings was significantly lower. It was 51.33%, 55.37%, and 48.40% lower than that of the control group (CK) in the same period, respectively. An amount of 40 μg/mL nano-silica treatment increased these values by 91.36%, 143.3%, and 96.80%, respectively, compared with the salt stress treatment in the same period. After the combined treatment of S + Si40 + C90, the increase rates were 85.97%, 143.9%, and 115.9%, respectively, compared with the salt stress treatment during the same period. Compared with the S + Si40 and S + C90 treatments in the same period, they were slightly higher.

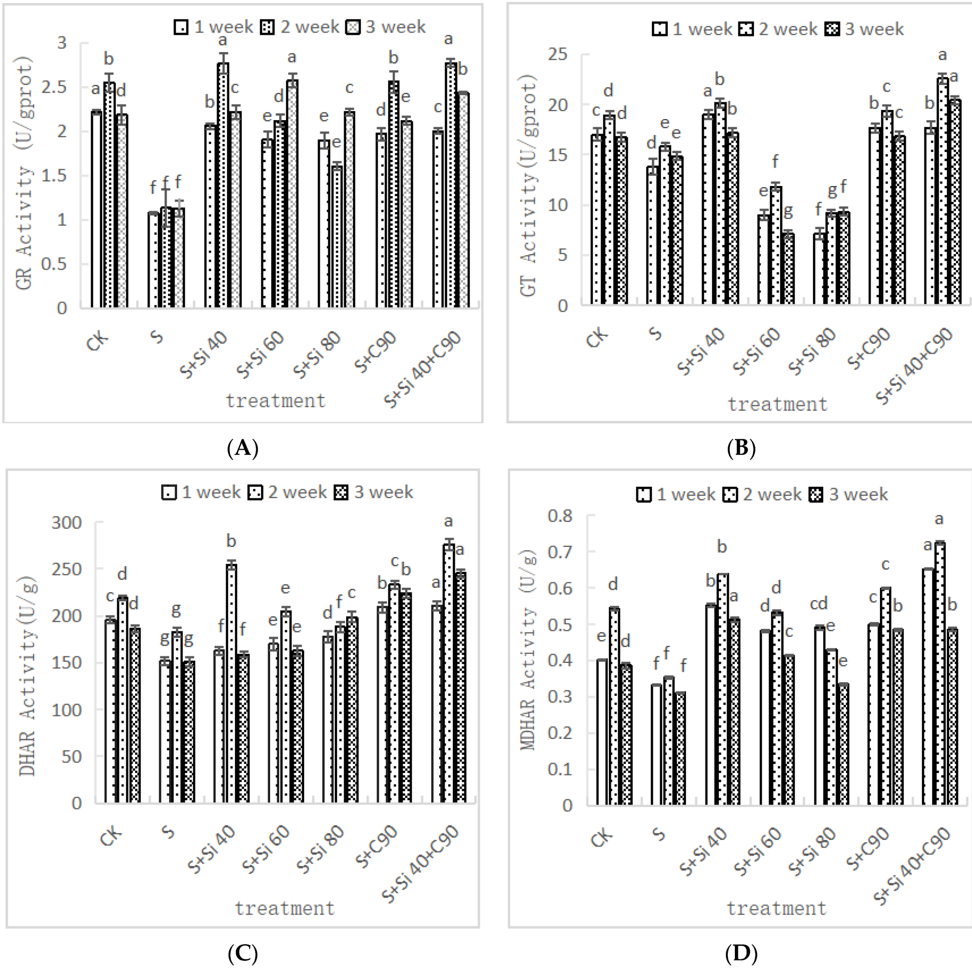


Figure 4. (**A**) Effects of different treatments on GR activity in leaves of grape seedlings; (**B**) Effects of different treatments on GT activity in leaves of grape seedlings; (**C**) Effects of different treatments on DHAR activity in leaves of grape seedlings; (**D**) Effect of different treatments on MDHAR activity in leaves of grape seedlings. Different small letters after data indicate the significance at $p < 0.05$ level.

According to Figure 4B, under the salt stress treatment (S), the GT activity in the leaves of grape seedlings decreased by 18.96%, 16.38%, and 11.36%, respectively, compared with that of the control group (CK) in the same period. An amount of 40 μg/mL nano-silica treatment increased these values by 37.78%, 27.30%, and 15.73%, respectively, compared to the salt stress treatment during the same period. After the combined treatment of S + Si40 + C90, the increase rates were 28.19%, 42.68%, and 37.75%, respectively, compared with the salt stress treatment in the same period. In comparison with the S + Si40 and S + C90 treatments during the same period, the increase was moderate.

The results in Figure 4C demonstrated that under the salt stress treatment (S), the dehydroascorbate reductase (DHAR) activity in the leaves of grape seedlings decreased by 22.36%, 16.57%, and 19.02%, respectively, compared to that of the control group (CK) during the same period. DHAR activity in the 40 μg/mL nano-silica treatment group increased by 6.92%, 39.36%, and 5.10%, respectively, compared to the group undergoing salt stress treatment during the same period. After the combined treatment of S + Si40 + C90, the increase was 38.42%, 51.40%, and 62.40%, respectively, compared with the salt stress treatment in the same period. The increase was 29.5%, 8.6%, and 54.5% compared with the S + Si40 treatment and 0.7%, 18.5%, and 9.6%, respectively, compared with the S + C90 treatment in the same period.

It can be seen from Figure 4D that salt stress treatment (S) decreased MDHAR activity in the leaves of grape seedlings by 17.41%, 34.99%, and 19.64%, respectively, compared to the CK group during the same period. The 40 μg/mL nano-silica treatment increased the MDHAR activity by 66.27%, 80.74%, and 64.95%, respectively, compared with that of the salt stress treatment. Meanwhile, the increase achieved by the combined treatment of S + Si40 + C90 was 96.39%, 104.82%, and 56.27%, respectively. Compared to the S + C90 treatment during the same period, the MDHAR activity of the S + Si40 + C90 group was 30.1%, 20.7%, and 0.4% higher, respectively. However, the increase was moderate compared to the S + Si40 treatment during the same period.

The experiment showed that the combined treatment of S + Si40 + C90 was the most beneficial to increasing the activities of related enzymes in the AsA-GSH cycle of grape seedling leaves under salt stress.

4. Discussion

As an important stage of plant growth, seed germination directly affects the growth and morphogenesis of plants in later stages and also influences yield in an indirect manner. Therefore, rapid and neat seed germination constitutes the basis for high and stable yields. The germination rate, fresh weight, dry weight, and root length of grape seeds treated with nano-silica and MWCNTs were all increased to different extents compared to salt control. The combined treatment of S + Si40 + C90 was the most effective in improving the germination of grape seeds under salt stress.

In recent years, the results of several studies have shown that different kinds of nanomaterials are able to improve seed germination and promote root growth to some extent [17,18]. Similarly to the effects of nano-silica and MWCNTs, this promotion may result from the penetration of nanomaterials into the plant seed coat, which favors water uptake by the seeds, and from the possible involvement of the nanomaterials in the regulation of water channel proteins in the seed coat and roots [19].

According to previous studies, it was found that 90 μg/mL MWCNTs could enhance the reducing ability of the antioxidant system in grape seedlings and alleviate salinity [2]. Compared with plants under salt stress, MWCNTs induce changes in the lipid composition, hardness, and permeability of the plasma membrane of broccoli roots, which enhance aquaporin transduction and water absorption and transportation, thus reducing the effect of salt stress on broccoli [20]. It was found that MWCNTs entered protoplasts and produced a response to the permeability of protoplasts, which was similar to that produced via electroporation, thus regulating the expression of aquaporins [21].

Alsaeedia et al. [22] used nano-silica to treat cucumber seeds subjected to high salt stress and found that the addition of nano-silica inhibited the Na^+ uptake of cucumber plants while promoting the uptake and utilization of K^+ and increasing the cytoplasmic K^+/Na^+ ratio in the cytoplasm, thus maintaining the intracellular ionic balance and reducing the osmotic stress caused by high salt concentration. The deposited silicon in shoots is condensed by transpiration and later transformed into amorphous silica localized in the cell wall of epidermal and vascular tissues, causing a reduction in water loss [23].

In this study, it is demonstrated that MWCNTs and nano-silica could interact to better increase the activities of three antioxidant enzymes (SOD, POD, CAT), reduce the content of malondialdehyde (MDA), and maximize the APX activity and DPPH· free radical scavenging activity in grape seedlings. In the AsA-GSH cycle, DHAR is directly related to ascorbate redox status and plant resistance [24]. Studies have shown that DHAR activity increased under various stress conditions that induced reactive oxygen species production [25,26]. Furthermore, altering DHAR expression in plants stably changes cytoplasmic and exosomal ascorbate redox status and affects plant stress resistance [27]. GR uses NADPH as a reducing agent to regenerate GSH from GSSG [28]. GT catalyzes the formation of glutathione derivatives from glutathione and induces other antioxidant enzymes to eliminate superoxide produced by oxidative stress [29]. In this study, the combined treatment of S + Si40 + C90 significantly increased the activities of DHAR, MDHAR, GR, and GT in grape seedlings.

Many mechanisms have been proposed explaining the substantial effect of Si on the development of plant crops regardless of growth conditions: (i) increase the phytohormone (i.e., GA1 and GA4) production [30]; (ii) maintain a high relative water content [31]; (iii) promote cell elongation and cell wall extensibility [32]; (iv) improve the nutrient uptake and alert the potassium–sodium ratio [22,33,34]; (v) increase the activity of antioxidant enzymes [35]; (vi) maintain the membrane integrity through reducing the permeability of plasma membrane of leaf cell [36]; (vii) enhance the ultrastructure of chloroplast [37].

However, in this study, the exact mechanism by which MWCNTs and nano-silica work together needs to be further investigated.

5. Conclusions

The co-treatment of 40 μg/mL nano-silica and 90 μg/mL MWCNTs delayed the reduction in seed fresh weight, reduced the MDA production, and minimized the damage to cell membranes. The activity of antioxidant enzymes in grape seedling leaves was significantly improved, and the stress effect of salt on grape seeds and seedlings was considerably reduced. Therefore, in the future, such amendments (MWCNTs and nano-silica) should be included in the guidelines for good agricultural practices, particularly in saline soils or in the case of using low-quality water to improve crop growth and productivity.

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