






Article

Form of Silica Improves Yield, Fruit Quality and Antioxidant Defense System of Tomato Plants under Salt Stress

Zeus H. Pinedo-Guerrero ¹, Gregorio Cadenas-Pliego ², Hortensia Ortega-Ortiz ², Susana González-Morales ³, Adalberto Benavides-Mendoza ⁴, Jesús Valdés-Reyna ⁵ and Antonio Juárez-Maldonado ^{5,*}

¹ Doctorado en Agricultura Protegida, Universidad Autónoma Agraria Antonio Narro, Saltillo, Coahuila 25315, Mexico; ing.zeuspinedo@gmail.com

² Centro de Investigación en Química Aplicada, Saltillo, Coahuila 25294, Mexico; gregorio.cadenas@ciqa.edu.mx (G.C.-P.); hortensia.ortega@ciqa.edu.mx (H.O.-O.)

³ CONACyT-Departamento de Horticultura, Universidad Autónoma Agraria Antonio Narro, Saltillo, Coahuila 25315, Mexico; qfb_sgm@hotmail.com

⁴ Departamento de Horticultura, Universidad Autónoma Agraria Antonio Narro, Saltillo, Coahuila 25315, Mexico; abenmen@gmail.com

⁵ Departamento de Botánica, Universidad Autónoma Agraria Antonio Narro, Saltillo, Coahuila 25315, Mexico; jvaldes.reyna@gmail.com

* Correspondence: antonio.juarez@uaaan.edu.mx; Tel.: +52-844-411-0317

Received: 23 July 2020; Accepted: 17 August 2020; Published: 19 August 2020



Abstract: Tomato crop is valuable worldwide thanks to its commercial and nutritional value, which plays a very important role in the human diet. However, in arid areas, tomato crops can be found with high salt content. Salinity is a major problem for agriculture, as it decreases productivity, lowers economic yields, and induces soil erosion. The application of silicon has been observed to increase tolerance to abiotic stress and specifically to salt stress. Therefore, the aim of this study is to evaluate the application of K_2SiO_3 and SiO_2 nanoparticles (SiO_2 NPs) on the growth, antioxidant content, and tolerance to saline stress of tomato plants. Plant growth, fruit quality parameters (pH, titratable acidity, total soluble solids, firmness), antioxidant capacity (ABTS, DPPH), enzymatic (SOD, PAL, APX, CAT, GPX) and non-enzymatic (flavonoids, phenols, vitamin C, β -carotene, lycopene) antioxidant compounds, chlorophylls, proteins, and H_2O_2 were evaluated. The application of SiO_2 NPs at 500 mg L^{-1} had positive effects on the plants that were not subjected to stress, increasing the average fruit weight, fruit yield, and chlorophyll, phenol, glutathione, and GPX activity. Meanwhile, in plants under salt stress, it helped to maintain the concentration of chlorophylls, GSH, PAL activity, and vitamin C. The application of SiO_2 NPs is more effective than K_2SiO_3 at inducing positive responses in tomato plants subjected to stress by NaCl.

Keywords: nanotechnology; environmental stress; antioxidants; biocompounds; reactive oxygen species

1. Introduction

Tomato crop is valuable worldwide thanks to its commercial and nutritional value. Furthermore, it is a good source of vitamins, minerals, and antioxidants, such as carotenoids (lycopene and β -carotene) and phenolic compounds, with positive effects on human health. The consumption of these fruits has been associated with a reduction in risks related to various diseases, such as cancer and non-communicable and chronic cardiovascular diseases [1,2]. However, in arid areas, tomato crops can be found with salinity problems [3]. Salinity is an important problem for agriculture, since it

decreases productivity, affecting the economy and also causing soil erosion, which affects the properties of the soil's electrical conductivity, moisture retention, permeability, cation exchange capacity, etc., and also impacts ecological aspects. It is estimated that a large area of the soil on the planet is affected by salinity, affecting up to 20% of the land area available for agriculture, which may reach up to 50% by the middle of this century [4]. The indiscriminate use of fertilizers, unsupervised irrigation, low precipitation, increased surface erosion, and industrial pollution are reasons that can be associated with the appearance of salinity [5]. Under salinity conditions, plants have two response phases: the first is the so-called fast phase, which is the water deficit, while the second is the accumulation of salt and toxicity, called the slow phase [6]. In both phases, transpiration and gas exchange (H_2O , CO_2) alter the photosynthetic process, causing oxidative stress by the generation of reactive species of oxygen and/or nitrogen [7].

Silicon (Si) is the second most abundant element in the lithosphere (27.7%), only behind oxygen (O_2) (47.4%) [8]. Despite being the second most abundant element in the lithosphere, Si combines with O_2 to form water-insoluble silicates or networks of Si dioxide polymers (quartz) and tends to exit circulation in an aerobic environment. The most abundant form of Si in the Earth's crust weathers so slowly that it is not an appreciable source of silicic acid for biota [9]. Silicon has shown several positive mechanical and physiological effects in plants, such as increased resistance to pathogens and insects, increased resistance to strong wind and rain, the alleviation of drought, alleviation of salt stress through the inhibition of Na^+ and Cl^- , alleviation of P deficiency, improvement of K, P, and Ca intake, reduction in the excess absorption of nutrients such as P, y, and N, and the alleviation of Mn, Cd, As, Al, and Zn toxicity [10–13]. Applications of nanotechnology in agriculture have grown in recent years, especially with the use of nanoparticles (NPs), since they have different characteristics from their bulk form due to their size of less than 100 nm [14]. Silicon in the form of nanoparticles (Si NPs) increases the growth of plants [15]. Si NPs can also diminish the negative effects of oxidative stress caused by environmental factors, such as with heavy metals, through antioxidant compounds, and can also improve the osmotic potential of the cell plant system [16]. In plants under salt stress, the increased expression of genes associated with the response to salt stress has been observed [17,18]. This also inhibits the negative effect caused by salinity on growth, increasing the K^+ concentration and the K^+/Na^+ ratio [19], and improves the accumulation of proline, free amino acids, the content of nutrients, proteins, phenolic components, ascorbic acid, α -tocopherol, and chlorophyll and also improves gas exchange and energy efficiency [19–21]. This also induces an increase in the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), peroxidases (POX), and ascorbate peroxidase (APX) [17,19]. All these responses taken together can increase tolerance to salt stress in crops and lessen the negative impact. For this reason, the aim of the present study is to determine the effect of the application of K_2SiO_3 and SiO_2 NPs on growth, antioxidant content, and salt stress tolerance in tomatoes.

2. Materials and Methods

2.1. Crop Growth

An indeterminate tomato crop variety, “El Cid” (Harris Moran, Davis, CA, USA), of the saladette type was established under greenhouse conditions. The seeds were sown in trays of 200 cavities, and the substrate was a mixture of peat moss and perlite (70:30). Once the seedlings had four true leaves, they were transplanted in polyethylene bags with a capacity of 12 L, with a mixture of perlite and peat moss (1:1) as substrate. Nutrition was provided with Steiner solution [22], applied with a directed irrigation system using drippers. The concentration applied was 25% for the first two weeks after the transplantation, 50% for the third and fourth weeks, 75% for the fifth and sixth weeks, and 100% for the rest of the crop cycle. The following micronutrients were used in chelated form in the nutrient solution: EDTA (2,2',2'',2'''-(ethane-1,2-diylidinitrilo)tetraacetic acid)/Fe EDTA = 3.75 mg L^{-1} ; Mn EDTA = 1.85 mg L^{-1} ; B = 0.35 mg L^{-1} ; Zn EDTA = 0.30 mg L^{-1} ;

Cu EDTA = 0.15 mg L⁻¹; Mo = 0.10 mg L⁻¹. The pH of the nutritive solution was adjusted to 6.5 with sulfuric acid each time it was prepared. The crop was managed to a single stem and, when there were between 10 and 11 clusters, the apex was cut to stop the growth of the plant at 18 weeks after transplantation. The tomato crop was developed by 21 weeks after transplantation.

2.2. Application of Treatments

Different doses of potassium silicate (K₂SiO₃) (250, 500 mg L⁻¹) and silicon dioxide nanoparticles (SiO₂ NPs) (250, 500 mg L⁻¹) were applied on the surface of the substrate 15 days after transplantation, applying 10 mL per plant every 15 days. The total number of applications was five, with a total of 50 mL of solution per plant. The application of salt stress in plants of treatments with stress was carried out with refined sea salt (NaCl) at a concentration of 50 mM. The salt was mixed with the nutritive solution and applied from transplanting and throughout the crop cycle. The electrical conductivity levels of the nutritive solutions were up to 3.4 dS m⁻¹ and 11 dS m⁻¹ without and with salt, respectively, when the nutrient solution was applied at 100% concentration. The substrate achieved electrical conductivity of 14 dS m⁻¹ at that point. A total of 10 treatments were applied, five without salt stress and five with salt stress. The SiO₂ NPs used were 10–20 nm in size, with a spherical morphology, a surface area of 160 m² g⁻¹, and a bulk density of 0.08–0.1 g cm⁻³ (SkySpring Nanomaterials Inc., Houston, TX, USA), as described by González-Moscóso et al. [23].

2.3. Crop Growth Variables

To determine the effect of the treatments on the growth of the tomato crop at 21 weeks after transplantation, the height of the plant, shoot fresh weight, stem diameter, number of clusters, and number of leaves were measured. In addition, the number of fruits per plant, the average weight of the fruits, and the yield of fruits per plant were quantified.

2.4. Sampling

Samples of fully developed young leaves were taken only once at 17 weeks after transplantation, placed in a freezer at −20 °C, and then lyophilized in a freeze dryer (Yamato Scientific Co. Ltd. freeze dryer, Model D401, Santa Clara, CA, USA). After this, samples were ground with a porcelain mortar and the biochemical variables were measured.

Tomatoes between the fourth and fifth bunches of the same size that were not damaged were selected; they were harvested in full red, according to the USDA scale (more than 90 percent of the surface in a red color sample). Six fruits were selected per treatment—each one from a different plant—and were washed and used immediately for quality variables. The samples were taken once at 17 weeks after transplantation.

2.5. Fruit Quality

The parameters that describe the quality of the fruit, such as hydrogen potential (pH), total soluble solids (SST), titratable acidity (AT), and firmness, were determined as described by López-Vargas et al. [24].

2.6. Biochemical Analysis

The biochemical analyses are fully described by González-Moscóso et al. [23] and López-Vargas et al. [24]. The chlorophyll content (mg 100 g⁻¹ dry weight (DW)) was determined according to the method of Nagata and Yamashita [25]. Phenols (mg g⁻¹ DW) were determined using the Folin–Ciocalteu reagent, as described in [26]. Flavonoids (mg 100 g⁻¹ DW) were determined with the method of Arvouet-Grand et al. [27]. Total protein (mg g⁻¹ of DW) determination was performed using Bradford's colorimetric technique [28]. Vitamin C (mg 100 g⁻¹ DW) content was determined as described by Padayatt et al. [29]. The glutathione (GSH) content

(mmol 100 g⁻¹ DW) was determined using the method of Xue et al. [30]. Lycopene and β -carotene content were determined according to the method of Nagata and Yamashita [25]. Antioxidant capacity by ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical was determined using the spectrophotometric method of Re et al. [31]. Antioxidant capacity by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was determined according to the method of Brand-Williams et al. [32]. The hydrophilic compounds were determined using a phosphate buffer for extraction and, for the lipophilic compounds, a hexane:acetone solution was used. The total antioxidant capacity was obtained from the sum of the hydrophilic and lipophilic compounds [33]. The antioxidant capacity was expressed as vitamin C equivalents ($\mu\text{g g}^{-1}$ DW). Hydrogen peroxide was carried out according to the methodology of Patterson et al. [34]. Ascorbate peroxidase (EC 1.11.1.11) was determined by the method of Nakano and Asada [35] and is expressed as U per gram of total proteins (U g⁻¹ TP), where U is equal to the μmol of oxidized ascorbate per milliliter per minute. Glutathione peroxidase (EC 1.11.1.9) (U per gram of total proteins (U TP⁻¹), where U is equal to the mM equivalent of reduced glutathione (GSH) per milliliter per minute), was determined by the method of Flohé and Günzler [30,36]. Catalase (EC 1.11.1.6) (U TP⁻¹, where U is equal to the mM equivalent of H₂O₂ consumed per milliliter per minute) was quantified by the method of Dhindsa et al. [37]. Phenylalanine ammonia lyase (PAL) (EC 4.3.1.5) was determined according to the method of Sykłowska-Baranek et al. [38]. Superoxide dismutase (SOD) (EC 1.15.1.1) (U mL⁻¹, where U is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical) was carried out using the Cayman kit (SOD Assay Kit 706002, Cayman Chemical, Ann Arbor, Michigan, USA).

2.7. Statistical Analysis

The variables of tomato growth were assessed using 10 replicates, where a plant represented a replicate. For each of the evaluated biochemical variables, five replicates per treatment were used. A completely random design was used. An analysis of variance and Fisher's least significant difference (LSD) mean test ($\alpha = 0.05$) were performed. All statistical procedures were performed using the software Infostat (v2018).

3. Results

Statistically significant differences in plant height, fresh weight, stem diameter, and the number of clusters were observed (Figure 1). However, in fresh weight and stem diameter, the significant difference was between treatments with and without saline stress only. The plants under salinity conditions showed a lower height, with the exception of those subjected to the A250 + NaCl treatment. In the number of clusters, a significant difference was only observed in the N250 treatment with and without salt stress, while the rest of the treatments were the same. The salinity affected the plants negatively, decreasing plant height, fresh weight, and stem diameter.

Differences were observed in the average weight of fruits and in the fruit yield per plant (Figure 2). The application of SiO₂ NPs showed a positive effect when there was no salt stress increasing the average weight of the tomato fruits. The N500 treatment registered 17.8% more weight than the control, while salinity decreased the average weight of the fruits in all treatments. In this condition, the application of SiO₂ NPs at 500 mg L⁻¹ also seems to induce a favorable response; however, it was not significantly different from its respective control.

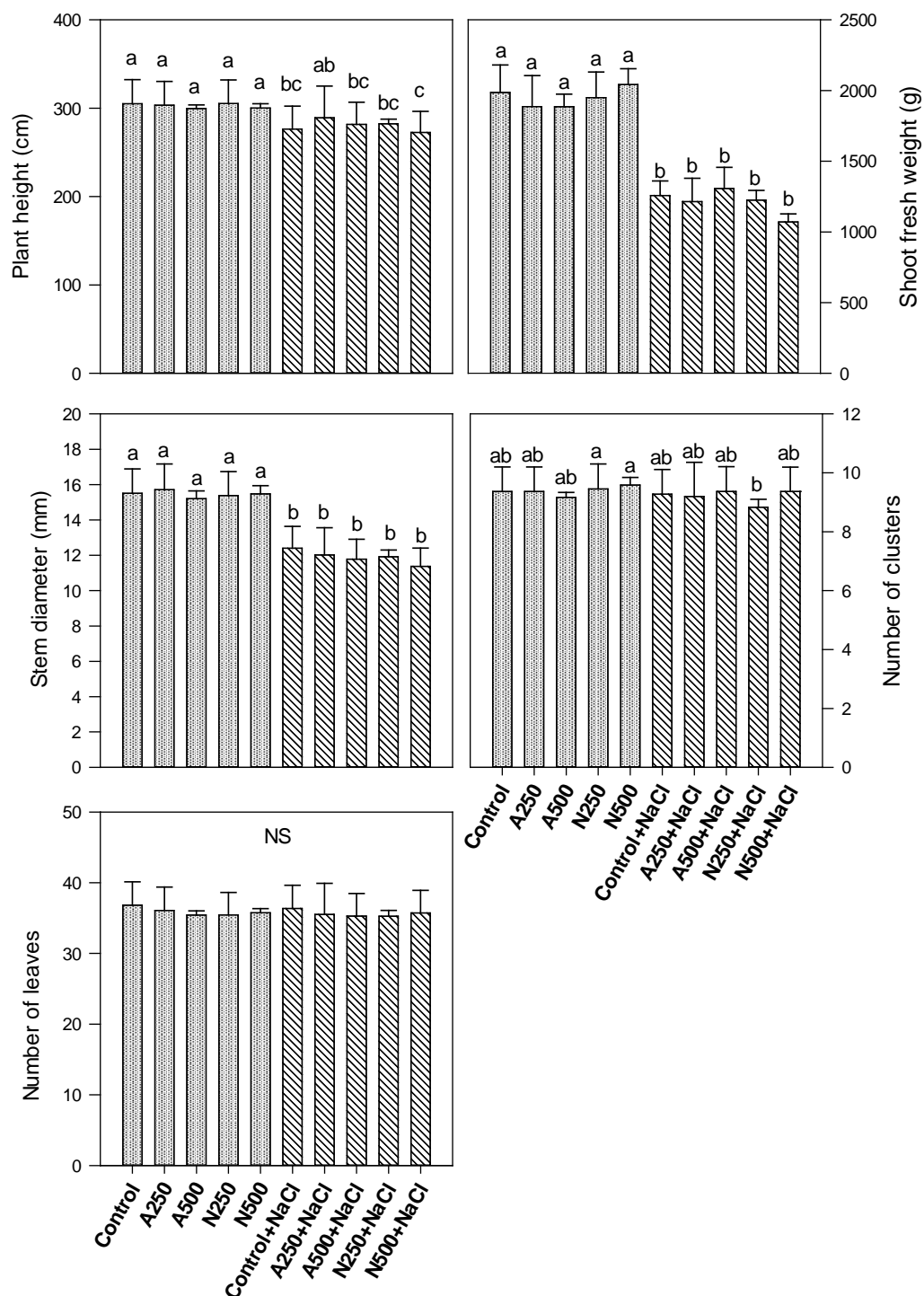


Figure 1. Plant height, shoot fresh weight, stem diameter, number of clusters, and number of leaves in tomato plants treated with K_2SiO_3 and SiO_2 NPs under salt (NaCl) stress. Different letters on the bars indicate significant differences according to Fisher's least significant difference test ($\alpha = 0.05$). $n = 10 \pm$ standard error. NS: No significant difference. A250, A500: K_2SiO_3 at 250 and 500 mg L^{-1} . N250, N500: SiO_2 NPs at 250 and 500 mg L^{-1} . NaCl: Plants developed under salt stress at 50 mM of NaCl applied via nutritive solution throughout the crop cycle.

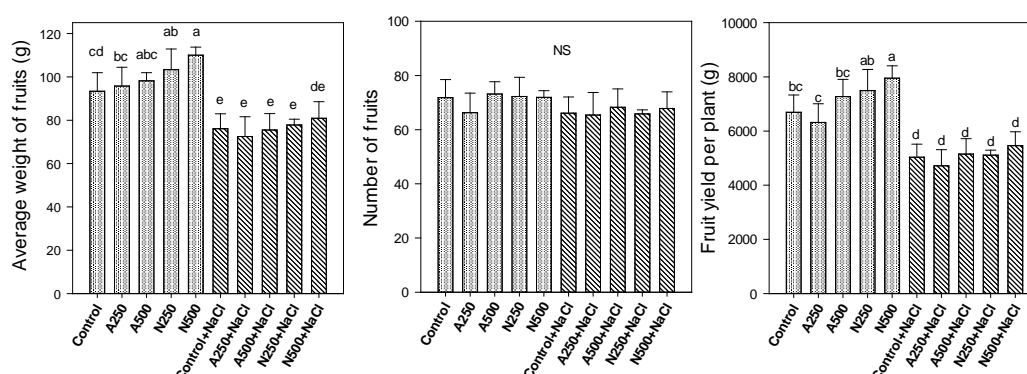


Figure 2. Average weight of fruits, number of fruits, and fruit yield in tomato plants treated with K_2SiO_3 and SiO_2 NPs under salt (NaCl) stress. Different letters on the bars indicate significant differences according to the Fisher's least significant difference test ($\alpha = 0.05$). $n = 10 \pm$ standard error. NS: No significant difference. A250, A500: K_2SiO_3 at 250 and 500 $mg\ L^{-1}$. N250, N500: SiO_2 NPs at 250 and 500 $mg\ L^{-1}$. NaCl: Plants developed under salt stress at 50 mM of NaCl applied via nutritive solution throughout the crop cycle.

The SiO_2 NPs positively impacted the fruit yield by inducing larger fruits; the treatment with 500 $mg\ L^{-1}$ of SiO_2 NPs registered 18.8% more fruit yield than the control. On the other hand, salinity decreased the yield in all treatments; the application of NaCl decreased the yield by 24.7%, while in the treatment with 500 $mg\ L^{-1}$ of SiO_2 NPs and NaCl, it only decreased by 18.38%.

Differences were observed in the content of chlorophyll *a*, chlorophyll *b*, and total chlorophyll (Figure 3). The application of SiO_2 NPs positively affected the chlorophyll content regardless of whether the plants were under salt stress. Without salt stress, the application of 500 $mg\ L^{-1}$ of SiO_2 NPs increased the content of chlorophyll *a* by 12.5%, chlorophyll *b* by 16.8%, and total chlorophyll by 15%. This same treatment under salinity increased the content of chlorophyll *a* by 17.3%, chlorophyll *b* by 15.1%, and total chlorophyll by 11.4%, compared to the control with salinity.

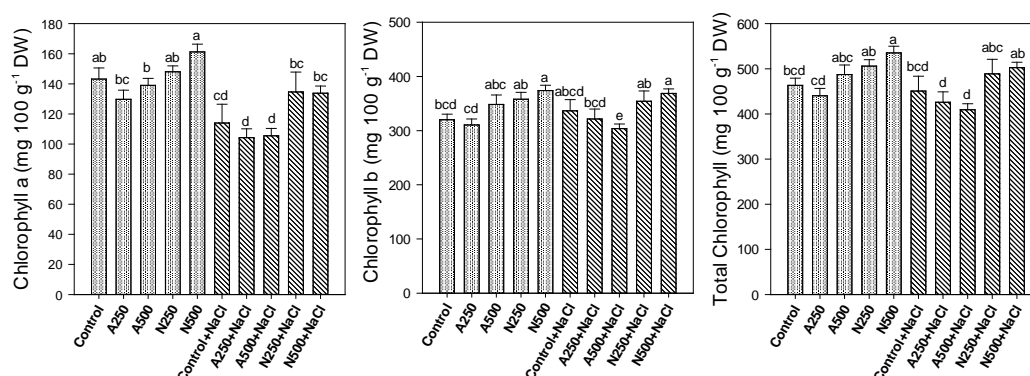


Figure 3. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll in the leaves of tomato plants treated with K_2SiO_3 and SiO_2 NPs under salt (NaCl) stress. Different letters on the bars indicate significant differences according to Fisher's least significant difference test ($\alpha = 0.05$). $n = 5 \pm$ standard error. A250, A500: K_2SiO_3 at 250 and 500 $mg\ L^{-1}$. N250, N500: SiO_2 NPs at 250 and 500 $mg\ L^{-1}$. NaCl: Plants developed under salt stress at 50 mM of NaCl applied via nutritive solution throughout the crop cycle.

Differences were observed in the content of flavonoids, phenols, vitamin C, total proteins, glutathione, and H_2O_2 (Figure 4). The flavonoid content was increased by 17.9% with the application of 500 $mg\ L^{-1}$ SiO_2 NPs when there was no salt stress; however, under salt stress, the application of 250 $mg\ L^{-1}$ of K_2SiO_3 presented the highest content of these compounds—8.99% more than their respective control.

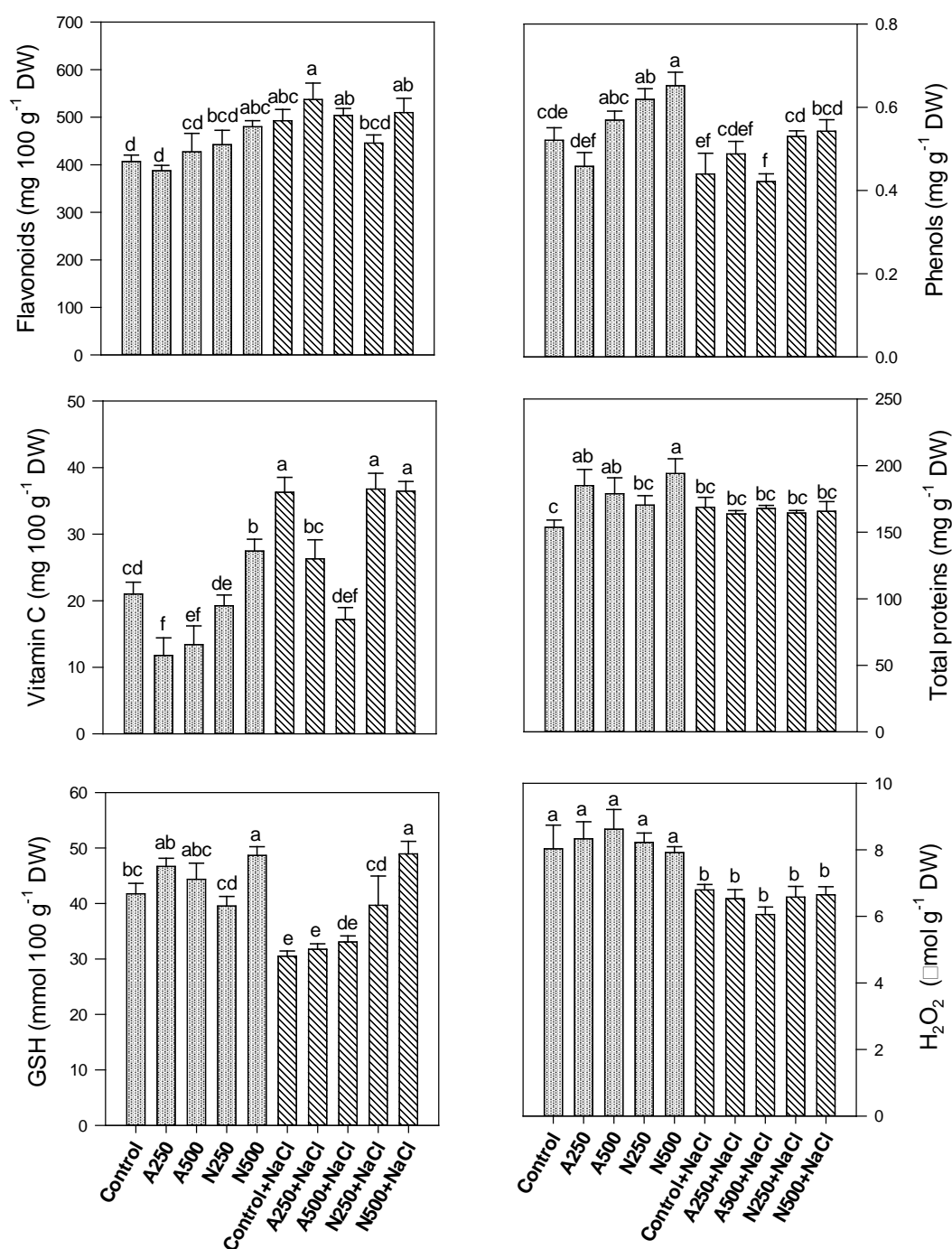


Figure 4. Flavonoids, phenols, vitamin C, total proteins, glutathione (GSH), and hydrogen peroxide (H_2O_2) in the leaves of tomato plants treated with K_2SiO_3 and SiO_2 NPs under salt (NaCl) stress. Different letters on the bars indicate significant differences according to Fisher's least significant difference test ($\alpha = 0.05$). $n = 5 \pm$ standard error. A250, A500: K_2SiO_3 at 250 and 500 mg L⁻¹. N250, N500: SiO_2 NPs at 250 and 500 mg L⁻¹. NaCl: Plants developed under salt stress at 50 mM of NaCl applied via nutritive solution throughout the crop cycle.

The phenol content showed different behavior since the salinity decreased the content. However, regardless of the stress condition, the application of SiO_2 NPs significantly increased the content of these compounds. Without salt stress, the application of 500 mg L⁻¹ of SiO_2 NPs increased the content of phenols by 25.19%, while, under salinity, the same treatment increased the content by 23.42% compared to its respective control.

Vitamin C was positively stimulated by salinity since the application of NaCl alone increased this compound by 72.67%. Without salt stress, only the application of 500 mg L⁻¹ of SiO₂ NPs increased the vitamin C content by 25.91%, while, under stress, the application of SiO₂ NPs did not induce changes regarding its control. However, regardless of the stress condition, the application of K₂SiO₃ decreased the vitamin C content in the leaves.

SiO₂ NPs had a positive effect on total protein content only when there was no salt stress, while, under stress, there was no difference between treatments. Without salt stress, the application of 500 mg L⁻¹ of SiO₂ NPs presented 26.27% more proteins than the control.

The glutathione content increased with the application of 500 mg L⁻¹ of SiO₂ NPs when there was no salt stress; it presented 16.55% more than the control. Under salt stress, the glutathione content decreased by 26.99% compared to the control. However, the application of SiO₂ NPs reversed the negative effect of salinity on glutathione content. Under salt stress, the application of 500 mg L⁻¹ of SiO₂ NPs increased the glutathione content by 60.49% compared to its respective control, while the application of 250 mg L⁻¹ of SiO₂ NPs increased the glutathione content by 30.15%.

Treatments did not have a significant effect on H₂O₂ content. The content of this compound was only affected by the application of salt stress, with the lowest content being observed in treatments with NaCl application.

Differences in leaves' enzymatic activity were observed (Figure 5). The enzymatic activity of SOD was affected by the application of the treatments, where the highest enzymatic activity was observed with the application of 250 mg L⁻¹ of SiO₂ NPs in the absence of salt stress, although it was not different from the control. The application of 500 mg L⁻¹ SiO₂ NPs decreased SOD activity by 17.85% under the same condition. Under salt stress conditions, there was an increasing trend in SOD activity due to the application of the treatments; however, the difference was not significant compared to its respective control.

PAL activity was increased with the application of SiO₂ NPs regardless of the stress condition, although it was greater under salt stress. Without stress, the application of 500 mg L⁻¹ SiO₂ NPs increased PAL activity by 94.48% compared to the control. Under saline stress conditions, both doses of SiO₂ NPs increased PAL activities, these being 81.20% more with 250 mg L⁻¹ and 77.48% more with 500 mg L⁻¹ compared to their respective controls.

The enzymatic activity of APX showed differences between treatments; under salt stress, the application of 500 mg L⁻¹ of K₂SiO₃ and 250 mg L⁻¹ of SiO₂ NPs significantly increased the activity of this enzyme, these being 60.06% and 53.27% more than their respective controls.

Catalase activity was decreased both by salt stress and by the application of silicon in both forms (K₂SiO₃ and SiO₂ NPs). The GPX activity was due to both the high doses of silicon (regardless of the form) and the application of NaCl. In the absence of salt stress, the application of 500 mg L⁻¹ of K₂SiO₃ and SiO₂ NPs increased GPX activity by 251% and 265% compared to the control. Under salt stress conditions, all silicon treatments decreased GPX activity compared to only NaCl application.

The antioxidant capacity evaluated in the leaves showed significant differences between treatments in both radicals studied (ABTS and DPPH) (Figure 6). Similar behaviors were not observed among the radicals; in the ABTS radical, the highest antioxidant activity of hydrophilic compounds and total antioxidant activity was observed in the control, while the antioxidant activity for lipophilic compounds was better in the treatment of 250 mg L⁻¹ of K₂SiO₃ and in the control under salinity. With the DPPH radical, the treatment with 250 mg L⁻¹ of SiO₂ NPs in hydrophilic compounds presented 3.5% more antioxidant activity than the control without stress. Additionally, the treatment with 500 mg L⁻¹ of K₂SiO₃ showed 6.3% more activity than the control in hydrophilic compounds when there was no salt stress. However, under salinity conditions, there were no treatments better than the control; in contrast, the application of 500 mg L⁻¹ of K₂SiO₃ decreased the antioxidant capacity compared to the control.

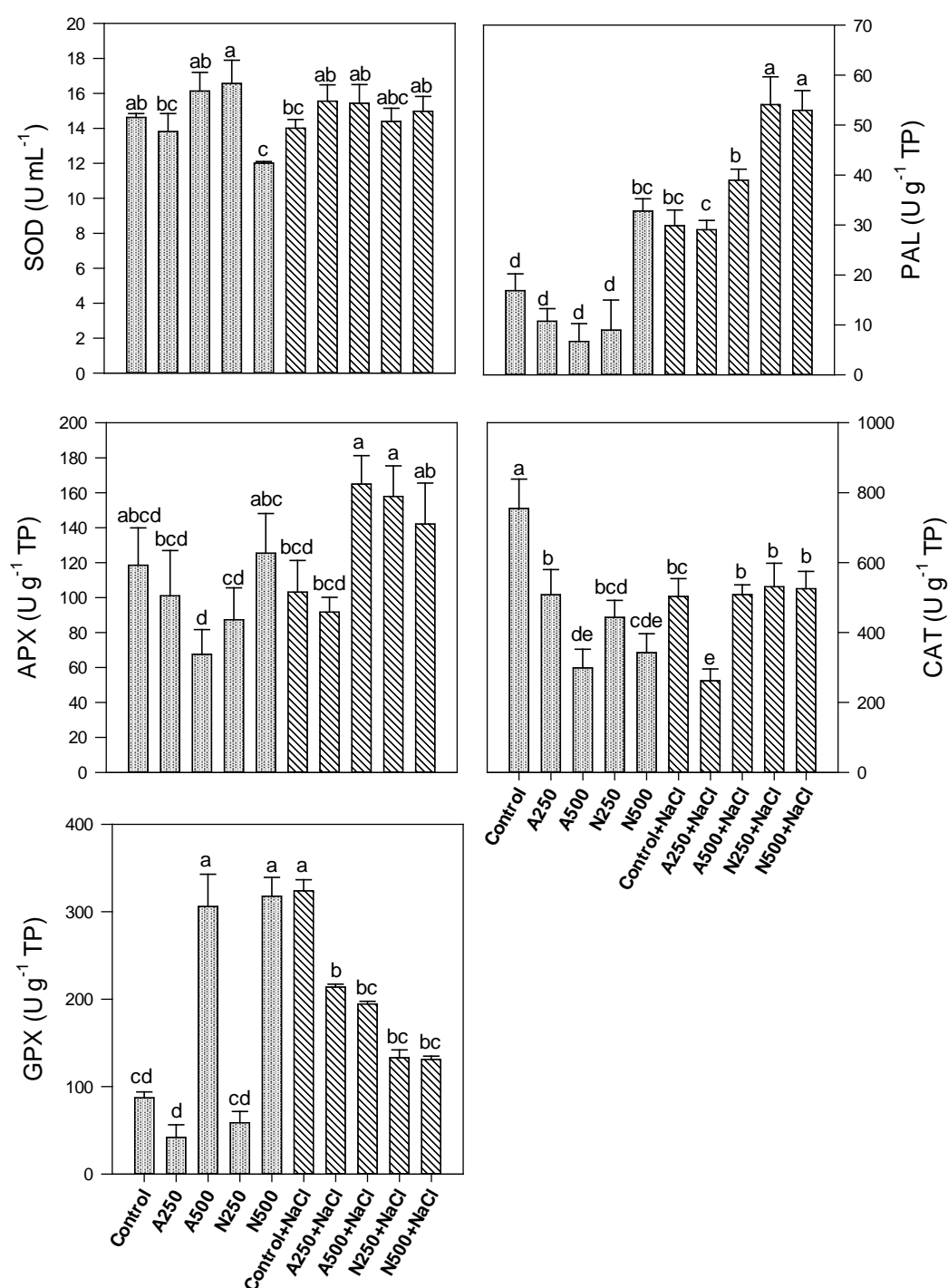


Figure 5. Enzymatic activity of superoxide dismutase (SOD), phenylalanine ammonia lyase (PAL), ascorbate peroxidase (APX), catalase (CAT), and glutathione peroxidase (GPX) in the leaves of tomato plants treated with K_2SiO_3 and SiO_2 NPs under salt (NaCl) stress. Different letters on the bars indicate significant differences according to Fisher's least significant difference test ($\alpha = 0.05$). $n = 5 \pm$ standard error. A250, A500: K_2SiO_3 at 250 and 500 mg L⁻¹. N250, N500: SiO_2 NPs at 250 and 500 mg L⁻¹. NaCl: Plants developed under salt stress at 50 mM of NaCl applied via nutritive solution throughout the crop cycle.

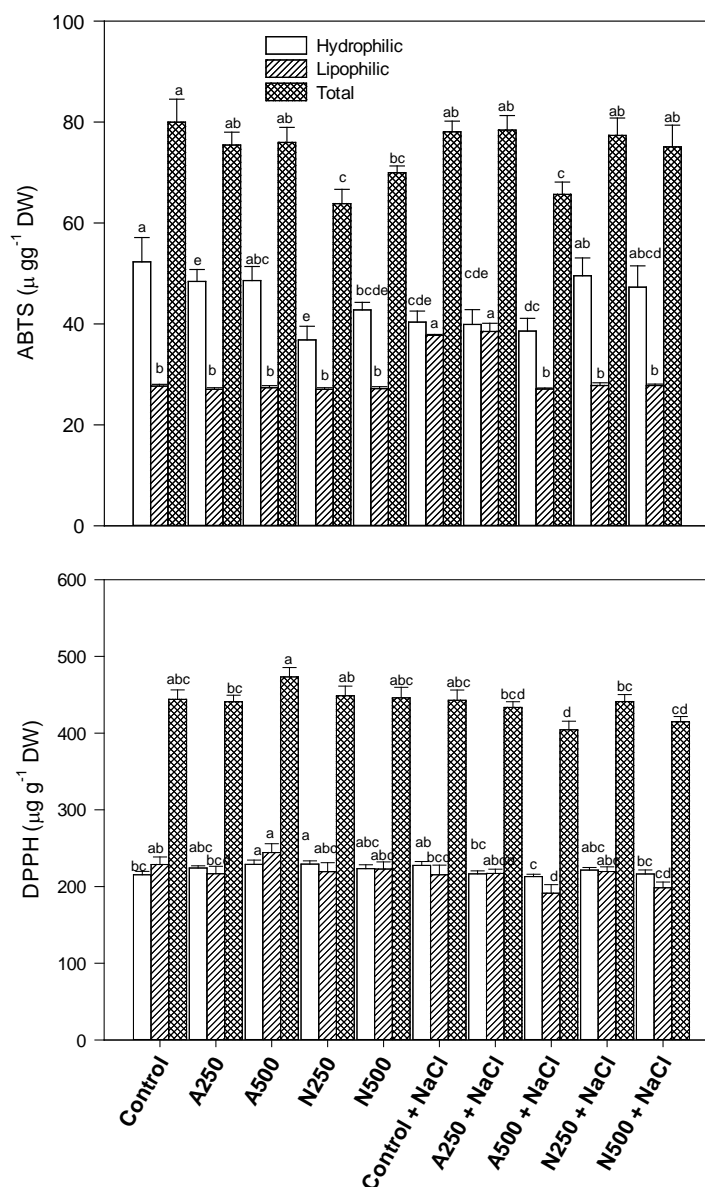


Figure 6. Antioxidant activity with the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (2,2-diphenyl-1-picrylhydrazyl) in the leaves of tomato plants treated with K_2SiO_3 and SiO_2 NPs under salt (NaCl) stress. Different letters on the bars indicate significant differences according to Fisher's least significant difference test ($\alpha = 0.05$). $n = 5 \pm$ standard error. A250, A500: K_2SiO_3 at 250 and 500 mg L^{-1} . N250, N500: SiO_2 NPs at 250 and 500 mg L^{-1} . NaCl: Plants developed under salt stress at 50 mM of NaCl applied via nutritive solution throughout the crop cycle.

In the antioxidants in tomato fruits, significant differences between treatments were observed in the content levels of flavonoids, phenols, vitamin C, and β -carotene. The GSH and lycopene were not affected by the treatments or salinity, as they did not show significant differences (Figure 7). The content levels of the flavonoids in fruit showed different behaviors from those found in the leaves, since these were decreased by salinity. In the absence of salt stress, the highest flavonoid content was found in the control. Phenols were not modified by the salt stress condition, while the application of silicon in both forms induced a positive effect. In the absence of salt stress, an increase in phenols was observed with the application of 500 mg L^{-1} of K_2SiO_3 , this being 70.3% more than the control. Under stress conditions, the application of 250 mg L^{-1} SiO_2 NPs induced the highest phenol content, although it was not significantly different from its respective control.

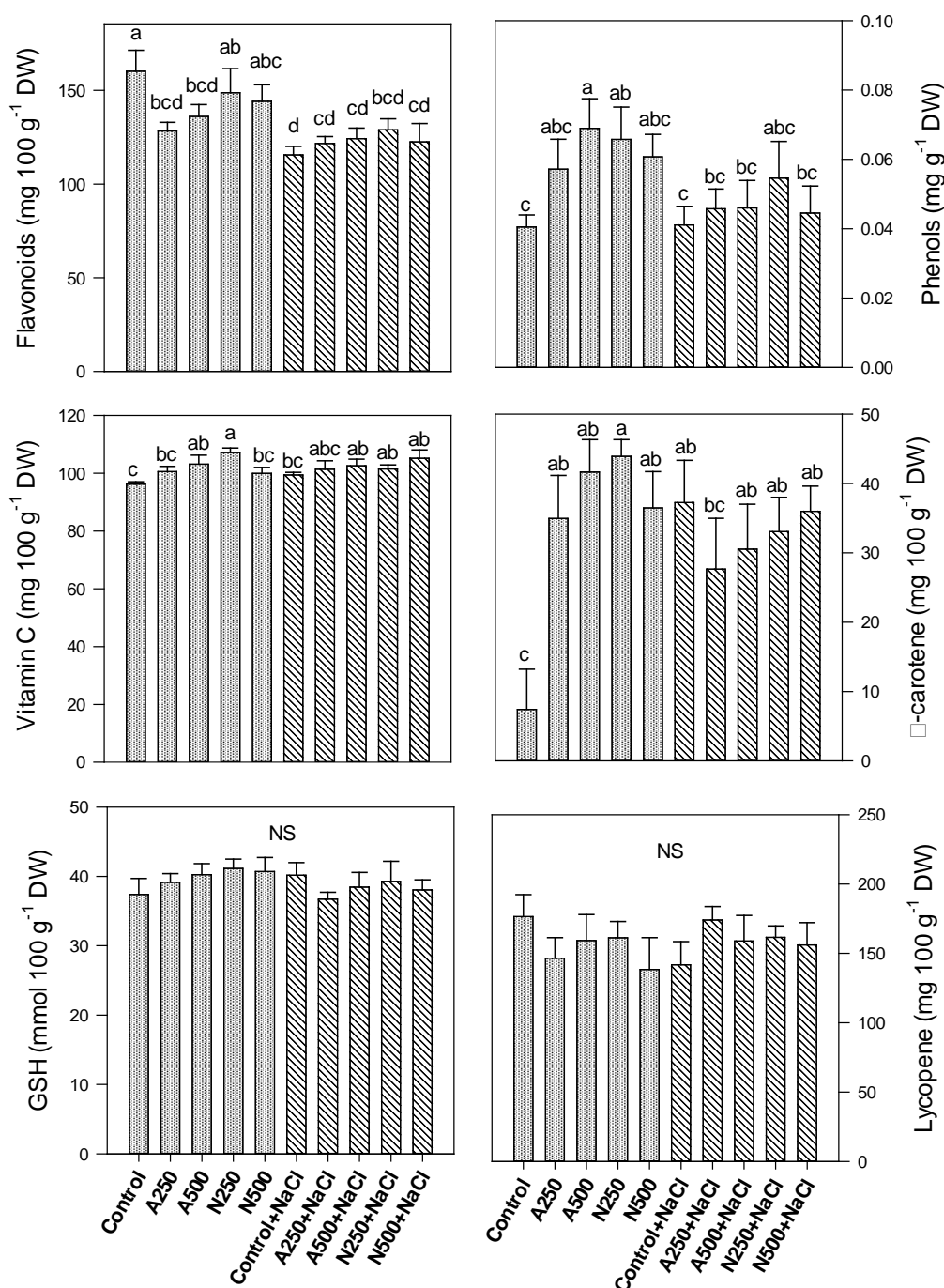


Figure 7. Flavonoids, phenols, vitamin C, β -carotene, glutathione (GSH), and lycopene in tomato fruits of plants treated with K_2SiO_3 and SiO_2 NPs under salt (NaCl) stress. Different letters on the bars indicate significant differences according to Fisher's least significant difference test ($\alpha = 0.05$). $n = 5 \pm$ standard error. NS: No significant difference. A250, A500: K_2SiO_3 at 250 and 500 mg L⁻¹. N250, N500: SiO_2 NPs at 250 and 500 mg L⁻¹. NaCl: Plants developed under salt stress at 50 mM of NaCl applied via nutritive solution throughout the crop cycle.

Vitamin C showed different behavior from that found in the leaves; the fruit was not directly affected by salinity. In the absence of salt stress, the application of 250 mg L⁻¹ of SiO_2 NPs induced the highest content of this compound, this being 11.3% more than the control. Under salt stress, the application of 500 mg L⁻¹ SiO_2 NPs induced the highest content of vitamin C; however, it was not different from its respective control.

β -carotene was stimulated by silicon in both forms in the absence of salt stress, increasing in a range of 371%–492% due to the effect of the treatments. However, salinity also induced an increase in this compound, this being 402% higher compared to the control. Under salt stress, the treatments did not show differences compared to the application of NaCl.

Differences in the antioxidant capacity of the fruit were observed in both radicals ABTS and DPPH (Figure 8). In the ABTS radical, the control showed the highest total antioxidant and lipophilic compound activity in the absence of salt stress. Under stress conditions, the application of 250 mg L⁻¹ of K₂SiO₃ induced the highest total antioxidant and hydrophilic compound activity compared to the respective controls.

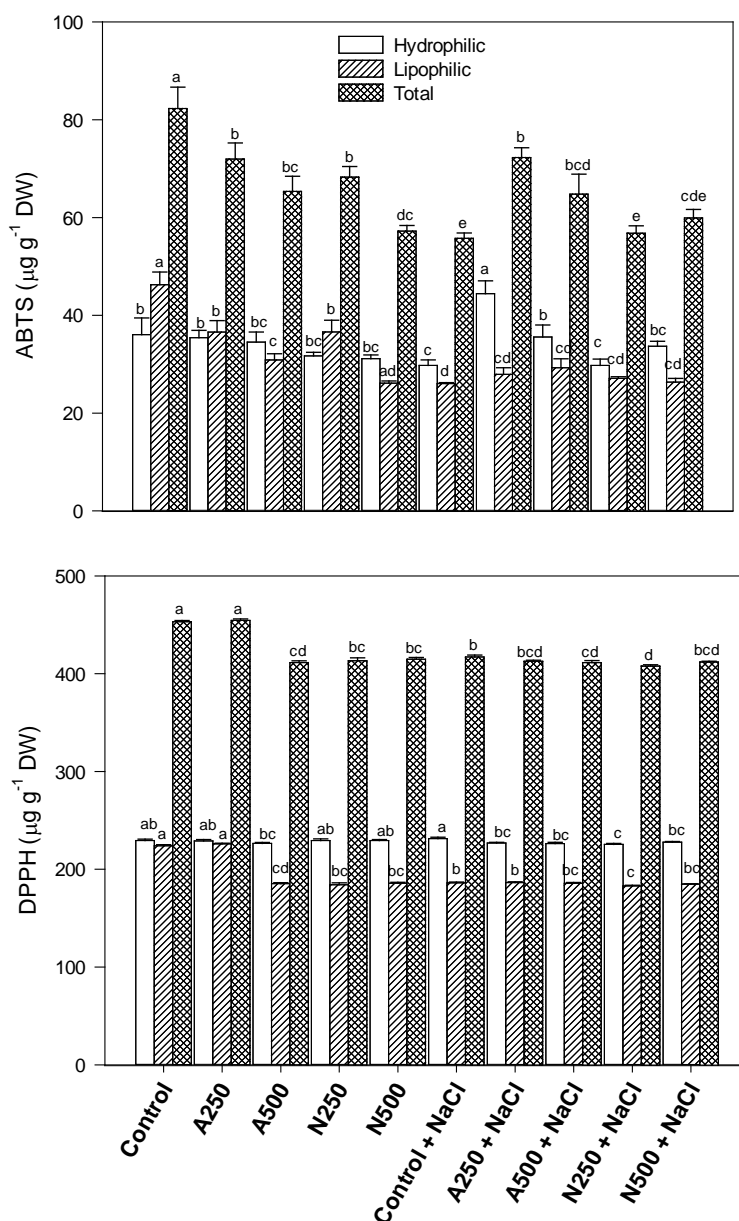


Figure 8. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant activity in tomato fruits of plants treated with K₂SiO₃ and SiO₂ NPs under salt (NaCl) stress. Different letters on the bars indicate significant differences according to Fisher's least significant difference test ($\alpha = 0.05$). $n = 5 \pm$ standard error. A250, A500: K₂SiO₃ at 250 and 500 mg L⁻¹. N250, N500: SiO₂ NPs at 250 and 500 mg L⁻¹. NaCl: Plants developed under salt stress at 50 mM of NaCl applied via nutritive solution throughout the crop cycle.

In the DPPH radical, the highest total antioxidant activity and lipophilic compounds were found in the control and with the application of 250 mg L^{-1} of K_2SiO_3 , while, in hydrophilic compounds, the highest antioxidant activity was obtained with the application of only NaCl.

Differences in fruit quality were observed (Figure 9). The highest pH was found in the A250 treatment, which was 1.1% higher than the control, while the lowest pH was shown by the N500 + NaCl treatment, having a pH 3.89% lower than the control. In firmness, a slight increase was observed in fruits of plants without salt stress and treated with NPs. Under salt stress, the greatest firmness improvements in treatments were found with the application of K_2SiO_3 , these being 8.4% greater than the control. The highest titratable acidity in plants without saline stress was found in the control treatment; under saline conditions, the highest titratable acidity was observed in the 250 mg L^{-1} of K_2SiO_3 treatment. The lowest titratable acidity was shown in the 250 mg L^{-1} of SiO_2 NPs treatment with and without salt stress. The TSS content was directly affected by salinity since the treatments under salt stress had the highest content.

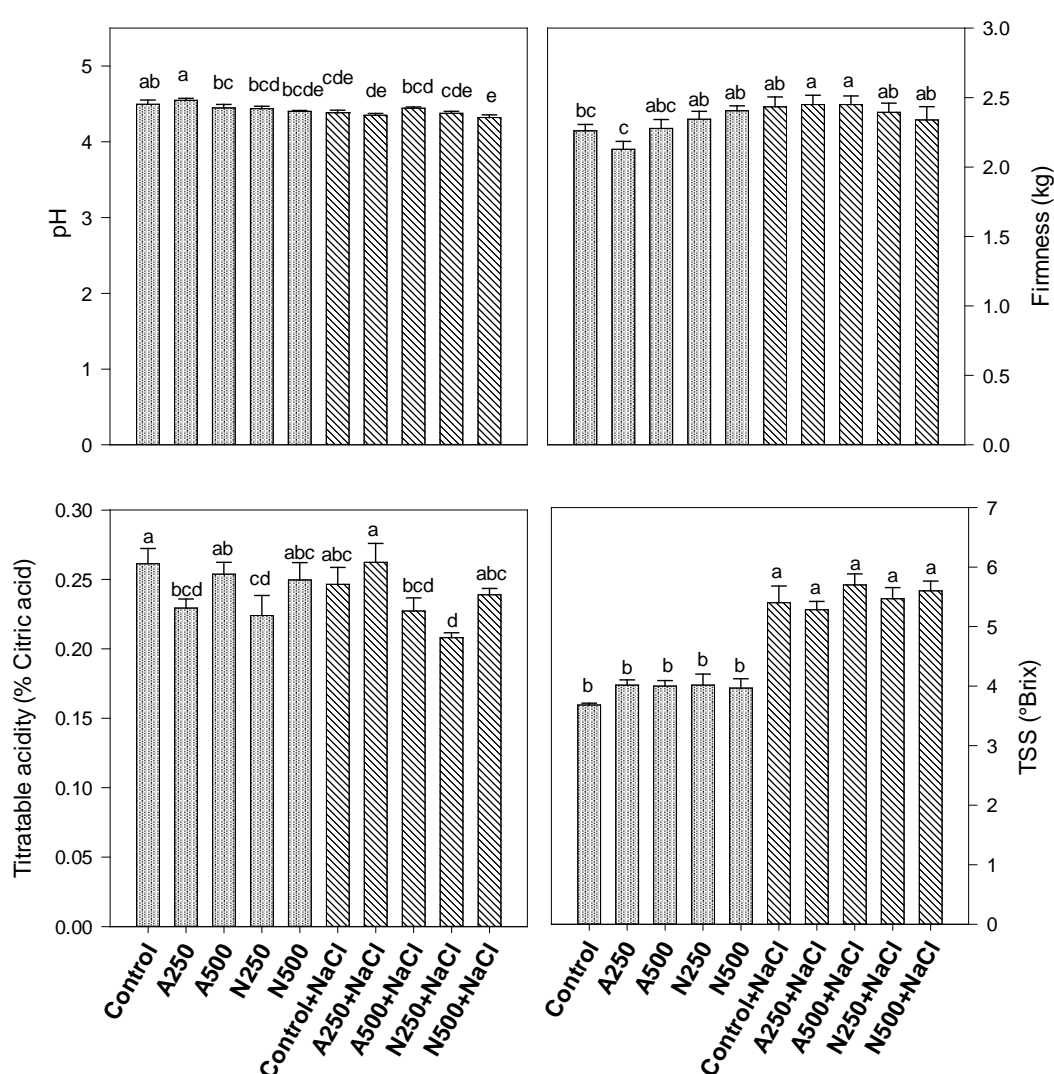


Figure 9. pH, firmness, titratable acidity, and total soluble solids (TSS) in tomato fruits of plants treated with K_2SiO_3 and SiO_2 NPs under salt (NaCl) stress. Different letters on the bars indicate significant differences according to Fisher's least significant difference test ($\alpha = 0.05$). $n = 5 \pm$ standard error. A250, A500: K_2SiO_3 at 250 and 500 mg L^{-1} . N250, N500: SiO_2 NPs at 250 and 500 mg L^{-1} . NaCl: Plants developed under salt stress at 50 mM of NaCl applied via nutritive solution throughout the crop cycle.

4. Discussion

High concentrations of salt in the soil solution decrease the acquisition of water by plants and induce the toxicity of specific ions and nutritional imbalances, affecting growth [6,39]. A decrease in biomass, leaf area, and growth has been observed in several crops [40,41]. Salinity causes an increase in the osmotic potential of plants, causing a reduction in water absorption and a decrease in water import to the fruit, reducing the weight of the fruits [42,43]. The yield was diminished due to the weight of the fruits and not to the number of fruits, similar to that reported by Sakamoto et al. [43]. Contrary to this, silicon nanoparticles improve water use efficiency, relative water content, and turgor pressure [44], which can increase the import of water to the fruit, increasing the weight and, therefore, the fruit yield.

Reducing the chlorophyll content is the fastest response to salinity [45] and it is a symptom of damage to plant growth and development. The decrease in chlorophyll is attributed to the inhibition of synthesis, together with its degradation by the enzyme chlorophyll [46], being a photoprotection mechanism to reduce the absorbance of light [47]. Matichenkov and Kosobrukhov [48] noticed an increase in the activity of the photosynthetic system with the application of silicon. Kalthe et al. [42] reported a decrease in the chlorophylls in basil plants under salt stress in the control treatment and in plants treated with Si; however, the application of SiO₂ NPs increased the concentration of chlorophylls. Haghighi and Pessaraklib [49] also found an increase in the chlorophyll with the application of Si and Si NPs in cherry tomato under salt stress—results similar to those found in the present investigation. Silicon is integrated into cycles, such as the Calvin, tricarboxylic acid, and pentose phosphate cycles, causing the stimulation of proteins related to photosynthesis and guaranteeing the photoprotection of plants [50].

Salt stress results in oxidative stress due to the generation of reactive oxygen species and/or reactive nitrogen species [7]. Furthermore, as a response to unfavorable environmental factors, secondary metabolites, such as phenolic compounds, are synthesized, which are crucial for the growth and reproduction of plants [51]. Flavonoids show antioxidant activity as they belong to phenolic compounds. Found in the leaves, flowers, and pollen, they generally accumulate in the vacuole as glycosides, although they also occur as exudates on the leaves and other parts of plants [52]. They play roles as signaling or protective molecules against reactive oxygen species. Therefore, it is common to observe an increase in phenolic compounds with salt stress [53,54]. Telesiński et al. [55] demonstrated an increase in flavonoid content and a decrease in phenols, similar to what happened in the leaves, while, in the fruit, both were diminished by salinity.

Vitamin C is the most abundant antioxidant and has the greatest reducing potential; it is considered the most powerful reactive oxygen species (ROS) detoxifier. It can directly quench reactive oxygen radicals (superoxide, hydroxyl, singlet oxygen) and can reduce the H₂O₂ to H₂O through activity of the ascorbate peroxidase enzyme. It can protect the membranes by regenerating tocopherols from tocopherol radicals. It is necessary to have high levels to compensate for oxidative stress and regulate metabolic processes [56–58]. It is also essential in the human diet, since the human body cannot produce it—the only way to obtain it is by ingesting it from fruits [29]. In the plant, it works as an antioxidant and cofactor in redox reactions; its levels can vary due to stress factors [59,60]. Telesiński et al. [55] positively correlate ascorbate with Cl[−] content in plants. This can be attributed to the increase in vitamin C content in plants that were subjected to NaCl stress. Farhangi-Abriz and Torabian [19] reported similar results, finding an increase in the content of vitamin C in soybeans under salt stress conditions with the application of SiO₂ NPs. On the part of the SiO₂ NPs, the increase in the vitamin C content could be due to the fact that Si under saline conditions increases the activity of the enzymes monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR), related to the cycle of ascorbic acid–glutathione, recycling vitamin C in an important way [61].

Proteins play an important role in tomato development and quality [62]. They are involved in the electron transport chain, Krebs cycle, carbon metabolism, and stress response [63]. The highest protein content levels were observed in the treatment of SiO₂ NPs in plants without salt stress; however, it has been observed that silicon has very little impact on the metabolism of non-stressed plants [64]. Nwugo

and Huerta [65] found that, in rice, silicon alters only four proteins in the absence of stress, compared to 57 proteins in plants stressed with cadmium. The stimulation could be due to the application of silicon in the form of NPs, since NPs can induce oxidative stress [66], and not silicon in its ionic form. Nazaralian et al. [67] also found an increase of 25% in the proteins when sodium silicate and Si NPs were added, which is different from the present work since only an increase was found in the application of Si NPs.

Hydrogen peroxide (H_2O_2) is a free radical derived from oxygen. It is the result of the reduction of O_2^- electrons and it has the highest half-life of ROS and smallest molecule size, allowing it to cross cell membranes and function as a signaling agent, particularly for acclimatization processes of stress and antioxidant defense [68]. It has been observed that the presence of H_2O_2 in the apoplast plays an active role in the transcription of resistance genes and, being toxic to pathogens, plays a positive role, limiting the spread of invading organisms by cell death around the infection [56,69]. It is common to observe an increase in H_2O_2 under salt stress; however, in the present work, a decrease in its content was observed in plants under salinity, which could be due to the observed increase in vitamin C content and the activity of the enzymes SOD and APX, the latter being dependent on vitamin C.

Glutathione (GSH) is necessary to maintain a normal state in the cells and to compensate for the harmful effects of stress. It can remove $^1\text{O}_2$, H_2O_2 , and the hydroxyl radical. GSH is the substrate for glutathione S-transferase and it plays an important role in the detoxification of dehydroascorbate reductase and xenobiotics. In combination with its oxidized form, glutathione maintains a redox balance in the cells, it being important to maintain the redox state under normal or stressful conditions. When stress increases, the concentration of GSH decreases, oxidizing the redox state [70–72]. Pérez-Labrada et al. [73] reported an increase in tomato GSH content under salinity with Cu NPs. The increase was observed in plants with and without salt stress. This is similar to what was found in the present study, which tells us that the increase in GSH may be due to NPs. One of the forms of action of the NPs is the stimulation and modulation of ROS [74]. Moreover, silicon improves the activity of glutathione reductase, an enzyme involved in the GSH recovery cycle, which can improve the levels of this compound [61].

There are different types of carotenoids in plants, such as β -carotene and β -cryptoxanthin, and non-provitamin A carotenoids, such as lutein and lycopene [75]. They are lipophilic organic compounds found in chloroplasts that perform many functions in plant metabolism, including a role in tolerance to oxidative stress. Carotenoids have different functions as light energy collectors and chlorophyll and O_2 triplet extinguishers and are essential elements of the xanthophyll cycle, among others [76]. In this work, a clear trend in the behavior of β -carotene was not observed, since an increase was shown in both forms of silicon with and without salinity, even by NaCl alone, which may indicate that all treatments induce this compound, which may allow for more stress tolerance.

The antioxidant enzymes through various chemical reactions eliminate intracellular ROS from organs, such as chloroplast, cytosol, apoplast, peroxisomes, and mitochondria [77,78]. Prolonged salinity stress causes oxidative stress, generating ROS harmful to biomolecules, such as proteins, nucleic acids, and membrane lipids [79]. One reaction to protect against excessive Na ion growth in tissues is to improve the activity of antioxidant enzymes [80]. SOD acts as the first line of defense against ROS, catalyzing the conversion of the superoxide or singlet oxygen radical into hydrogen peroxide (which is less harmful) and molecular oxygen [81]. It is one of the most important enzymes in the defense system against oxidative stress and is found in all the cells of the plant. The increase in SOD activity at the cellular level, when there is environmental stress, is a clear indicator of its function in the defense system in charge of neutralizing oxidative stress [52]. CAT plays an important role in the defense system of plants against oxidative stress. CAT plays a positive role by cooperating with SOD to eliminate ROS, degrading H_2O_2 into water and oxygen [82]. Similarly, APX transforms H_2O_2 into H_2O , using ascorbate as a hydrogen donor, producing monodehydroascorbate, which is found in various cellular compartments. It is the key enzyme to remove hydrogen peroxide in the chloroplast and cytosol from plant cells [83]. GPX is a class of enzymes in the antioxidant defense

system that catalyzes reactions in the ascorbate–glutathione cycle, contributing to the elimination of ROS and using glutathione as a reducing agent from H_2O_2 to H_2O through processes mediated by a thiol group independent of ascorbate [84,85]. The activities of glutathione reductase (GR) and GPX maintain a balanced state of reduced glutathione/oxidized glutathione (GSH/GSSG) [86]. An increase in the activity of enzymes, such as SOD, APX, and GPX, has been reported in plants with the application of Si in dill, barley, cucumber, and tomato plants [87–90]. PAL catalyzes the non-oxidative deamination of L-phenylalanine to form trans-cinnamic acid and a free ammonium ion. The conversion of phenylalanine into transcinnamic acid is the step required to channel the carbon input from primary metabolism to secondary phenylpropanoid metabolism. Its importance in metabolism is due to the large amounts of phenylpropanoid products found in plants [91]. The general metabolism of phenylpropanoid generates an enormous variety of secondary metabolites, and phenylpropanoids contribute to the responses to biotic and abiotic factors [92]. PAL catalyzes the velocity-determining step in the phenylpropanoid pathway responsible for phenolic compounds [93]. PAL activity mediates the discouragement of phenylalanine and, together with trans-cinnamic acid, they are precursors of phenolic compounds, such as lignin [94]. Lignin is a complex mixture of phenolic compounds and is essential in the structure of the vascular cell wall and the mechanical support of the plant and water transport [95]. Silicon regulates the expression of many genes, including the activity of enzymes such as PAL, although there is still no clarity on the specific mechanisms [67]. Furthermore, silicon, by improving the ascorbic acid–glutathione cycle and improving the activity of enzymes related to it, significantly improves the activity of antioxidant enzymes, such as APX and GPX [61]. The increase in SOD enzyme activity may be related to the fact that silicon positively regulates the expression of mRNA [Cu=Zn] SOD [96]. Nazaralian et al. [67] found an increment of 60% PAL by the application of sodium silicate and Si NPs in fenugreek.

There are different methods to measure antioxidant capacity—the two most used are ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (2,2-diphenyl-1-picrylhydrazyl) [97]. The total antioxidant activity in tomato is commonly classified as hydrophilic and lipophilic. Hydrophilic compounds are mainly phenolic compounds and vitamin C, comprising 83% of the total antioxidant activity, while lipophilic compounds are conferred by carotenoids, vitamin E, and lipophilic phenols, comprising 17% of the total antioxidant capacity [98]. No clear trend was observed in the ABTS radical, in which greater antioxidant activity was observed in the control. However, for the DPPH radical, an increase in antioxidant capacity was observed by K_2SiO_3 . That being said, it is clear that the application of silicon in both forms can influence the antioxidant capacity of tomato plants by modifying the different enzymatic and non-enzymatic antioxidants.

The pH was kept between 4 and 4.5; its changes are due to the transformation of organic acids into simple sugars [24]. Costan et al. [99] point out that the titratable acidity of tomato fruit increases when there is saline stress; however, in the present work, this did not happen. A clear trend was not observed, nor was an effect marked either by salinity or the application of treatments. Salinity causes an increase in the osmotic potential of plants, causing a reduction in water absorption and a decrease in water flux into the fruit [43]. This causes an increase in the concentration of TSS and an accumulation of solutes and organic molecules typically generated in plants under salt stress [100]. In tomato fruits under salinity, Sakamoto et al. [43] reported less water in the fruit and a higher dry weight, thus modifying the firmness of the fruit, since an increase in firmness was observed in the fruits that were under stress due to salinity.

5. Conclusions

The application of SiO_2 NPs at 500 mg L^{-1} had positive effects on the plants that were not subjected to salt stress, increasing the weight of the fruits and, therefore, increasing the fruit yield. In addition, the concentration of chlorophylls, phenols, GSH, and GPX activity were increased. In plants subjected to salt stress, the application of SiO_2 NPs helped to maintain the concentration of chlorophylls and the

content levels of GSH, PAL, and vitamin C. In addition to this, the application helped to maintain fruit size and fruit quality.

The results showed that the application of silicon in both forms (SiO_2 NPs and K_2SiO_3) induced positive responses in tomato plants under salt stress. However, SiO_2 NPs showed more favorable results in tomato plants under this stress condition.

Author Contributions: Conceptualization, A.J.-M., A.B.-M., and G.C.-P.; methodology, Z.H.P.-G. and H.O.-O.; formal analysis, Z.H.P.-G. and S.G.-M.; writing—original draft preparation, Z.H.P.-G. and S.G.-M.; writing—review and editing, A.J.-M. and J.V.-R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Canene-Adams, K.; Campbell, J.K.; Zaripheh, S.; Jeffery, E.H.; Erdman, J.W. The Tomato as a Functional Food. *J. Nutr.* **2005**, *135*, 1226–1230. [[CrossRef](#)] [[PubMed](#)]
- Frusciante, L.; Carli, P.; Ercolano, M.R.; Pernice, R.; Di Matteo, A.; Fogliano, V.; Pellegrini, N. Antioxidant nutritional quality of tomato. *Mol. Nutr. Food Res.* **2007**, *51*, 609–617. [[CrossRef](#)] [[PubMed](#)]
- Murillo-Amador, B.; Reyes-Pérez, J.J.; Hernández-Montiel, L.G.; Rueda-Puente, E.O.; De Lucia, B.; Beltrán-Morales, F.A.; Ruiz-Espinoza, F.H. Physiological responses to salinity in *Solanum lycopersicum* L. varieties. *Pak. J. Bot.* **2017**, *49*, 809–818.
- Hu, Y.; Schmidhalter, U. Limitation of Salt Stress to Plant Growth. In *Plant Toxicology*; Hock, B., Elstner, E.F., Eds.; CRC Press: Boca Raton, FL, USA, 2004; pp. 205–238.
- Shrivastava, P.; Kumar, R. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi J. Biol. Sci.* **2015**, *22*, 123–131. [[CrossRef](#)]
- Machado, R.; Serralheiro, R. Soil Salinity: Effect on Vegetable Crop Growth. Management Practices to Prevent and Mitigate Soil Salinization. *Horticultrae* **2017**, *3*, 30. [[CrossRef](#)]
- Ashraf, M.; Harris, P.J.C. Photosynthesis under stressful environments: An overview. *Photosynthetica* **2013**, *51*, 163–190. [[CrossRef](#)]
- Epstein, E. Silicon: Its manifold roles in plants. *Ann. Appl. Biol.* **2009**, *155*, 155–160. [[CrossRef](#)]
- Exley, C. Silicon in life: A bioinorganic solution to bioorganic essentiality. *Inorg. Biochem.* **1998**, *69*, 139–144. [[CrossRef](#)]
- Guntzer, F.; Keller, C.; Meunier, J.D. Benefits of plant silicon for crops: A review. *Agron. Sustain. Dev.* **2012**, *32*, 201–213. [[CrossRef](#)]
- Zhu, Y.; Gong, H. Beneficial effects of silicon on salt and drought tolerance in plants. *Agron. Sustain. Dev.* **2014**, *34*, 455–472. [[CrossRef](#)]
- Shi, Y.; Wang, Y.; Flowers, T.J.; Gong, H. Silicon decreases chloride transport in rice (*Oryza sativa* L.) in saline conditions. *J. Plant Physiol.* **2013**, *170*, 847–853. [[CrossRef](#)] [[PubMed](#)]
- Savvas, D.; Ntatsi, G. Biostimulant activity of silicon in horticulture. *Sci. Hortic.* **2015**, *196*, 66–81. [[CrossRef](#)]
- Juárez-Maldonado, A.; Ortega-Ortiz, H.; González-Morales, S.; Morelos-Moreno, Á.; Cabrera-de la Fuente, M.; Sandoval-Rangel, A.; Cadenas-Pliego, G.; Benavides-Mendoza, A. Nanoparticles and Nanomaterials as Plant Biostimulants. *Int. J. Mol. Sci.* **2019**, *20*, 162. [[CrossRef](#)] [[PubMed](#)]
- Yuvakkumar, R.; Elango, V.; Rajendran, V.; Kannan, N.S.; Prabu, P. Influence of nanosilica powder on the growth of maize crop (*Zea Mays* L.). *Int. J. Green Nanotechnol. Biomed.* **2011**, *3*, 180–190. [[CrossRef](#)]
- Sanglard, L.M.V.P.; Martins, S.C.V.; Detmann, K.C.; Silva, P.E.M.; Lavinsky, A.O.; Silva, M.M.; Detmann, E.; Araújo, W.L.; DaMatta, F.M. Silicon nutrition alleviates the negative impacts of arsenic on the photosynthetic apparatus of rice leaves: An analysis of the key limitations of photosynthesis. *Physiol. Plant.* **2014**, *152*, 355–366. [[CrossRef](#)]
- Gowayed, S.M.H.; Al-Zahrani, H.S.M.; Metwali, E.M.R. Improving the Salinity Tolerance in Potato (*Solanum tuberosum*) by Exogenous Application of Silicon Dioxide Nanoparticles. *Int. J. Agric. Biol.* **2017**, *19*, 183–192. [[CrossRef](#)]
- Almutairi, Z.M. Effect of nano-silicon application on the expression of salt tolerance genes in germinating tomato (*Solanum lycopersicum* L.) seedlings under salt stress. *Plant Omics* **2016**, *9*, 106–114.

19. Farhangi-Abriz, S.; Torabian, S. Nano-silicon alters antioxidant activities of soybean seedlings under salt toxicity. *Protoplasma* **2018**, *255*, 953–962. [\[CrossRef\]](#)
20. Xie, Y.; Li, B.; Tao, G.; Zhang, Q.; Zhang, C. Effects of nano-silicon dioxide on photosynthetic fluorescence characteristics of *Indocalamus barbatus* McClure. *J. Nanjing For. Univ. Nat. Sci. Ed.* **2012**, *36*, 59–63.
21. Suriyaprabha, R.; Karunakaran, G.; Yuvakkumar, R.; Prabu, P.; Rajendran, V.; Kannan, N. Growth and physiological responses of maize (*Zea mays* L.) to porous silica nanoparticles in soil. *J. Nanoparticle Res.* **2012**, *14*, 1–14. [\[CrossRef\]](#)
22. Steiner, A.A. A universal method for preparing nutrient solutions of a certain desired composition. *Plant Soil* **1961**, *15*, 134–154. [\[CrossRef\]](#)
23. González-MoscOSO, M.; Martínez-Villegas, N.V.; Cadenas-Pliego, G.; Benavides-Mendoza, A.; Del Carmen Rivera-Cruz, M.; González-Morales, S.; Juárez-Maldonado, A. Impact of silicon nanoparticles on the antioxidant compounds of tomato fruits stressed by arsenic. *Foods* **2019**, *8*, 612. [\[CrossRef\]](#)
24. Lopez-Vargas, E.R.; Ortega-ortiz, H.; Cadenas-pliego, G.; De-Alba-Romenus, K.; Cabrera-De-La-Fuente, M.; Benavides-Mendoza, A.; Juarez-Maldonado, A. Foliar Application of Copper Nanoparticles Increases the Fruit Quality and the Content of Bioactive Compounds in Tomatoes. *Appl. Sci.* **2018**, *8*, 1020. [\[CrossRef\]](#)
25. Nagata, M.; Yamashita, I. Simple Method for Simultaneous Determination of Chlorophyll and Carotenoids in Tomato Fruit. *J. Jpn. Soc. Food Sci. Technol. Shokuhin Kagaku Kogaku Kaishi* **1992**, *39*, 925–928. [\[CrossRef\]](#)
26. Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178.
27. Arvouet-Grand, A.; Vennat, B.; Pourrat, A.; Legret, P. Standardization of a propolis extract and identification of the main constituents. *J. Pharm. Belg.* **1994**, *49*, 462–468.
28. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [\[CrossRef\]](#)
29. Padayatty, S.J.; Katz, A.; Wang, Y.; Eck, P.; Kwon, O.; Lee, J.-H.; Chen, S.; Corpe, C.; Dutta, A.; Dutta, S.K.; et al. Vitamin C as an Antioxidant: Evaluation of Its Role in Disease Prevention. *J. Am. Coll. Nutr.* **2003**, *22*, 18–35. [\[CrossRef\]](#)
30. Xue, T.; Hartikainen, H.; Piironen, V. Antioxidative and growth-promoting effect of selenium on senescing lettuce. *Plant Soil* **2001**, *237*, 55–61. [\[CrossRef\]](#)
31. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol.* **1999**, *26*, 1231–1237. [\[CrossRef\]](#)
32. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a Free Radical Method to Evaluate Antioxidant Activity. *Food Sci. Technol.* **1995**, *28*, 25–30. [\[CrossRef\]](#)
33. Arnao, M.B.; Cano, A.; Acosta, M. The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem.* **2001**, *73*, 239–244. [\[CrossRef\]](#)
34. Patterson, B.D.; MacRae, E.A.; Ferguson, I.B. Estimation of hydrogen peroxide in plant extracts using titanium(IV). *Anal. Biochem.* **1984**, *139*, 487–492. [\[CrossRef\]](#)
35. Nakano, Y.; Asada, K. Purification of ascorbate peroxidase in spinach chloroplasts; its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. *Plant Cell Physiol.* **1987**, *28*, 131–140. [\[CrossRef\]](#)
36. Flohé, L.; Günzler, W.A. Assays of glutathione peroxidase. *Methods Enzymol.* **1984**, *105*, 114–120. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Dhindsa, R.S.; Plumb-dhindsa, P.; Thorpe, T.A. Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.* **1981**, *32*, 93–101. [\[CrossRef\]](#)
38. Sykłowska-Baranek, K.; Pietrosiuk, A.; Naliwajski, M.R.; Kawiak, A.; Jeziorek, M.; Wyderska, S.; Łojkowska, E.; Chinou, I. Effect of l-phenylalanine on PAL activity and production of naphthoquinone pigments in suspension cultures of *Arnebia euchroma* (Royle) Johnst. *Vitr. Cell. Dev. Biol. Plant* **2012**, *48*, 555–564. [\[CrossRef\]](#)
39. Hossain, Z.; Mustafa, G.; Komatsu, S. Plant Responses to Nanoparticle Stress. *Int. J. Mol. Sci.* **2015**, *16*, 26644–26653. [\[CrossRef\]](#)
40. Zribi, L.; Fatma, G.; Fatma, R.; Salwa, R.; Hassan, N.; Néjib, R.M. Application of chlorophyll fluorescence for the diagnosis of salt stress in tomato "*Solanum lycopersicum* (variety Rio Grande)". *Sci. Hort.* **2009**, *120*, 367–372. [\[CrossRef\]](#)

41. Giuffrida, F.; Scuderi, D.; Giurato, R.; Leonardi, C. Physiological response of broccoli and cauliflower as affected by NaCl salinity. *Acta Hortic.* **2013**, 435–441. [\[CrossRef\]](#)
42. Kalteh, M.; Alipour, Z.T.; Ashraf, S.; Aliabadi, M.M.; Nosratabadi, A.F. Effect of silica nanoparticles on basil (*Ocimum basilicum*) under salinity stress. *J. Chem. Health Risks* **2014**, 4, 49–55. [\[CrossRef\]](#)
43. Sakamoto, Y.; Watanabe, S.; Nakashima, T.; Okano, K. Effects of salinity at two ripening stages on the fruit quality of single-truss tomato grown in hydroponics. *J. Hortic. Sci. Biotechnol.* **1999**, 74, 690–693. [\[CrossRef\]](#)
44. Rawson, H.; Long, M.; Munns, R. Growth and Development in NaCl-Treated Plants. I. Leaf Na⁺ and Cl⁻ Concentrations Do Not Determine Gas Exchange of Leaf Blades in Barley. *Funct. Plant Biol.* **1988**, 15, 519. [\[CrossRef\]](#)
45. Munns, R.; Termaat, A. Whole-plant responses to salinity. *Aust. J. Plant Physiol.* **1986**, 13, 143–160. [\[CrossRef\]](#)
46. Santos, C.V. Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. *Sci. Hortic.* **2004**, 103, 93–99. [\[CrossRef\]](#)
47. Elsheery, N.I.; Cao, K.F. Gas exchange, chlorophyll fluorescence, and osmotic adjustment in two mango cultivars under drought stress. *Acta Physiol. Plant.* **2008**, 30, 769–777. [\[CrossRef\]](#)
48. Matichenkov, V.V.; Kosobrukhov, A.A. Si effect on the plant resistance to salt toxicity. *Conserv. Soil Water Soc. Shar. Solut.* **2004**, 2, 1–4.
49. Haghighi, M.; Pessarakli, M. Influence of silicon and nano-silicon on salinity tolerance of cherry tomatoes (*Solanum lycopersicum* L.) at early growth stage. *Sci. Hortic.* **2013**, 161, 111–117. [\[CrossRef\]](#)
50. Soundararajan, P.; Manivannan, A.; Ko, C.H.; Jeong, B.R. Silicon Enhanced Redox Homeostasis and Protein Expression to Mitigate the Salinity Stress in *Rosa hybrida* ‘Rock Fire’. *J. Plant Growth Regul.* **2018**, 37, 16–34. [\[CrossRef\]](#)
51. Valentine, I.K.; Maria, V.K.; Bruno, B. Phenolic Cycle in Plants and Environment. In *Mechanisms of Landscape Rehabilitation and Sustainability*; Palavan-Unsal, N., Kefeli, V., Blum, W., Eds.; Bentham Science Publishers: Sharjah, UAE, 2012; Volume 13, pp. 75–78.
52. Ashraf, M. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnol. Adv.* **2009**, 27, 84–93. [\[CrossRef\]](#)
53. Petropoulos, S.A.; Levizou, E.; Ntatsi, G.; Fernandes, Â.; Petrotos, K.; Akoumianakis, K.; Barros, L.; Ferreira, I.C.F.R. Salinity effect on nutritional value, chemical composition and bioactive compounds content of *Cichorium spinosum* L. *Food Chem.* **2017**, 214, 129–136. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Ahmed, I.M.; Cao, F.; Han, Y.; Nadira, U.A.; Zhang, G.; Wu, F. Differential changes in grain ultrastructure, amylase, protein and amino acid profiles between Tibetan wild and cultivated barleys under drought and salinity alone and combined stress. *Food Chem.* **2013**, 141, 2743–2750. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Telesiński, A.; Nowak, J.; Smolik, B.; Dubowska, A.; Skrzypiec, N. Effect of soil salinity on activity of antioxidant enzymes and content of ascorbic acid and phenols in bean (*Phaseolus vulgaris* L.) plants. *J. Elem.* **2008**, 13, 401–409.
56. Smirnoff, N. Ascorbic acid: Metabolism and functions of a multi-facetted molecule. *Curr. Opin. Plant Biol.* **2000**, 3, 229–235. [\[CrossRef\]](#)
57. Yamamoto, A.; Bhuiyan, M.N.H.; Waditee, R.; Tanaka, Y.; Esaka, M.; Oba, K.; Jagendorf, A.T.; Takabe, T. Suppressed expression of the apoplastic ascorbate oxidase gene increases salt tolerance in tobacco and Arabidopsis plants. *J. Exp. Bot.* **2005**, 56, 1785–1796. [\[CrossRef\]](#)
58. Athar, H.R.; Khan, A.; Ashraf, M. Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. *Environ. Exp. Bot.* **2008**, 63, 224–231. [\[CrossRef\]](#)
59. Fenech, M.; Amaya, I.; Valpuesta, V.; Botella, M.A. Vitamin C Content in Fruits: Biosynthesis and Regulation. *Front. Plant Sci.* **2019**, 9, 1–21. [\[CrossRef\]](#)
60. Leiva-Brondo, M.; Valcárcel, M.; Cortés-Olmos, C.; Roselló, S.; Cebolla-Cornejo, J.; Nuez, F. Exploring alternative germplasm for the development of stable high vitamin C content in tomato varieties. *Sci. Hortic.* **2012**, 133, 84–88. [\[CrossRef\]](#)
61. Hasanuzzaman, M.; Nahar, K.; Rohman, M.M.; Anee, T.I.; Huang, Y.; Fujita, M. Exogenous Silicon Protects Brassica napus Plants from Salinity-Induced Oxidative Stress Through the Modulation of AsA-GSH Pathway, Thiol-Dependent Antioxidant Enzymes and Glyoxalase Systems. *Gesunde Pflanz.* **2018**, 70, 185–194. [\[CrossRef\]](#)
62. Cai, J.; Wang, P.; Tian, S.; Qin, G. Quantitative proteomic analysis reveals the involvement of mitochondrial proteins in tomato fruit ripening. *Postharvest Biol. Technol.* **2018**, 145, 213–221. [\[CrossRef\]](#)

63. Qin, G.; Wang, Q.; Liu, J.; Li, B.; Tian, S. Proteomic analysis of changes in mitochondrial protein expression during fruit senescence. *Proteomics* **2009**, *9*, 4241–4253. [[CrossRef](#)] [[PubMed](#)]
64. Van Bockhaven, J.; De Vleeschauwer, D.; Höfte, M. Towards establishing broad-spectrum disease resistance in plants: Silicon leads the way. *J. Exp. Bot.* **2013**, *64*, 1281–1293. [[CrossRef](#)] [[PubMed](#)]
65. Nwugo, C.C.; Huerta, A.J. The effect of silicon on the leaf proteome of rice (*Oryza sativa* L.) plants under cadmium-stress. *J. Proteome Res.* **2011**, *10*, 518–528. [[CrossRef](#)] [[PubMed](#)]
66. Mosa, K.A.; El-Naggar, M.; Ramamoorthy, K.; Alawadhi, H.; Elnaggar, A.; Wartanian, S.; Ibrahim, E.; Hani, H. Copper Nanoparticles Induced Genotoxicity, Oxidative Stress, and Changes in Superoxide Dismutase (SOD) Gene Expression in Cucumber (*Cucumis sativus*) Plants. *Front. Plant Sci.* **2018**, *9*, 1–13. [[CrossRef](#)] [[PubMed](#)]
67. Nazaralian, S.; Majd, A.; Irian, S.; Najafi, F.; Ghahremaninejad, F.; Landberg, T.; Greger, M. Comparison of silicon nanoparticles and silicate treatments in fenugreek. *Plant Physiol. Biochem.* **2017**, *115*, 25–33. [[CrossRef](#)]
68. Hossain, M.A.; Bhattacharjee, S.; Armin, S.-M.; Qian, P.; Xin, W.; Li, H.-Y.; Burritt, D.J.; Fujita, M.; Tran, L.-S.P. Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: Insights from ROS detoxification and scavenging. *Front. Plant Sci.* **2015**, *6*, 1–19. [[CrossRef](#)]
69. Horemans, N.; Foyer, C.H.; Asard, H. Transport and action of ascorbate at the plant plasma membrane. *Trends Plant Sci.* **2000**, *5*, 263–267. [[CrossRef](#)]
70. May, M.J.; Vernoux, T.; Leaver, C.; Montagu, M.V.; Inze, D. Glutathione homeostasis in plants: Implications for environmental sensing and plant development. *J. Exp. Bot.* **1998**, *49*, 649–667. [[CrossRef](#)]
71. Tausz, M. The glutathione system as a stress marker in plant ecophysiology: Is a stress-response concept valid? *J. Exp. Bot.* **2004**, *55*, 1955–1962. [[CrossRef](#)]
72. Noctor, G.; Foyer, C.H. Ascorbate and glutathione: Keeping active oxygen under control. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1998**, *49*, 249–279. [[CrossRef](#)]
73. Pérez-Labrada, F.; López-Vargas, E.R.; Ortega-Ortiz, H.; Cadenas-Pliego, G.; Benavides-Mendoza, A.; Juárez-Maldonado, A. Responses of Tomato Plants under Saline Stress to Foliar Application of Copper Nanoparticles. *Plants* **2019**, *8*, 151. [[CrossRef](#)] [[PubMed](#)]
74. Abdel-Halim, M.E.F.; Hegazy, H.S.; Hassan, N.S.; Naguib, D.M. Effect of silica ions and nano silica on rice plants under salinity stress. *Ecol. Eng.* **2017**, *99*, 282–289. [[CrossRef](#)]
75. Rao, A.; Rao, L. Carotenoids and human health. *Pharmacol. Res.* **2007**, *55*, 207–216. [[CrossRef](#)] [[PubMed](#)]
76. Demmig-Adams, B. Antioxidants in Photosynthesis and Human Nutrition. *Science* **2002**, *298*, 2149–2153. [[CrossRef](#)]
77. Foyer, C.H.; Noctor, G. Ascorbate and glutathione: The heart of the redox hub. *Plant Physiol.* **2011**, *155*, 2–18. [[CrossRef](#)]
78. Hasanuzzaman, M.; Fujita, M. Selenium pretreatment upregulates the antioxidant defense and methylglyoxal detoxification system and confers enhanced tolerance to drought stress in rapeseed seedlings. *Biol. Trace Elem. Res.* **2011**, *143*, 1758–1776. [[CrossRef](#)]
79. Ahmad, P.; Jaleel, C.A.; Sharma, S. Antioxidant defense system, lipid peroxidation, proline-metabolizing enzymes, and biochemical activities in two *Morus alba* genotypes subjected to NaCl stress. *Russ. J. Plant Physiol.* **2010**, *57*, 509–517. [[CrossRef](#)]
80. Torabian, S.; Farhangi-Abriz, S.; Zahedi, M. Efficacy of FeSO₄ nano formulations on osmolytes and antioxidative enzymes of sunflower under salt stress. *Ind. J. Plant Physiol.* **2018**, *23*, 305–315. [[CrossRef](#)]
81. Mittler, R. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* **2002**, *7*, 405–410. [[CrossRef](#)]
82. Che, Y.; Zhang, N.; Zhu, X.; Li, S.; Wang, S.; Si, H. Enhanced tolerance of the transgenic potato plants overexpressing Cu/Zn superoxide dismutase to low temperature. *Sci. Hortic.* **2020**, *261*, 108949. [[CrossRef](#)]
83. Asada, K. The water–water cycle as alternative photon and electron sinks. *Philos. Trans. R. Soc. London. Ser. B Biol. Sci.* **2000**, *355*, 1419–1431. [[CrossRef](#)] [[PubMed](#)]
84. Hossain, M.S.; Dietz, K.-J. Tuning of Redox Regulatory Mechanisms, Reactive Oxygen Species and Redox Homeostasis under Salinity Stress. *Front. Plant Sci.* **2016**, *7*. [[CrossRef](#)] [[PubMed](#)]
85. Gill, S.S.; Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* **2010**, *48*, 909–930. [[CrossRef](#)] [[PubMed](#)]
86. Ghasemnezhad, M.; Sherafati, M.; Payvast, G.A. Variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (*Capsicum annum*) fruits at two different harvest times. *J. Funct. Foods* **2011**, *3*, 44–49. [[CrossRef](#)]

87. Shekari, F.; Abbasi, A.; Mustafavi, S.H. Effect of silicon and selenium on enzymatic changes and productivity of dill in saline condition. *J. Saudi Soc. Agric. Sci.* **2017**, *16*, 367–374. [[CrossRef](#)]
88. Al-aghabary, K.; Zhu, Z.; Shi, Q. Influence of silicon supply on chlorophyll content, chlorophyll fluorescence, and antioxidative enzyme activities in tomato plants under salt stress. *J. Plant Nutr.* **2004**, *27*, 2101–2115. [[CrossRef](#)]
89. Liang, Y.; Chen, Q.; Liu, Q.; Zhang, W.; Ding, R. Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L.). *J. Plant Physiol.* **2003**, *160*, 1157–1164. [[CrossRef](#)]
90. Zhu, Z.; Wei, G.; Li, J.; Qian, Q.; Yu, J. Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). *Plant Sci.* **2004**, *167*, 527–533. [[CrossRef](#)]
91. Hyun, M.W.; Yun, Y.H.; Kim, J.Y.; Kim, S.H. Fungal and Plant Phenylalanine Ammonia-lyase. *Mycobiology* **2011**, *39*, 257–265. [[CrossRef](#)]
92. Vogt, T. Phenylpropanoid Biosynthesis. *Mol. Plant* **2010**, *3*, 2–20. [[CrossRef](#)]
93. Wanner, L.A.; Li, G.; Ware, D.; Somssich, I.E.; Davis, K.R. The phenylalanine ammonia-lyase gene family in *Arabidopsis thaliana*. *Plant Mol. Biol.* **1995**, *27*, 327–338. [[CrossRef](#)] [[PubMed](#)]
94. Weng, J.-K.; Chapple, C. The origin and evolution of lignin biosynthesis. *New Phytol.* **2010**, *187*, 273–285. [[CrossRef](#)] [[PubMed](#)]
95. Boerjan, W.; Ralph, J.; Baucher, M. Lignin biosynthesis. *Annu. Rev. Plant Biol.* **2003**, *54*, 519–546. [[CrossRef](#)] [[PubMed](#)]
96. Khan, A.; Bilal, S.; Khan, A.L.; Imran, M.; Al-Harrasi, A.; Al-Rawahi, A.; Lee, I.J. Silicon-mediated alleviation of combined salinity and cadmium stress in date palm (*Phoenix dactylifera* L.) by regulating physio-hormonal alteration. *Ecotoxicol. Environ. Saf.* **2020**, *188*, 109885. [[CrossRef](#)]
97. Vinha, A.F.; Alves, R.C.; Barreira, S.V.P.; Castro, A.; Costa, A.S.G.; Oliveira, M.B.P.P. Effect of peel and seed removal on the nutritional value and antioxidant activity of tomato (*Lycopersicon esculentum* L.) fruits. *LWT Food Sci. Technol.* **2014**, *55*, 197–202. [[CrossRef](#)]
98. Kotíková, Z.; Lachman, J.; Hejtmánková, A.; Hejtmánková, K. Determination of antioxidant activity and antioxidant content in tomato varieties and evaluation of mutual interactions between antioxidants. *LWT Food Sci. Technol.* **2011**, *44*, 1703–1710. [[CrossRef](#)]
99. Costan, A.; Stamatakis, A.; Chrysargyris, A.; Petropoulos, S.A.; Tzortzakis, N. Interactive effects of salinity and silicon application on *Solanum lycopersicum* growth, physiology and shelf-life of fruit produced hydroponically. *J. Sci. Food Agric.* **2020**, *100*, 732–743. [[CrossRef](#)]
100. Munns, R. Comparative physiology of salt and water stress. *Plant Cell Environ.* **2002**, *25*, 239–250. [[CrossRef](#)]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).