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Silicon and Nitric Oxide Applications Allow Mitigation of Water Stress in Myrobalan 29C Rootstocks (*Prunus cerasifera* Ehrh.)

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Abstract: (1) Background: Silicon (Si) and nitric oxide (NO) have been proven to protect against cellular injury caused by stress conditions, mostly by salinity and water stress in agriculture. (2) Methods: The goal was to study the effect of soil applications of NO, Si, and their combination (Si+NO) on the response of Myrobalan 29C rootstocks (*Prunus cerasifera* Ehrh.) subjected to water stress and well-watered conditions. (3) Results: The results showed that water stress decreased growth parameters (i.e., leaf area, shoot dry weight, root fresh weight, shoot fresh weight, root dry weight, and relative shoot diameter), physio-biochemical parameters (i.e., chlorophyll and relative water content in leaves), and leaf and root minerals (i.e., P, Ca, Fe, and Zn in leaves and N, K, Ca, and Zn in roots), compared to well-watered plants. Under these conditions, all treatments mitigated the detrimental effects of water stress on Myrobalan 29C rootstocks, being the most effective the Si+NO treatment. (4) Conclusions: These findings briefly highlight that the combination of silicon and nitric oxide may provide greater tolerance to water stress in Myrobalan 29C rootstocks.

Keywords: abiotic stress; lipid peroxidation; Myrobalan plum; proline; soil fertilization

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1. Introduction

Many plant species generally grow under abiotic (frost, flood, metals, salt, drought, heat, and cold) and biotic (pathogens, such as fungi, viruses, and bacteria) stress, affecting plant growth and threatening world's food security [1–3]. Plants are altered in their physiology, metabolism, and gene regulation in response to abiotic and biotic stress conditions [4]. Water stress may affect plant crop quality, productivity, and several metabolic functions via altering the molecular, physiological, and morphological aspects of the plant [5]. Some plant biological processes which occur in the cytosol are disturbed by water stress, affecting photosynthesis, respiration rate, turgor pressure, carbon assimilation rate, mineral nutrition, and gas exchange on leaves [6]. The physiological response mechanisms of plants that are activated by water stress are complex and closely related to the length and extent of drought and differ between plant species [7].

Plants subjected to water stress activate several physiological mechanisms that allow them to tolerate these conditions by maintaining cell turgor. Currently, some materials, such as silicon (Si) and nitric oxide (NO), are used to alleviate the effects of drought on several plant species, such as wheat, cowpea, maize, pea, and strawberry, among others [2,8–12]. Si is currently not considered an essential nutrient for plants, but it is the second-most abundant element in the agricultural cultivation areas in the world [2]. Si induces compartmentation and complexation of metal ions, immobilization of toxic metal ions in growth conditions, coprecipitation of toxic metal ions, and activation of antioxidant enzyme activity, reducing lipid peroxidation in plant roots [13]. Si ameliorates abiotic stress in plants by regulating growth, morphology, antioxidant enzyme activities, osmolytes accumulation, photosynthesis, and nutrient uptake and by modulating the expression of phytohormones [14]. Si mitigates the effects of drought stress, enhancing water uptake and transport, regulating stomatal conductance and water loss by transpiration, accumulating solutes and osmoregulatory substances, and maintaining whole-plant water balance [15]. NO is a water- and lipid-soluble free radical that plays a key role in cytoprotection [16]. NO induces high reactive oxygen species (ROS) production in plant cells, and it plays a key role as a signaling molecule in the response of plants to abiotic stresses [17]. Simontacchi et al. [18] reviewed the role of NO in adaptative plant responses, indicating its positive effects against abiotic stress, such as heavy metals, drought, extreme temperatures, and salinity. However, few studies support the role of NO in drought response, and currently, research by specialists continues [16].

To our knowledge, studies about the effects of the combination of silicon and nitric oxide (Si+NO) on alleviating abiotic stress in plants are scarce, and there are no studies about its effects on the mitigation of water stress on plants. Most of the published studies are focused on studying its effects on heavy metals and salinity stress in agriculture [19–22]. Therefore, the aim of this research was to study the effect of NO, Si, and Si+NO application on growth parameters, physio-biochemical parameters, and leaf and root minerals as well as membrane permeability, temperature, proline content, and lipid peroxidation in Myrobalan 29C rootstocks (*Prunus cerasifera* Ehrh.) subjected to water stress and well-watered conditions.

2. Materials and Methods

2.1. Experimental Site and Plant Material

The research trial was conducted at the Research Center of the Harran University of Agriculture Faculty in 2019. In this trial, the Myrobalan 29C *Prunus cerasifera* Ehrh.) rootstock was used as plant material. Myrobalan 29C rootstocks were propagated in a tissue culture medium. Then, three buds were left on the main stem, and the rest of the buds were removed. Subsequently, the rootstocks were planted into 12 L plastic pots filled with peat moss (Klasmann, TS1, Klasmann-Deilman Gmbh, Lower Saxony, Germany) on 15 March. The peat growing medium possessed an electrical conductivity of 35 mS m^{-1}, a pH of 6.5, and 1.0 kg m^3 14:10:18 N:P:K. Sodium silicate (Si) and sodium nitroprusside (SNP), which breaks down in circulation to release nitric oxide, (NO) were added to the peat solution.

2.2. Treatments

Rootstock were irrigated equally for 8 weeks from March 15 to May 15. Water stress applications were carried out at the end of this period, considering plant pot capacity (i.e., field capacity). Pot capacity (PC) was determined by weighing the pot with dry soil, which was subsequently saturated with water. The pot was fully covered with aluminum foil to avoid evaporation and was kept for 48 h to drain out water. The pot was re-weighed, and PC was measured according to the equation exposed by Liyanage et al. [23]. A water stress program and Si and NO applications to mitigate the negative effects of the stress on Myrobalan 29C rootstock were started on 15 May. The amount of water required for a rootstock in each plastic pot was determined according to their PC. Plastic pots were weighed at two-day intervals to determine the water consumption by the plants via evapotranspiration, and thus when to refill the consumed water. The pot moisture mixture was filled to 100% (non-water-stress) and 50% (water stress) of the PC. The onset of the irrigation period during water stress was adjusted based on the level at which 40% of the useful moisture is consumed.

The experiment was planned with a randomly arranged split-plot trial design. The study was designed with 6 replications considering one plant per replicate. The main plot was designed at two different irrigation levels (non-water-stress and water stress), and the sub-plots were defined by the application of four solutions that corresponded to control, silicon (Si), nitric oxide (NO), and the simultaneous application of Si and NO (Si+NO). Control corresponded to water application; Si corresponded to the application of 10 mM of Si; NO corresponded to the application of 200 µM of NO, and Si+NO corresponded to the application of 10 mM of Si and 200 µM of NO. Si and NO dosages were defined according to studies by different authors on abiotic stress in woody plant species [24–26].

A total of 48 plants were used in this study and subjected to two different irrigation levels and four applications for a total period of 9 weeks. Thus, eight different combinations of factors were conducted in this trial: (i) non-water-stress + control; (ii) non-water-stress + Si; (iii) non-water-stress + NO; (iv) non-water-stress + Si+NO; (v) water stress + control; (vi) water stress + Si; (vii) water stress + NO; (viii) water stress + Si+NO. Chemical solutions were applied to soil in the root zone every two weeks using 200 mL of the solution, adding 50% Hoagland solution [27].

2.3. Plant Growth Parameters

Before evaluations, the rootstocks were harvested on 23 July, carefully removing the roots from the pots using water. Root dry weight (RDW), root fresh weight (RFW), shoot dry weight (SDW), and shoot fresh weight (SFW) per plant were measured. Shoot and root samples were dried at 65 to 70 °C for 48 h. A day before harvest, three leaves located in the middle of the apical shoot of the plant were removed, and their leaf area was measured by the ImageJ program [28]. Leaf area was calculated as cm^2 according to the methodology exposed by Klamkowski and Treder [29]. Relative shoot diameter (RSD) and relative shoot length (RSL) measurements (%) were performed at the beginning of water stress treatments (on 15 May) and at the end of the trial (on 23 July), according to the exposed by Bolat et al. [30].

After the leaves were kept in water for 24 h, relative water content of the leaf (L-RWC) of the samples was measured. They were then dried at 70 °C for 48 h in a ventilated oven. L-RWC was expressed as percent, and leaf proportional water content and turgor loss were calculated according to the methodology exposed by Yamasaki and Dillenburg [31].

2.4. Physio-Biochemical Parameters

Leaf stomatal conductance was performed between 12.00–14.00 h at the solar zenith, according to the protocol performed by several authors [32–34]. Briefly, a leaf porometer (Model SC-1, Steady-State Diffusion Porometer, Decagon Devices Inc., WA, USA) was used to determine this parameter on the 3rd and 5th leaves, located in the same positions between the apical and middle regions of the terminal shoot of the plant [33].

Chlorophyll (Chl) content in leaves was measured with a SPAD-502 Plus (Konica Minolta Optics, Inc., Tokyo, Japan). The SPAD index was determined by two measurements performed on the 3rd and 5th leaves in the same positions between the apical and middle regions of the terminal shoot of the samples. Subsequently, the mean of the values obtained from the SPAD readings was registered and calculated [35]. Leaf temperature (LT) was measured with an infrared thermometer on the 3rd and 5th leaves in the same positions between the apical and middle regions of the terminal shoot of the plant. The measurement was performed on sunny days at noon when the clouds did not block the sun [36].

2.5. Mineral Analysis in Roots and Leaves

After harvest, roots and leaves were washed three times with distilled water and were placed on blotting paper. Then, the samples were kept for 48 h in an oven that was set at 65–70 °C, and the dry leaves were ground in a porcelain mortar. The N content of the samples was expressed as a percentage using the modified Kjeldahl procedure [37], and K, Ca, Fe, Zn, and Mg contents were analyzed using an atomic absorption spectrophotometer. P was determined via spectrophotometry (Shimadzu UV-1700), according to the methodology reported by Kacar and Inal [37].

2.6. Proline and Lipid Peroxidation Analysis in Samples

Proline content of the leaves collected at harvest was determined based on the methodology published by Bates et al. [38]. Briefly, leaf samples (0.5 g) were ground in 10 mL of 3% of sulfosalicylic acid, which allows the extraction of proline. Then, the mixture was centrifuged at 10,000 rpm for 10 min. Subsequently, 2 mL of the supernatant was added and mixed into test tubes with 2 mL of the freshly prepared acid–ninhydrin and 2 mL of glacial acetic acid solution. The tube was incubated in a water bath for 1 h at 90 °C for reaction. Then, samples were extracted using 5 mL toluene and were vortexed for 15 s. After this, the samples were held at room temperature in the dark for at least 20 min to separate toluene and the aqueous phase. The toluene phase was carefully collected, and the absorbances of the samples were read at 520 nm by a spectrophotometer (Shimadzu UV-1700, Kyoto, Japan). The standard curve for the proline calculation was prepared using L-proline, and the values were measured as μg g^{-1} of fresh weight.

Lipid peroxidation of samples was measured according to the methodology stated by Jalel et al. [39] using the thiobarbituric acid test. The absorbances of samples were read at 532 and 600 nm by the Shimadzu UV-1700 spectrophotometer. Malondialdehyde (MDA) value in leaves was calculated according to Jalel et al. [39].

2.7. Statistical Analysis

Data analysis was performed using SPSS software (SPSS Version 23, IBM, VA, USA). The variables were subjected to an analysis of variance (ANOVA), and the separation of the means was performed using Tukey's test ($p \leq 0.01$). A principal component analysis (PCA) was performed to determine relationships between variables according to water regimen and chemical solutions, using the average data in each case. The analysis was performed using the SPSS software (SPSS Version 23). The R software (The R Foundation, Vienna, Austria) was used to perform the correlation matrix of the variables.

3. Results

3.1. Plant Growth Parameters

Figure 1 shows the effects of water stress and chemical applications on Myrobolan 29C rootstock growth parameters. Based on this figure, water stress significantly ($p \leq 0.01$) reduced leaf area, shoot dry weight, root fresh weight, shoot fresh weight, root dry weight, and relative shoot diameter of Myrobalan 29C rootstocks compared to non-water-stress at harvest. Shoot fresh weight was only affected by the silicon (Si) treatment under non-water-stress; this parameter was higher in Si than in control. Si, nitric oxide (NO), and simultaneous application of silicon and nitric oxide (Si+NO) induced higher shoot dry weight, root dry weight, and root shoot length than control under non-water-stress conditions. Similar effects were found for control and NO for root fresh weight and for control and Si for leaf area and relative shoot diameter under these conditions (Figures 1 and 2). Chemical applications to Myrobolan 29C rootstocks induced higher root fresh weight, root dry weight, relative shoot length, and relative shoot diameter than control under water stress conditions (Figures 1 and 2). Simultaneous application of Si and NO induced higher levels of the studied parameters than control under water stress conditions and higher levels of most of the studied parameters except shoot fresh weight than control in non-water-stress conditions.

3.2. Physio-Biochemical Parameters

Figure 2 shows the effects of water stress and chemicals applications on Myrobolan 29C rootstock physio-biochemical parameters. Based on this figure, water stress significantly ($p \leq 0.01$) reduced chlorophyll and relative water content of leaves of Myrobalan 29C rootstocks compared to non-water-stress at harvest. Leaf relative water content was not affected by the treatments in the well-watered rootstocks. Chemicals solutions induced lower membrane permeability and higher stomatal conductance than control under non-water-stress conditions. Simultaneous application of silicon and nitric oxide (Si+NO) induced higher chlorophyll content and lower leaf temperature than control under non-water-stress conditions. Chemicals solutions induced lower membrane permeability and leaf temperature, and higher stomatal conductance and leaf chlorophyll content than control under water stress conditions. Si+NO treatment induced higher leaf relative water content than control and enhanced leaf chlorophyll and leaf temperature compared the rest of the treatments under water stress conditions.

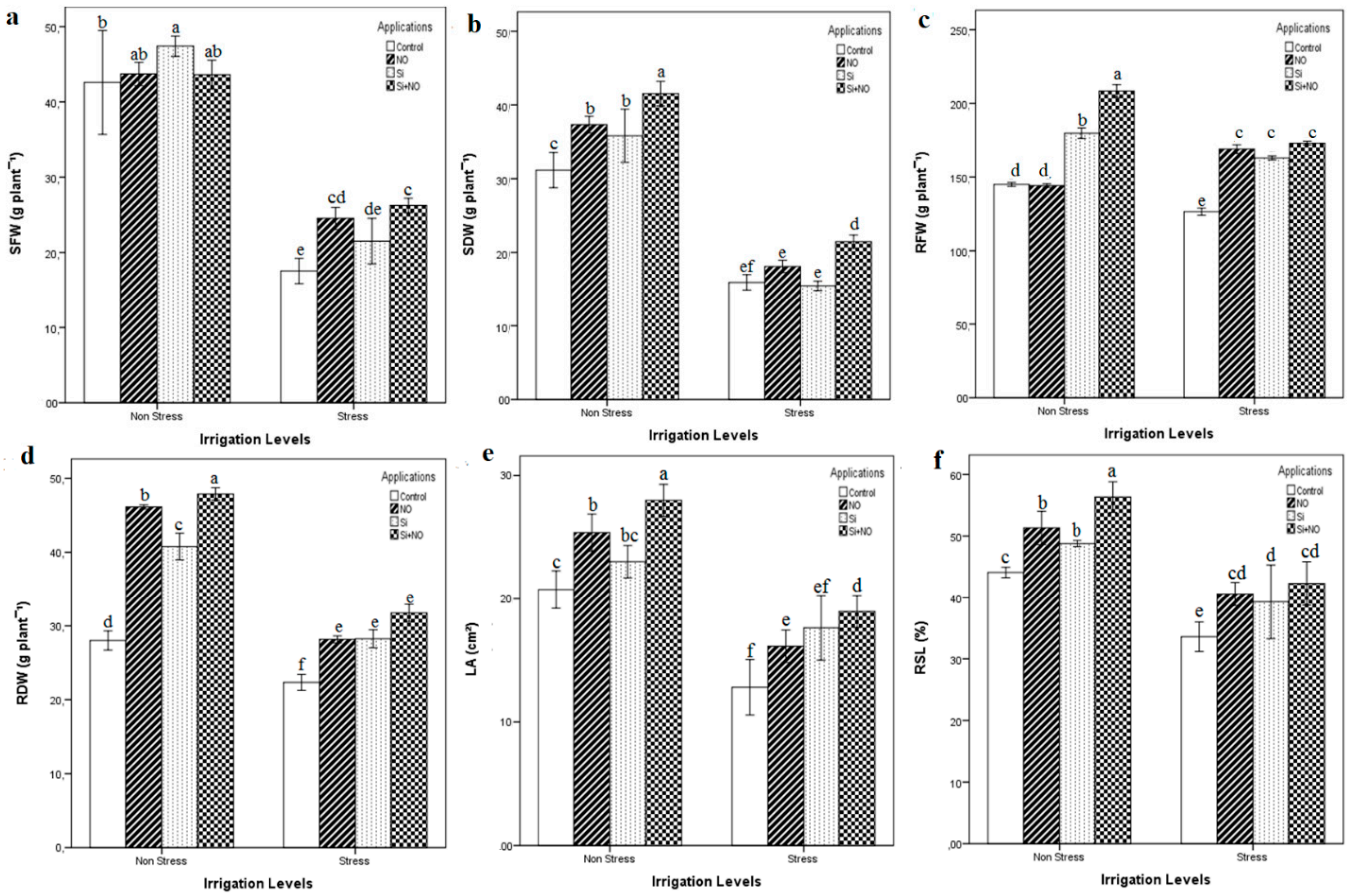

Figure 1. Effects of water stress and chemicals applications on Myrobolan 29C growth parameters determined at harvest. Different lowercase letters indicate significant differences (Tukey test; $p \leq 0.01$) between irrigation levels and chemicals applications; SFW: shoot fresh weight (**a**); SDW: shoot dry weight (**b**); RFW: root fresh weight (**c**); RDW: root dry weight (**d**); LA: leaf area (**e**); RSL: relative shoot length (**f**). Error bars on figures represent standard error (SE).

3.3. Minerals in Roots and Leaves

Table 1 shows the content of N, P, K, Ca, Mg, Fe, and Zn of Myrobolan 29C leaves subjected to different irrigation regimes and chemicals treatments. Irrigation regime and chemicals applications factors significantly affected the content of the studied minerals. The water stressed plants presented lower P, Ca, Fe, and Zn content in leaves than the non-water-stressed ones. Interaction of the factors considerably affected the content of most of the studied minerals in the leaves, except K. Leaf K content significantly increased in the presence of Si+NO under both non-stress and water deficit stress conditions (Supplementary Table S1), but in interaction, none of the factors affected the content of K in the leaves. The Si+NO applications to Myrobalan 29C rootstocks growing under non-water-stress conditions presented the highest contents of N, P, Ca, Fe, and Zn. Under non-water-stress, Si+NO treatment significantly increased the leaf content of N, P, Ca, Fe, and Zn compared the rest of the treatments and control. Additionally, under water stress conditions, Si+NO treatment to Myrobolan 29 C rootstocks increased the leaves' content of Ca, Mg, Fe, and Zn compared the other applications.

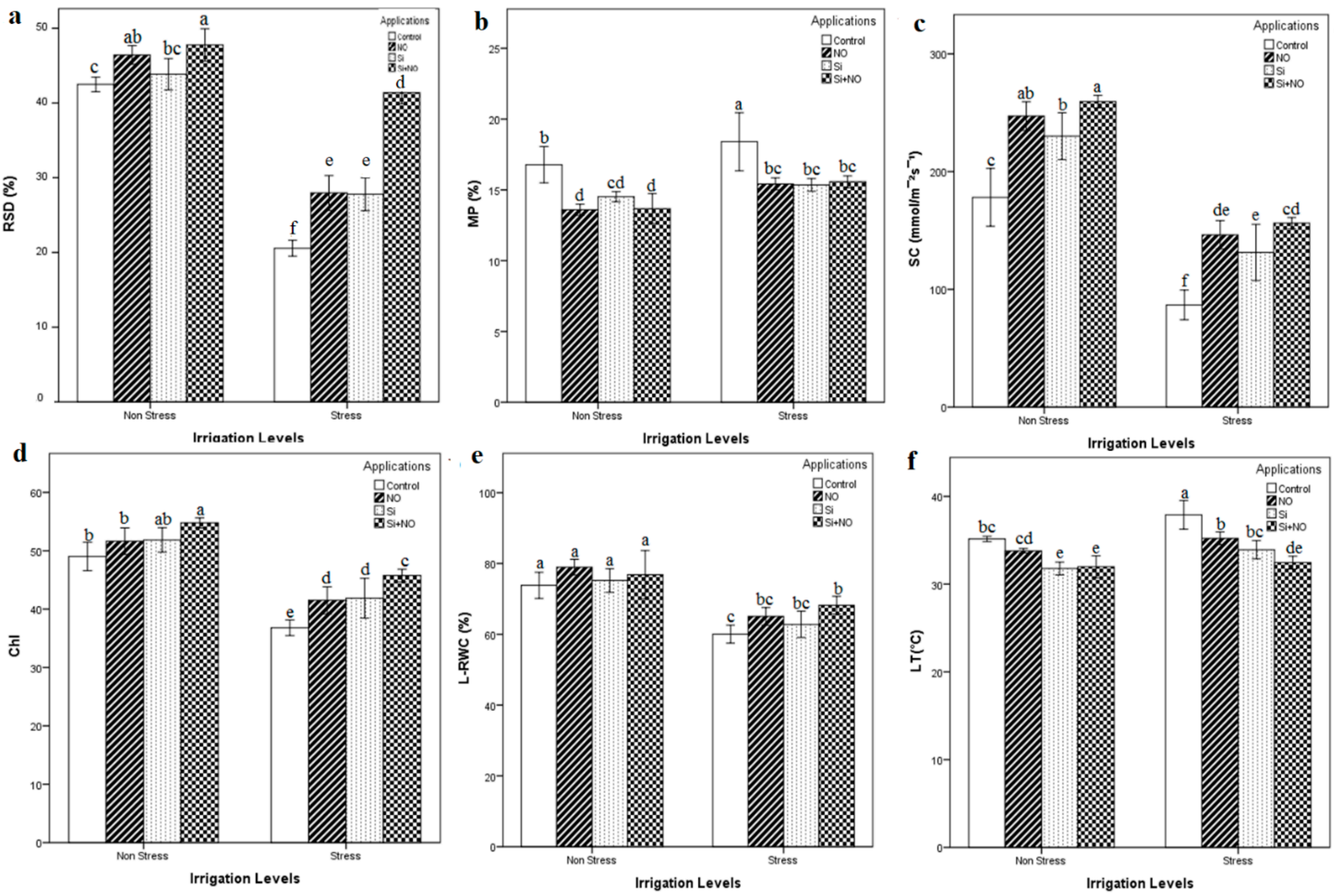

Figure 2. Effects of water stress and chemicals applications on relative shoot diameter and physio-biochemical parameters determined at harvest in Myrobolan 29C rootstocks. Different lowercase letters indicate significant differences (Tukey test; $p \leq 0.01$) between irrigation levels and chemicals applications; RSD: Relative shoot diameter (**a**); MP: Membrane permeability (**b**); SC: Stomata conductance (**c**) Chl: Chlorophyll content (**d**); L-RWC: Leaf relative water content (**e**); LT: Leaf temperature (**f**). Error bars on figures represent standard error (SE).

Table 2 shows the contents of N, P, K, Ca, Mg, Fe, and Zn of Myrobolan 29C roots subjected to different irrigation regimes and chemicals treatments. Irrigation regime, chemical applications, and their interaction affected N, P, K, Ca, Mg, and Zn. Root Fe content significantly increased in the presence of Si, NO, and Si+NO applications under both non-stress and water deficit stress conditions (Supplementary Table S1), but in interaction, none of the factors affected the content of Fe in the roots. Si+NO applications to Myrobalan 29C rootstocks growing under non-water-stress conditions induced the highest contents of N and Mg compared to the rest of the interactions. Si+NO samples presented higher K, Ca, and Zn root content than control. Si treatment applied to plants resulted in the highest content of P compared to the rest of treatments. Under non-water-stress, control presented the lowest of N, P, K, and Zn in roots, whereas Si+NO samples showed the highest content of N and Mg in the roots. In addition, Si+NO samples presented higher Ca root content than control and NO samples. Under water stress conditions, chemical applications to Myrobolan 29C roots induced higher P, K, Ca, and Zn than control, whereas Si and Si+NO treatments resulted in higher N root content than control.

Table 1. Mineral content determined at harvest in leaves of Myrobolan 29C rootstocks subjected to different irrigation regimes and chemicals applications.

Irrigation Regime	Chemicals Applications	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Zn (ppm)
Non-water-stress	Control	2.80 bc	0.19 b	2.8	1.5 c	0.41 c	75.5 d	27.4 c
	Si	2.89 b	0.19 b	2.9	1.6 b	0.41 c	77.2 c	28.4 b
	NO	2.86 b	0.19 b	2.7	1.6 b	0.79 a	83.2 b	28.5 b
	Si+NO	3.54 a	0.22 a	3.2	1.7 a	0.84 a	94.7 a	29.4 a
Water stress	Control	2.50 d	0.15 c	2.1	1.1 g	0.20 d	65.2 h	22.5 g
	Si	2.59 cd	0.16 c	2.2	1.2 f	0.25 d	65.3 g	23.3 f
	NO	2.65 bcd	0.15 c	2.3	1.3 e	0.43 c	70.5 f	24.4 e
	Si+NO	2.72 bcd	0.16 c	2.4	1.4 d	0.54 b	72.1 e	25.1 d
Irrigation regime		**	**	**	**	**	**	**
Chemicals applications		**	**	**	**	**	**	**
Interaction		**	**	ns	**	**	**	**

For a given factor and significance, different letters within a column represent significant differences (Tukey's test, $p < 0.01$). ns: non-significant. **: Significant difference ($p < 0.01$). Si: silicon. NO: nitric oxide. Si+NO: silicon + nitric oxide. Interaction: irrigation regime × chemicals applications.

Table 2. Mineral content in roots determined at harvest of Myrobolan 29C rootstocks subjected to different irrigation regimes and chemicals applications.

Irrigation Regime	Chemicals Applications	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Zn (ppm)
Non-water-stress	Control	1.63 c	0.19 c	0.91 c	1.7 bc	0.16 c	12.3	1.0 c
	Si	1.83 b	0.22 a	1.05 ab	1.9 ab	0.17 bc	14.0	1.3 b
	NO	1.79 b	0.20 b	1.01 b	1.8 b	0.19 b	14.2	1.4 a
	Si+NO	2.38 a	0.20 b	1.08 a	2.0 a	0.22 a	15.2	1.4 a
Water stress	Control	1.30 f	0.16 e	0.46 e	1.1 f	0.13 d	5.0	0.6 f
	Si	1.46 de	0.18 cd	0.75 d	1.4 e	0.15 cd	5.8	0.7 e
	NO	1.40 ef	0.17 d	0.76 d	1.5 de	0.17 bc	5.4	0.8 d
	Si+NO	1.60 cd	0.18 cd	0.78 d	1.6 d	0.17 bc	6.7	0.8 d
Irrigation regime		**	**	**	**	**	*	**
Chemical applications		**	**	**	**	**	*	**
Interaction		**	**	**	**	**	ns	**

For a given factor and significance, different letters within a column represent significant differences (Tukey's test, $p < 0.01$). ns: non-significant. **: Significant difference ($p < 0.01$). *: Significant difference ($p < 0.05$). Si: silicon. NO: nitric oxide. Si+NO: silicon + nitric oxide. Interaction: irrigation regime x chemicals applications.

3.4. Proline and Lipid Peroxidation Levels, and Principal Component Analysis (PCA)

Figure 3 shows the effects of water stress and chemical applications on L-proline and lipid peroxidation (MDA) in Myrobolan 29C rootstock leaves. Chemicals did not affect L-proline content in the leaves of the rootstocks growing under non-water-stress conditions. However, these solutions considerably increased its content under water stress conditions. Lipid peroxidation was lower in the Myrobolan 29C rootstocks growing under both irrigation regimes.

Principal component analysis (PCA) was performed using data of plant growth parameters, physio-biochemical parameters, minerals found in leaves and roots, L-proline, and lipid peroxidation values evaluated at harvest. The first two components represented 84.75 % of the total variance (80.1% and 4.74% for components 1 and 2, respectively). Most of the variables were close—located in the first quadrant of the PCA—except for lipid peroxidation, proline, membrane permeability, and leaf temperature. Regarding the Pearson correlation coefficient, there was a negative and weak correlation between L-proline and lipid peroxidation with P, N, Ca, K, Fe, Zn, and Mg contents of leaves and roots. There also was a positive correlation between stomatal conductance and root fresh weight, L-proline, and root Fe content, whereas there was a negative correlation between chlorophyll; leaf temperature; leaf area; shoot fresh weight; shoot dry weight; root dry weight; relative shoot length; relative shoot diameter; lipid peroxidation; N, P, K, Ca, Mg, Fe, and Zn contents of leaves; and N, P, K, Ca, Mg, and Zn contents of roots. Additionally, a positive correlation was observed between L-proline and membrane permeability, stomatal conductance, leaf area, and root dry weight, whereas a negative correlation was observed between them and leaf relative water content, chlorophyll, leaf temperature, shoot fresh weight, shoot dry weight, root fresh weight, relative shoot length, and relative shoot diameter. In addition, there was a negative correlation between membrane permeability and stomatal conductance, leaf temperature, root fresh weight, L-proline, leaf K content, and root Fe content (Figure 3d).

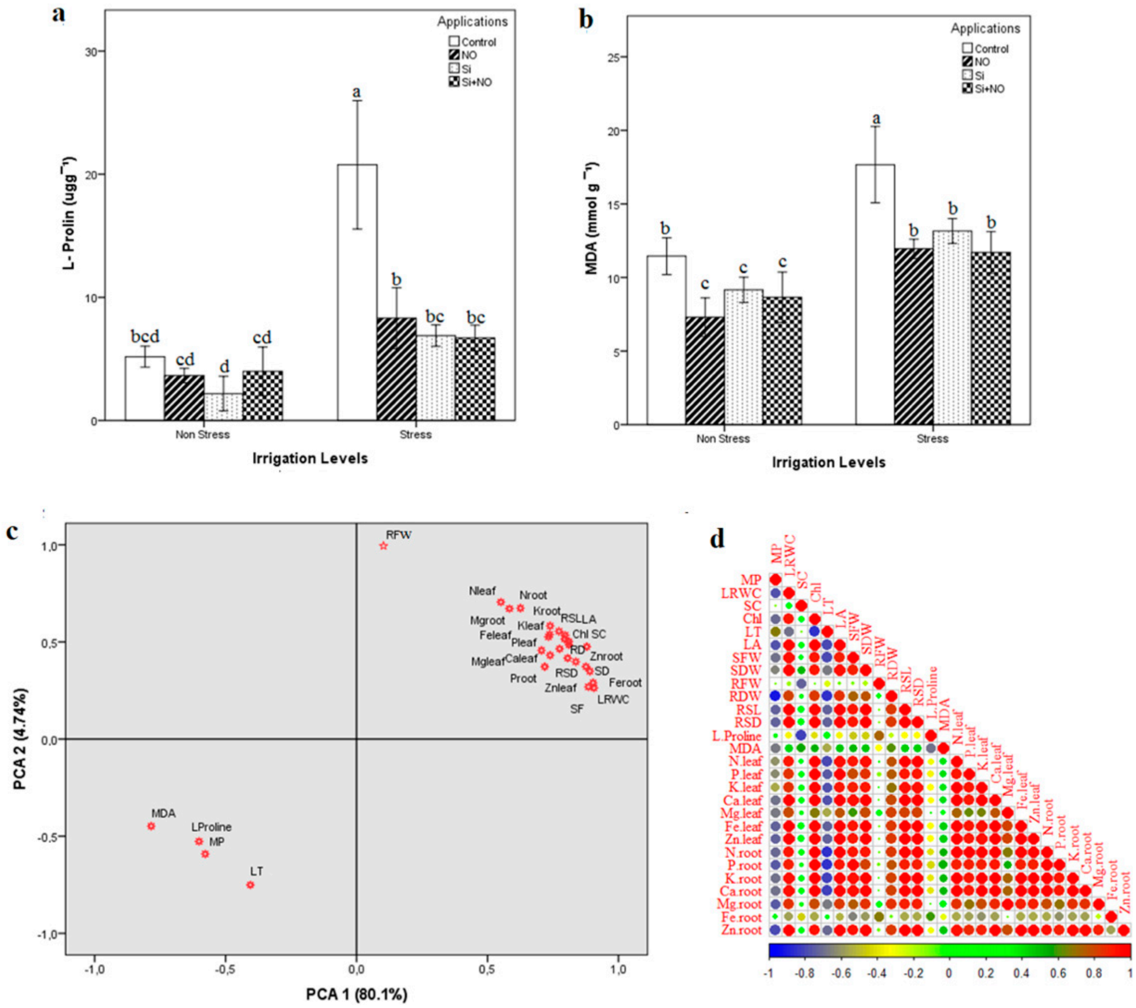

Figure 3. Effects of water stress and chemicals applications on L-proline and lipid peroxidation (MDA) determined at harvest in Myrobolan 29C rootstock leaves. Different lowercase letters indicate significant differences (Tukey test; $p \leq 0.01$) between irrigation levels and chemicals applications; L-proline (**a**), lipid peroxidation (**b**) (MDA) expressed as malondialdehyde content. The loading plot of all the measured variables included in PCA for morphological, physiological, biochemical, and mineral variables (**c**). Pearson's correlation between all traits (p-value ≤ 0.05) where, correlation coefficients are indicated by color (green to red shows positive correlation from 0 to 1, and yellow to blue shows negative correlation from 0 to −1) (**d**). SFW: shoot fresh weight, SDW: shoot dry weight, RFW: root fresh weight, RDW: root dry weight, LA: leaf area, RSL: relative shoot length, RSD: relative shoot diameter, MP: membrane permeability, Chl: chlorophyll content, L-RWC: leaf relative water content, LT: leaf temperature, MDA: lipid peroxidation. Error bars on figures represent standard error (SE).

4. Discussion

To date, significant contributions have been published to understand the response of plant crops to water stress by applying silicon (Si) and nitric oxide (NO) [8–10,12,18]. Nevertheless, there is scarce available information regarding the effects of simultaneous application of Si and NO, whose fundamental function is to establish the root structure for the scion, on rootstocks. The results showed above reported that the combination of Si and NO allowed the improvement of several biochemical and growth parameters of Myrobalan 29C rootstock growing under stress conditions compared their individual application. Based on this, we suggest an additive effect of Si and NO when applied on Myrobalan 29 C rootstocks. Badem and Söylemez [20] reported that Si and NO application at 14-day intervals could increase marketable yield of pepper under saline environments. Synergistic effects of Si and NO were also reported by Ahmad et al. [40], who showed that their application promoted plant growth, oxidative stress tolerance, and reduction in arsenic uptake in *Brassica juncea*. Similar results have been published by different authors for other horticultural crops [19,41]. In this report, chemical applications to Myrobalan 29C rootstock enhanced most of the plant's growth parameters, physio-biochemical parameters, and minerals under both irrigation regimes. Similar to this, some authors have indicated improvements in plant growth parameters after the simultaneous applications of Si and NO to horticultural crops [20,22]. Based on this, it is possible to suggest that Si and NO hold a synergistic effect that could modulate secondary metabolism of plants through upregulation of gene expression and enzymes related to both non-stress and water stress conditions.

Deficit irrigation affects leaf turgor and water use efficiency, altering the plant's photosynthesis process [34,42]. In this study, water stress induced an increase in membrane permeability, L-proline content, leaf temperature, and lipid peroxidation as well as a decrease in chlorophyll, stomatal conductance, and leaf relative water, which was also reported by some authors [34,43,44]. Proline protects plants by stabilizing enzymes that are activated by biotic and abiotic stresses [45,46]. Under both water conditions, Si, NO, and Si+NO applications had similar effects on membrane permeability, L-proline content, and lipid peroxidation, improving their levels in leaves compared to control. Some studies have reported that NO applications decreased arsenic As^{3+} toxicity in different crops by decreasing reactive oxygen species (ROS) and reversing As-induced antioxidant enzymes [47,48]. Si has also been reported to decrease the levels of lipid peroxidation in maize and barley, decreasing the permeability and maintaining the integrity of membranes [13,49]. In this study, chemical applications induced significant improvements on chlorophyll content and temperature in the leaves. Agarie [50] reported that Si increased leaf area, improving photosynthesis and preventing chlorophyll degradation. Si plays a key role in water stress tolerance by causing a rise in carotenoid and chlorophyll content in leaves, leading to a continuous supply of carbon assimilation [43]. NO has an important role in the regulation of osmotic pressure by protecting chlorophyll pigments and chloroplast membrane [26].

Water scarcity affects the transfer of essential nutrients from the soil to the plant, altering plant metabolic activities [51]. In the present study, Myrobalan 29C rootstock growing under water stress conditions absorbed lesser amount of minerals than the well-watered plants, similar to the results reported by Dehghanipoodeh et al. [2]. Generally, chemical applications to the plants under both water conditions improved mineral uptake in Myrobalan 29C rootstocks. Sonobe et al. [52] suggested that the increase in leaf and root mineral contents in plants subjected to water stress could be attributed to an increase on osmolytes that support osmotic regulation. Zhu and Gong [53] reported that Si induced a balance in nutrients of plants, improving mineral uptake. In addition, lamellar systems and cell division in cell organelles are maintained by Ca, whereas numerous enzymes are stimulated by K to regulate various metabolic processes in plants subjected to water stress [54].

The results mentioned above showed some relationships between growth, physio-biochemical parameters, and other parameters. Bhardwaj and Kapoor [55] reported that lipid peroxidation, proline, membrane permeability, and leaf temperature are related to the adjustment of plant biochemical events under osmotic stress. Leaf chlorophyll content decreases under water stress due to the activity of oxygen radicals [56]. In this context, it is possible that chlorophyll degradation could be associated with the increases in the levels of lipid peroxidation and proline. Zhu et al. [57] reported that proline is a key factor in Si-induced salt tolerance, and it may be implicated in the cytokinin metabolism. On the other hand, stomatal size decreased under water stress conditions, but the number of stomata was positively related to stomatal permeability, net CO_2 assimilation, and water use efficiency [58]. In addition, it was reported that Si enhanced root water uptake in salt-stressed plants through up-regulating aquaporin gene expression [59]. Therefore, silicon and nitric oxide could be an interesting agronomical strategy in horticulture due to the functionality of a rootstock, since these elements could provide to the roots a better adaptation to water stress, a condition for which more and more rootstocks are selected.

5. Conclusions

Silicon (Si), nitric oxide (NO), and simultaneous application of Si and NO (Si+NO) to Myrobalan 29C rootstocks mitigated the negative effects of water stress. Deficit irrigation negatively affected leaf area, shoot dry weight, root fresh weight, shoot fresh weight, root dry weight, relative shoot diameter, leaf chlorophyll content, leaf relative water content, P, Ca, Fe, and Zn content in leaves and N, K, Ca, and Zn content in roots compared to well-watered plants. Si+NO increased all the growth parameters and demonstrated the best improvement in stomatal conductance, leaf chlorophyll content, and leaf temperature compared the rest of the treatments under water stress conditions. In addition, Si+NO applications to Myrobalan 29C rootstocks increased the leaf contents of Ca, Mg, Fe, and Zn compared the other applications. Under water stress conditions, chemical applications (Si, NO and Si+NO) to Myrobolan 29C roots induced higher P, K, Ca, and Zn than control, whereas Si and Si+NO treatments resulted in higher N root content than control. In addition, these solutions improved proline and lipid peroxidation in the rootstocks growing under both irrigation regimes. Therefore, Si+NO applications to rootstocks could be an interesting strategy to improve plant material adaptation to drought and to the negative effects of climate change in agriculture. Lastly, further research should be developed to understand the possible synergistic effect of silicon and nitric oxide on plant biostimulation elucidating upregulation of gene expression, enzymes activity, and crosstalk amongst phytohormones that could be altered.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12081273/s1, Table S1. K content in leaves and Fe content in roots of Myrobolan 29C rootstocks subjected to different irrigation regimes and chemicals applications.

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