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Silicon Nanoparticles Mitigate Hypoxia-Induced Oxidative Damage by Improving Antioxidants Activities and Concentration of Osmolytes in Southern Highbush Blueberry Plants

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Abstract: Climate change exacerbates flooding problems due to hurricanes followed by heavy rains, particularly in sub-tropical regions. Consequently, submerged plants experience hypoxia stress which limits agronomic and horticultural crop growth and production. Hypoxia causes oxidative damage by accelerating the lipid peroxidation associated with O_2- and H_2O_2 levels. Additionally, hypoxia increases the accumulation of organic osmoprotectants and antioxidant activity, whereas it decreases the macronutrient (N, P, K, and Zn) uptake. This study aimed at investigating the effects of flooding-induced hypoxia stress on the growth and the physiological, biochemical, and nutritional characteristics of the hydroponically grown southern highbush blueberry (cv. Jewel) plants. In addition, the hypoxia-mitigating effects of conventional silicon (Si-C) and silicon nanoparticles (SiNPs) and their application methods (foliar vs. foliar and rootzone application) were also appraised. Both the Si-C and the SiNPs efficiently alleviated hypoxia-induced oxidative and osmotic damage to cells by enhancing the activities of the enzymatic antioxidants (ascorbate peroxidase, catalase, dehydroascorbate reductase, superoxide dismutase, peroxidase, guaiacol peroxidase, monodehydroascorbate, reductase); the non-enzymatic antioxidants (ascorbic acid and glutathione contents); and the accumulation of compatible solutes (proline and glycinebetaine) in leaves and roots. However, the SiNPs were more effective than Si-C at improving antioxidant activities and osmolytes formation. A strong negative correlation between the antioxidant activities and the lipid peroxidation rate was observed in the SiNP-treated plants under hypoxia stress. The SiNPs also regulated nutrient uptake by increasing the K, N, P, and Zn concentrations while decreasing Fe and Mn concentrations to a less toxic level. Blueberry plants treated with SiNPs responded more effectively to hypoxia stress by maintaining higher antioxidant and osmoprotectant concentrations than blueberry plants treated with Si-C. Additionally, the foliar and rootzone applications yielded better results than the foliar applications only.

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1. Introduction

Blueberries are referred to as a "super fruit" due to their high antioxidant capacity, particularly their anthocyanin content, which contributes to the prevention of a variety of diseases, including cardiovascular disease, neurological disorders, and type 2 diabetes mellitus [1]. In recent years, the consumption of blueberries (*Vaccinium spp.*) has attained particular interest due to their nutritious and health-beneficial features [1,2]. For these reasons, blueberry cultivation has been expanding rapidly from traditional cultivating areas to new production areas [3]. The annual global production of blueberries is 823,328 tonnes, and the cultivation area is 119,472 hectares [4]. The main blueberry producing countries are in the American continent (United States, Canada, Peru, and Mexico), followed by the European countries (Spain, Germany, Poland, Holland, and Portugal) and, lastly, the Asian and African countries [4].

However, in the new and the conventional areas crop sustainability is reduced as there is a wider range of abiotic stresses, including high temperatures, salinity, drought, flooding, heavy metal toxicity, etc. Overall, a reduction of about 16% in agriculture production is caused by flooding [5], and it is expected to be more severe in the changing climate scenario [6]. Flooding in the form of waterlogging or submergences causes a reduction in oxygen in the root zone, inducing anaerobic fermentation instead of the usual aerobic respiration, which negatively affects the development of plants, particularly during their vegetative cycle. So, flooding causes a decrease in the leaf water potential, the net assimilation rate of CO_2, nutritional imbalances, hormonal alterations, and a higher accumulation of heavy metals and toxic substances, such as alcohols and aldehydes, as well as reactive oxygen species (ROS) [7,8]. Although plants are equipped with an antioxidative defense system, which alleviates oxidative damage under stressed conditions, under more severe and prolonged stressed conditions, the antioxidant system does not remain strong enough to prevent the cell damage caused by lipid peroxidation and methyl-glyoxal accumulation [9].

As with many fruit crops, blueberries are very sensitive to hypoxia stress [10]. The reports depicted that blueberry plants exhibited a significant reduction in stomatal conductance right after four days of flooding, while plant growth ceased after seven days of flooding [10]. The blueberry plants also showed a reduction in the net photosynthetic rate (Pn) by limiting stomatal conductance during the early days of flooding. However, prolonged flooding caused a reduction in the Pn by decreasing the quantum and carboxylation efficiency in the leaves and increasing cellular respiration [11]. Prolonged periods of flooding also caused physiological, biochemical, and morphological modulations in the blueberry plants similar to those observed in other perennial fruit crops [12]. However, it has not been studied yet the physiological and biochemical responses of blueberry plants to tackle the flooding induced oxidative stress. Although some reports indicated blueberry cultivars can face oxidative stress caused by high temperature tolerance, this is associated with oxidative protein-repairing genes and high transcript levels of antioxidative genes [13]. A comparison of the physiological and morpho-anatomical characteristics of two apple cultivars revealed that physiological performance, particularly the Pn and Gs, increased as leaf thickness and chlorophyll content increased during the leaf expansion period. Because chlorophyll helps to capture more light, the possibility of a greater Pn may be associated with the conversion of light energy to chemical energy [14]. It is possible that Si exerts a similar mechanism in order to improve the Gs and Pn ratios in blueberry plants under hypoxia stress.

In view of the sensitivity of various crops to hypoxia stress and its growth-limiting effect, there is a dire need to investigate and develop new strategies to alleviate the ad-

verse effects of flooding in commercially important specialty crops such as blueberries. Nutrient management is one of the approaches used for approving abiotic stress tolerance. Silicon (Si) is a beneficial plant element and has been found effective in alleviating various abiotic stresses. The stress-mitigating action of Si is related to its role in improving epidermis thickening; regulating the chemical stability of DNA, RNA, and chlorophylls; the functional activation of organelles; the optimization of transport and the distribution of metabolites; reducing the uptake of the toxic element by the roots; and activating the antioxidants [15–17]. However, the Si-induced improvement in abiotic stress tolerance in crops is mainly associated with the accelerated activities of the antioxidants. In wheat, the application of Si under flooding increased the leaf water potential and superoxide dismutase (SOD) activity while decreasing the activities of acid phospholipase and lipoxygenase, resulting in improved production and harvest quality [18]. Some reports indicated that Si also alleviated oxidative damage without any modulations in the activities of the antioxidant enzymes [19]. Although Si is present in an abundant amount in soil, intensive agricultural practices, runoff and leaching cause a reduction in its concentration, particularly in tropical and subtropical regions. Therefore, it is necessary to apply Si as a fertilizer or a supplement to improve a crop's tolerance to various biotic and abiotic stresses for sustainable production [20].

NPs are extremely small (1–100 nm) particles of various elements with unique physical, chemical, and electrical properties that make them highly reactive. Synthetic nanoparticles have been widely used as nano fertilizers, pesticides, and herbicides in recent years to manage a variety of abiotic and biotic stresses in crops. As a result, NPs contribute significantly to increasing food security and productivity in the face of climate change [21]. NPs have also been found very effective in enhancing seed germination, photosynthetic activities, carbohydrate and nitrogen metabolism, the activities of antioxidants, green pigments, and regulating gene expression [22]. Recent studies have shown that application of Si in the form of NPs can be beneficial against various abiotic stresses in a variety of plant species [23–27].

The study described here was planned in order to investigate the modulations in the physiological, biochemical, and antioxidant systems of flooding-stressed blueberry plants and to appraise whether the application of Si in conventional or NP form (20–30 nm) as a foliar spray and/or supplementation in the nutrient solution can improve flooding tolerance. Additionally, we evaluated the hypothesis that Si-NPs are more effective at mitigating osmotic and oxidative stress in hypoxia-stressed blueberry plants than Si-C.

2. Materials and Methods

2.1. Plant Material and Treatments

The experiments were conducted in a greenhouse at the Environmental Horticulture Department, University of Florida, Gainesville, FL, USA. Six-month-old blueberry seedlings (cv. Jewel) were provided by AgriStart Apopka, FL, USA, and were transferred to a hydroponic system. Each hydroponic unit consisted of a 20 L bucket with two plants in it. Following the shifting to the hydroponic system, plants were supplemented with half-strength Hoagland solution for one week to alleviate any stress or transplanting shock. The composition of the Hoagland solution has already been described [27].

During the experiment, the average day and night temperatures were 26 °C and 21 °C, respectively, the relative humidity was 90–95%, and the photoperiod of 12 h was maintained by employing fluorescent lamps (490 µmol m^{-2} s^{-1}). Afterward, plants were applied with six treatments i.e., (i) control (aerated plants), (ii) hypoxia stress (no aeration), (iii) hypoxia stress + foliar application of conventional Si (Si-C) (500 ppm), (iv) hypoxia stress + foliar Si-C (250 ppm) + rootzone Si-C (250 ppm), (v) hypoxia stress + foliar application of silicon nanoparticles (SiNPs) (500 ppm), (vi) hypoxia stress + foliar application of SiNPs (250 ppm) + rootzone application of SiNPs (250 ppm). In our preliminary study (data not shown), a concentration of 250 ppm of conventional Si effectively boosted the physiology and growth of blueberry plants, so an equal concentration of Si-NPs was used to maintain

uniformity. The experiment was conducted as a randomized complete block design (RCBD) with five blocks. There were two plants per treatment in each block (one hydroponic unit with two plants per treatment). Hypoxia stress was created by skipping oxygen supply to the nutrient solution. In the control treatment, the aeration was provided by using the air pumps (Deluxe LGPUMPAIR38). Pumps were kept working throughout the entire duration of the experiment.

The conventional Si (SiO_2) and its nanoparticles of the size 20–30 nm were received from Sigma-Aldrich and US Research Nanomaterials Inc., Houston, TX, USA, respectively. A preliminary study using different sized silicon nanoparticles was conducted. All the nanoparticles were applied at 250 ppm twice a week (3-day interval) and data was collected 10 days after treatment application. The foliar application of the size 20–30 nanoparticles was found to be more efficacious in improving leaf greenness, photosynthetic activity, and plant dry biomass (Figure 1).

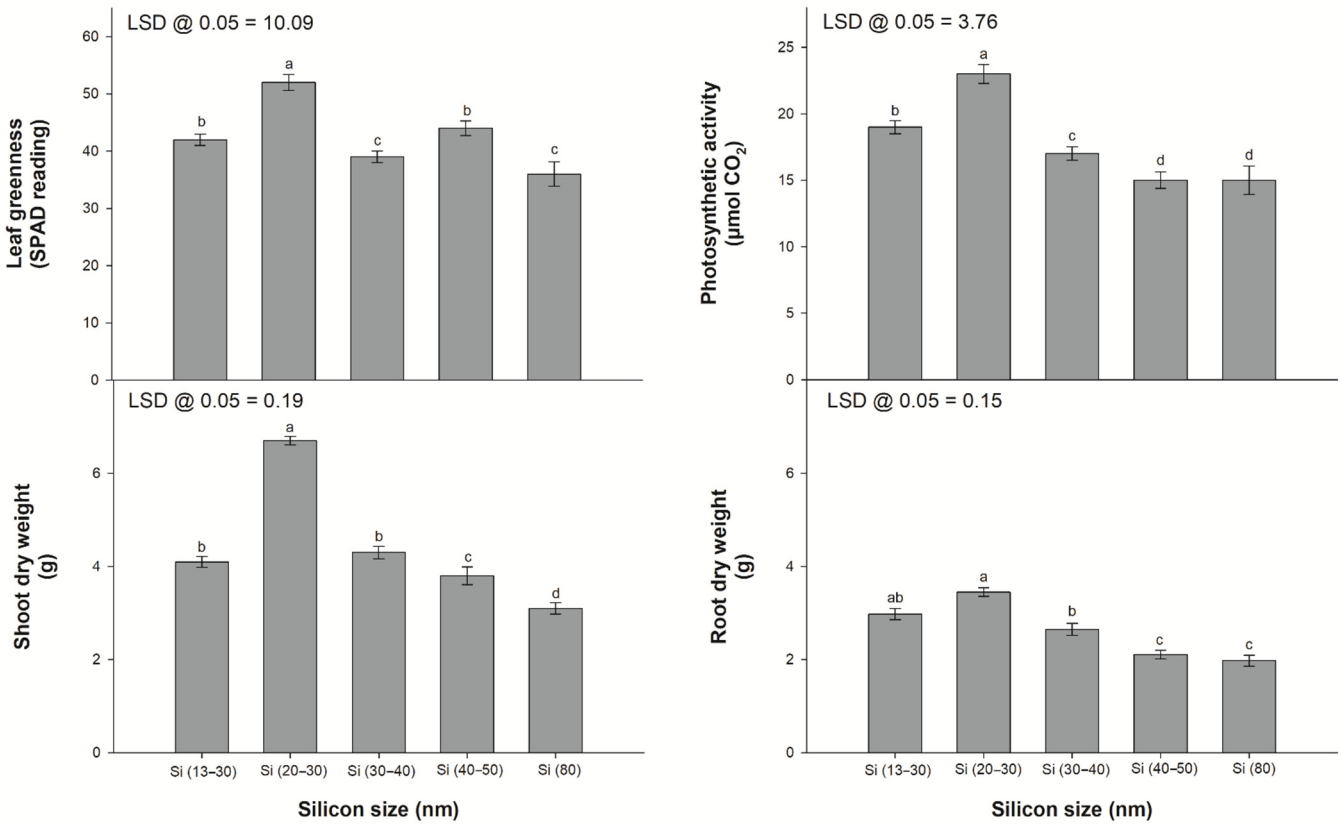

Figure 1. Effect of silicon nanoparticles of different sizes on leaf greenness, photosynthetic activity, and root/shoot plant biomass. Nanoparticles were foliar applied at the rate of 250 ppm two times in a week, and data were collected 10 days after application. Values are mean of four independent replicates ($n = 4$) ± SE (shown in vertical bars), and values denoted by different letters (in small alphabets) differ significantly at $p < 0.05$, using the LSD test.

The nanoparticles had >98% purity, brown-yellow dispersion color, polycrystalline structure, were spherical in morphology, and had a 180–600 m^2 g^{-1} specific surface area (SSA). Based on this preliminary work, similar size (20–30 nm) silicon nanoparticles were used in our recently published study [27]. The foliar applications were made by using handheld sprayers. Each plant was treated with 30 mL of solution, while control plants were treated with distilled water. The foliar application was made carefully by covering the bucket lids with aluminum foil to prevent the entry of foliar solution into the buckets. There were three foliar application events, the first on the day of starting the hypoxia stress, and the second and third at 7-day intervals following the first application. The nutrient solution in all of the hydroponic units was replaced after the 7-day interval. The

Si application via irrigation (rootzone application) was applied on the day of the hypoxia initiation, and the remaining two were applied on the day of nutrient solution replacement. The pH of the solution was maintained between 4.5-to-5.0 in hydroponic units and tested daily using a portable pH meter (HI9124, Hanna Instruments, Smithfield, RI, USA). Any pH fluctuation was adjusted with NaOH or HCl. The oxygen concentration in both aerated and non-aerated hydroponic units was monitored on a daily basis by using an oxygen meter (HI98193, Hanna Instruments, Smithfield, RI, USA). In aerated and hypoxia-stressed (non-aerated) hydroponic units, the average dissolved oxygen concentration was 7.96 mg/L and 1.47 mg/L, respectively. One week after the third application of foliar Si application, data on leaf greenness and photosynthetic activity was determined. After determining leaf greenness and photosynthesis, destructive sampling was done to determine enzymatic, osmolyte, and nutrient attributes.

2.2. Leaf Gas Exchange Parameters and Chlorophylls

Net photosynthetic rate (Pn) and stomatal conductance (gs) of fully developed healthy leaves were measured using LiCor (LI 6400 XT, Licor Corporation, Lincoln, NE, USA) as described earlier [27]. The following were the conditions determining gas exchange characteristics: molar airflow per unit leaf area 395 mmol m^{-2} s^{-1}, leaf temperature 24.2 °C, air CO_2 concentration 386 µmol mol^{-1}, while other conditions of atmospheric pressure and photosynthetic photon flux density (PPFD) were the same as mentioned previously [28]. Leaf greenness was measured using a SPAD meter (SPAD-501, Minolta, Inc., Kyoto, Japan) on the adaxial side of leaves [27]. SPAD measurements of leaf greenness were taken on three sides (left, right, and centre) of the plant canopy using 15 leaves per plant and four plants per treatment, totaling 60 leaves per treatment.

2.3. Antioxidant Capacity Determination

Leaf and root samples of each plant were subjected to determine the antioxidant enzyme activities. Approximately 0.5 g of tissue was used to assay the activities of ascorbate pe-roxidase (APX), catalase (CAT), dehydroascorbate reductase (DHAR), guaiacol peroxidase (GPX), glutathione reductase (GRT), superoxide dismutase (SOD), peroxidase (POD), and monodehydroascorbate reductase (MDAR) and detailed procedures have already been described [27,28]. The SOD activity was determined as described by Giannopolitis and Ries (1977) [29], and the CAT and POD activities were assayed using the method demon-strated by Maehly (1954) [30] with some modifications [28]. APX activity was assayed ac-cording to Nakano and Asada (1981) [31], GPX activity was determined as described by Urbanek et al. (1991) [32], GRT activity was assayed according to the procedure of Es-terbauer and Grill (1978) [33], MDAR activity was measured by determining the decrease in absorbance at 340 nm caused by NADH oxidation [31], and DHAR was assayed by Hossain and Asada, (1984) method [34].

AsA and GSH concentrations were measured after following the procedure described by Arakawa et al. (1981) [35] and Griffiths (1980) [36], respectively, and a detailed procedure has been discussed earlier [27].

2.4. Glycinebetaine, Lipid Peroxidation, Proline, and ROS Concentrations

The detailed procedure to determine the concentrations of osmolytes, lipid peroxidation, and ROS has been discussed earlier [27,28]. The superoxide (O_{2-}) rate was determined by following the method of Elstner and Heupel (1976) [37], Hydrogen peroxide (H_2O_2) was assayed according to the protocol of Patterson et al. (1984) [38], lipid peroxidation was determined as described by Heath and Packer (1968) [39]. The free proline contents were determined using the Bates et al. (1973) [40] method and the glycinebetaine (GB) contents using the Grieve and Grattan (1983) method [41].

2.5. Mineral Nutrient Concentration in Plant Tissue

To assess the concentrations of various nutrients such as iron (Fe), manganese (Mn), nitrogen (N), phosphorus (P), and potassium (K), leaf samples were initially oven-dried at 65 °C then 0.5 g of dried tissues were ground in a pestle and mortar. The concentration of N was determined using an analyzer (PE2400 CHN, Perkin-Elmer, Waltham, MA, USA). To determine Fe, K, Mn and P concentrations, tissues were ashed in a Muffle furnace (Thermo Fisher Scientific, Waltham, MA, USA) at 500 °C/4 h, then dissolved in 1 M HCl, and analyzed for P [42]. The concentration of K was determined by atomic emission spectrometry and the concentrations of Fe, Mn and Zn were determined by atomic absorption spectrometry.

2.6. Statistical Analysis

A one-way ANOVA was performed with STATISTICA 9.0 (Stat-Soft, Inc., Palo Alto, CA, USA). When the variables were found to be significant ($p < 0.05$), the treatments were separated with Fisher's least-significance-difference (LSD). The experiment was conducted as a RCBD with five blocks. There were two plants per treatment in each block (one hydroponic unit with two plants per treatment).

3. Results

3.1. Leaf Gas Exchange and Leaf Greenness Parameters

The plants grown under hypoxia stress with no supplemented Si had a significant reduction in leaf gas exchange traits, i.e., the net photosynthetic rate (Pn), the stomatal conductance (gs), and the leaf greenness relative to the control plants (aerated; Figure 2). The hypoxia caused a reduction by 76%, 44%, and 52% in the Pn, gs, and leaf greenness, respectively, relative to the aerated plants. All the Si (Si-C and/or SiNPs) treatments improved the Pn, gs, and leaf greenness and there were significant differences between the Si treatments. The Pn, gs, and leaf greenness were increased in the following order: Si-C (F) < Si-C (F & R) < SiNPs (F) < SiNPs (F & R). The plants treated with Si-C (F) and Si-C (F & R) had a lower Pn, gs, and leaf greenness than the control plants. However, the plants treated with the SiNPs (F) and the SiNPs (F & R) maintained Pn, gs, and leaf greenness values similar to those of the control plants. In the case of Si-C, the plants treated by the F & R application method had greater leaf greenness and Pn values compared to those treated by the F method. However, in the case of the SiNP, there were no considerable differences in the Pn, gs, and leaf greenness values between the F and the F & R application methods.

3.2. Enzymatic Activities

The hypoxia conditions without the Si applications increased the activities of the SOD, POD, CAT, APX, MDAR, DHAR, ASC, GPX, GTR, and GSH in the plant tissue, except for the MDAR and ASC content in the leaves and the POD in the roots (Figures 3–5). Regardless of the form (Si-C or SiNP), the supplemented silicon significantly improved all these enzymes and substrate activities. The increase followed in the order of Si-C (F) < Si-C (F & R) < SiNPs (F) < SiNPs (F & R) both in the leaves and the roots. The enzymatic activities of the GTR and DHAR within the ascorbate-glutathione cycle were also highly increased. For example, in the SiNPs (F & R) plants the GTR and DHAR activities were increased by 3.2- and 5.8-fold, respectively, in the leaves, and 6.8- and 10-fold in the roots (Figures 3 and 4), relative to the control plants. There was also a significant increase in the ASC and GSH substrates both in the leaves and the roots with the Si application. These increases were greater in the roots in the case of ASC and in the leaves for GSH. Regardless of the form of Si, it was also observed that the plants receiving silicon by F & R maintained higher enzymatic activities and substrate content in their leaves and roots than the Si application by F. Overall, the SiNPs applied as F & R were found to be more effective in maintaining the greater enzymatic activities and substrate contents.

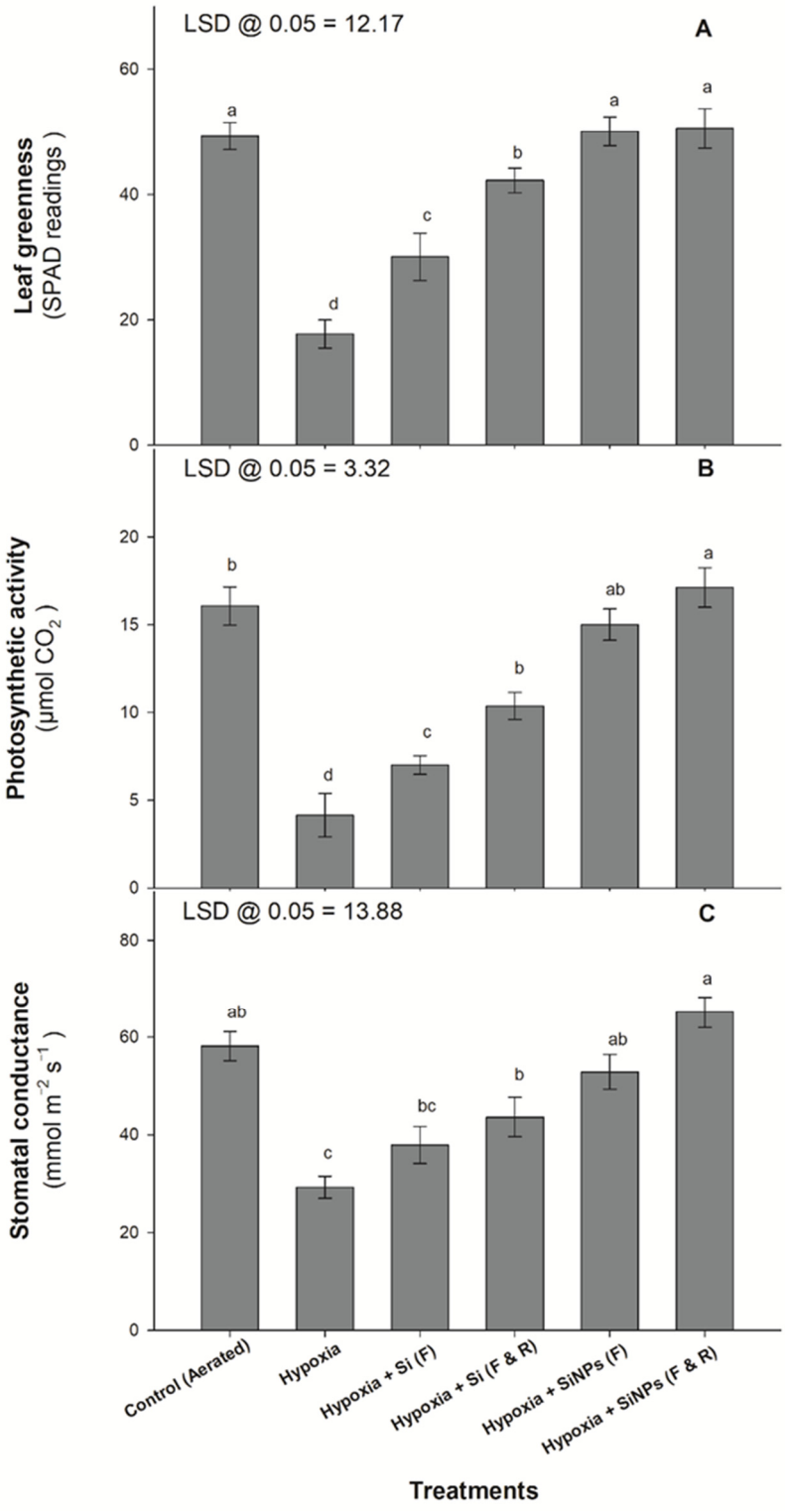

Figure 2. Leaf greenness (**A**), photosynthetic activity (**B**), and stomatal conductance (**C**) in leaves of blueberry plants in response to six treatments i.e., (i) control (aerated), (ii) hypoxia, (iii) hypoxia + Si (F), (iv) hypoxia + Si (F & R), (v) hypoxia + SiNP (F), (vi) hypoxia + SiNPs (F & R). Values are mean of four independent replicates (n = 4) ± standard error (SE; shown in vertical bars), and values denoted by different letters (in small alphabets) differ significantly at $p < 0.05$, using the Least Significant Difference (LSD) test. The abbreviations used are F: foliar application of Si or SiNP; F & R: combined foliar and root zone application of Si; R: root zone application of Si or SiNP; Si: conventional silicon; SiNPs: silicon nanoparticles.

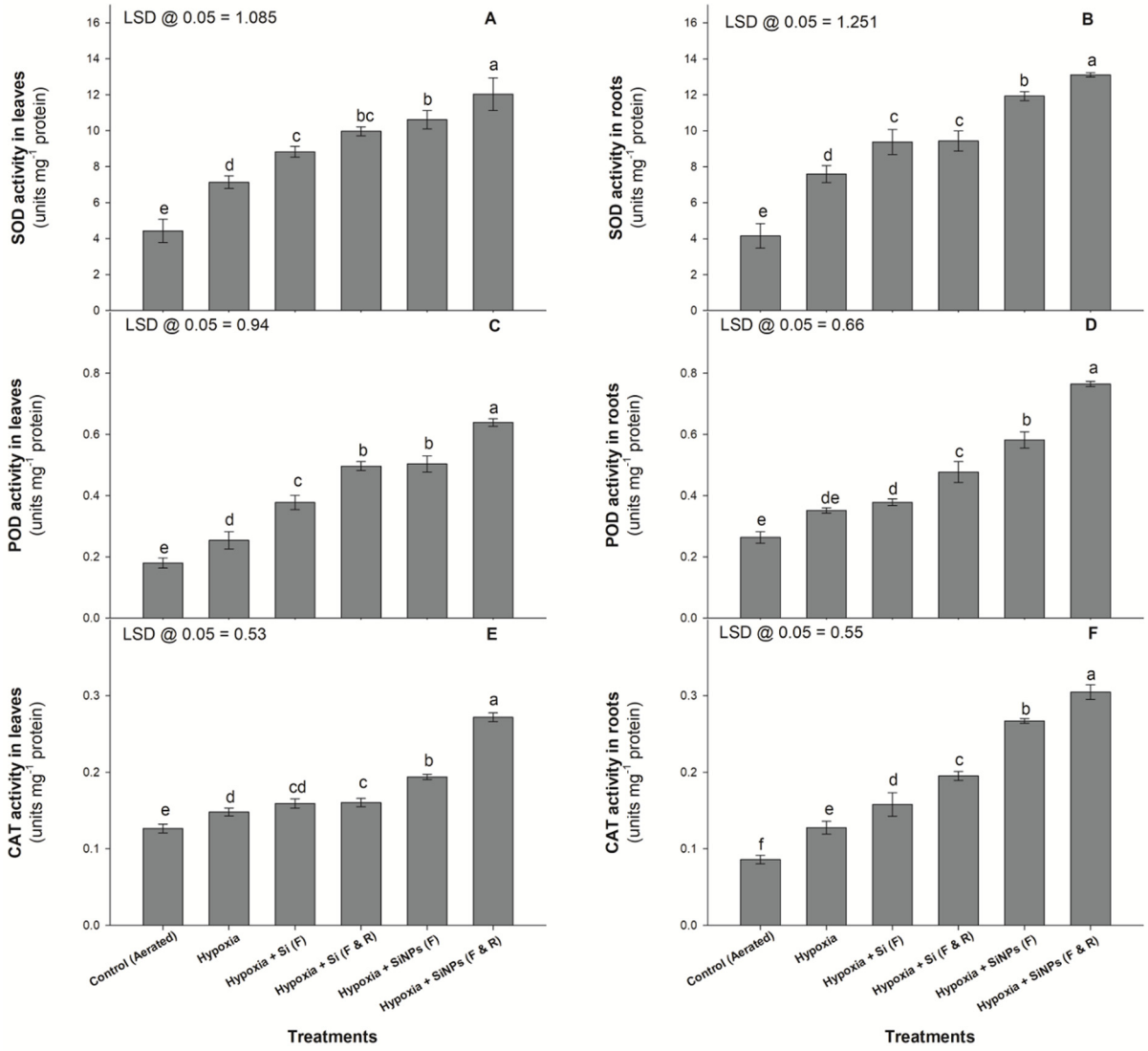

Figure 3. SOD (**A**,**B**), POD (**C**,**D**), and CAT (**E**,**F**) activities in leaves and roots of blueberry plants in response to six treatments i.e., (i) control (aerated), (ii) hypoxia, (iii) hypoxia + Si (F), (iv) hypoxia + Si (F & R), (v) hypoxia + SiNP (F), (vi) hypoxia + SiNPs (F & R). Values are mean of four independent replicates ($n = 4$) ± SE (shown in vertical bars), and values denoted by different letters (in small alphabets) differ significantly at $p < 0.05$, using the LSD test. The abbreviations used are CAT: catalase; F: foliar application of Si or SiNP; F & R: combined foliar and root zone application of Si; POD: peroxidase; R: root zone application of Si or SiNP; Si: conventional silicon; SiNPs: silicon nanoparticles; SOD: superoxide dismutase.

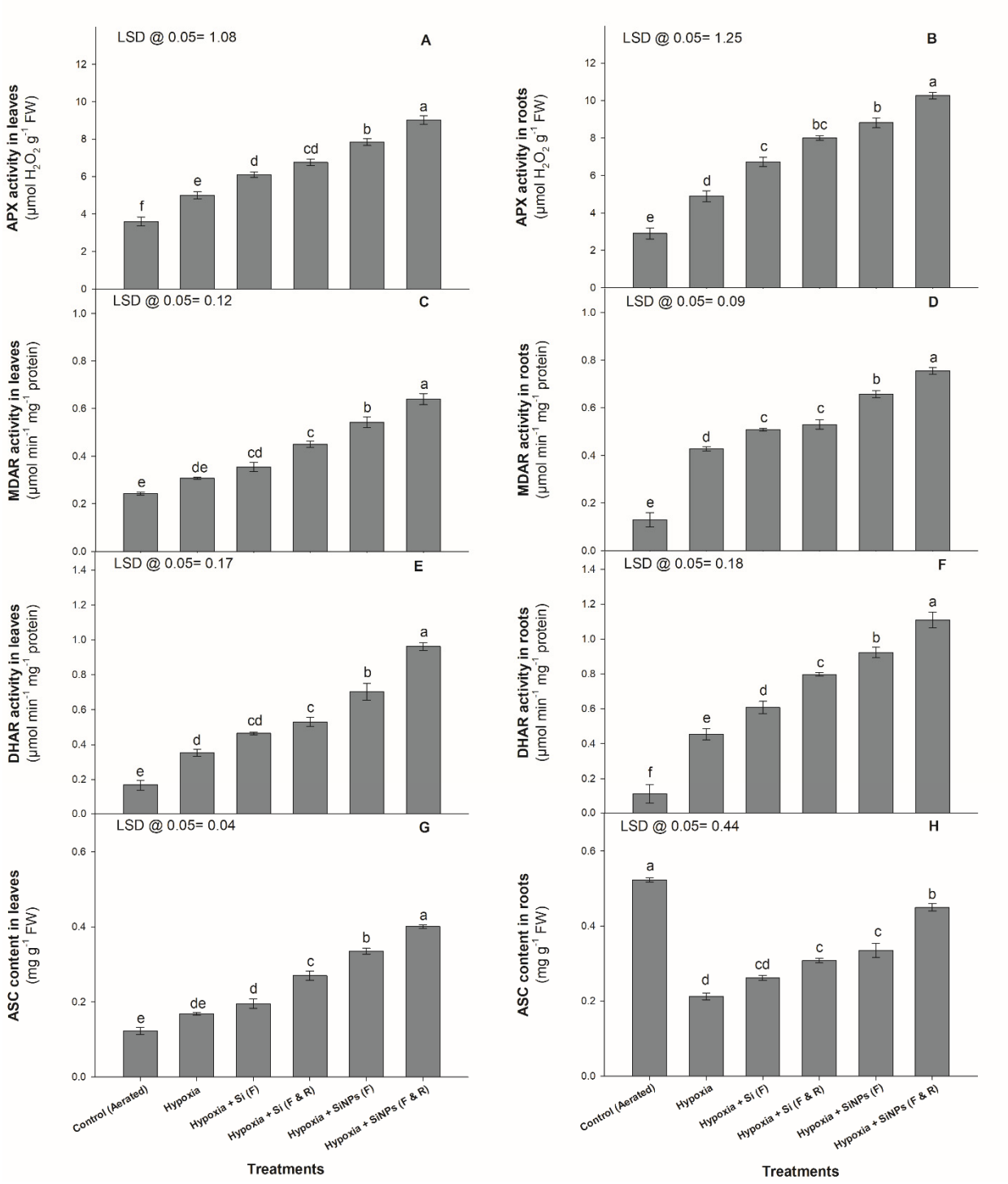

Figure 4. APX activity (**A**,**B**), MDAR activity (**C**,**D**), DHAR activity (**E**,**F**), and ASC content (**G**,**H**) in leaves and roots of blueberry plants in response to six treatments i.e., (i) control (aerated), (ii) hypoxia, (iii) hypoxia + Si (F), (iv) hypoxia + Si (F & R), (v) hypoxia + SiNP (F), (vi) hypoxia + SiNPs (F & R). Values are mean of four independent replicates (n = 4) ± SE (shown in vertical bars), and values denoted by different letters (in small alphabets) differ significantly at $p < 0.05$, using the LSD test. The abbreviations used are ASC: ascorbic acid; APX: ascorbate peroxidase; DHAR: dehydroascorbate reductase; F: foliar application of Si or SiNP; F & R: combined foliar and root zone application of Si; MDAR: monodehydroascorbate reductase; R: root zone application of Si or SiNP; Si: conventional silicon; SiNPs: silicon nanoparticles.

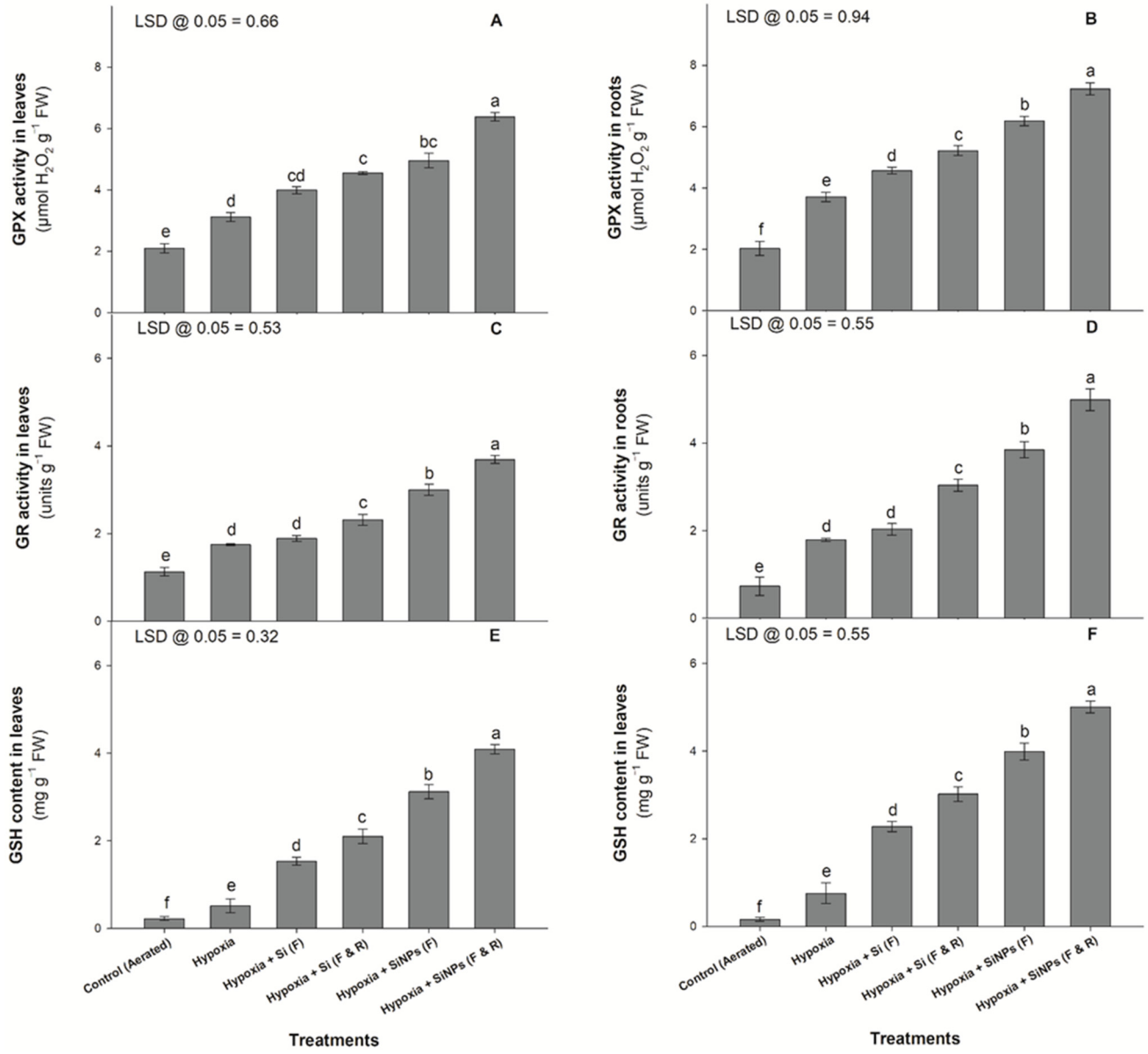

Figure 5. GPX activity (**A**,**B**), GR activity (**C**,**D**), and GSH content (**E**,**F**) in leaves and roots of blueberry plants in response to six treatments i.e., (i) control (aerated), (ii) hypoxia, (iii) hypoxia + Si (F), (iv) hypoxia + Si (F & R), (v) hypoxia + SiNP (F), (vi) hypoxia + SiNPs (F & R). Values are mean of four independent replicates (n = 4) ± SE (shown in vertical bars), and values denoted by different letters (in small alphabets) differ significantly at $p < 0.05$, using the LSD test. The abbreviations used are F: foliar application of Si or SiNP; F & R: combined foliar and root zone application of Si; GPX: guaiacol peroxidase; GR: glutathione reductase; GSH: glutathione; R: root zone application of Si or SiNP; Si: conventional silicon; SiNPs: silicon nanoparticles.

And 6.8 and 10-fold in the roots (Figures 4 and 5), relative to control plants. There was also a significant increase in ASC and GSH substrates both in leaves and roots with the Si application. These increases were greater in roots in the case of ASC and in leaves for GSH. Regardless of the form of Si, it was also observed that plants receiving silicon by F & R maintained higher enzymatic activities and substrate content in their leaves and roots than Si application by F. Overall, SiNPs applied as F & R was found to be more effective in maintaining the greater enzymatic activities and substrate contents.

3.3. Osmolyte Concentration

In this study, concerning the control plants, the plants grown under hypoxia stress without the supplemented Si showed increased proline and GB contents in their leaves and roots. (Figure 6). The Si treatments improved the accumulation of both compatible solutes by several folds greater than the control plants (aerated). The highest values of proline and GB were recorded in the plants treated with the SiNP (F & R) followed by the SiNPs (F), the Si-C (F & R) and the Si-C (F). Regardless of the Si type (Si-C or SiNP), the application method F & R was more effective than F in increasing the proline and GB contents in both the leaves and the roots.

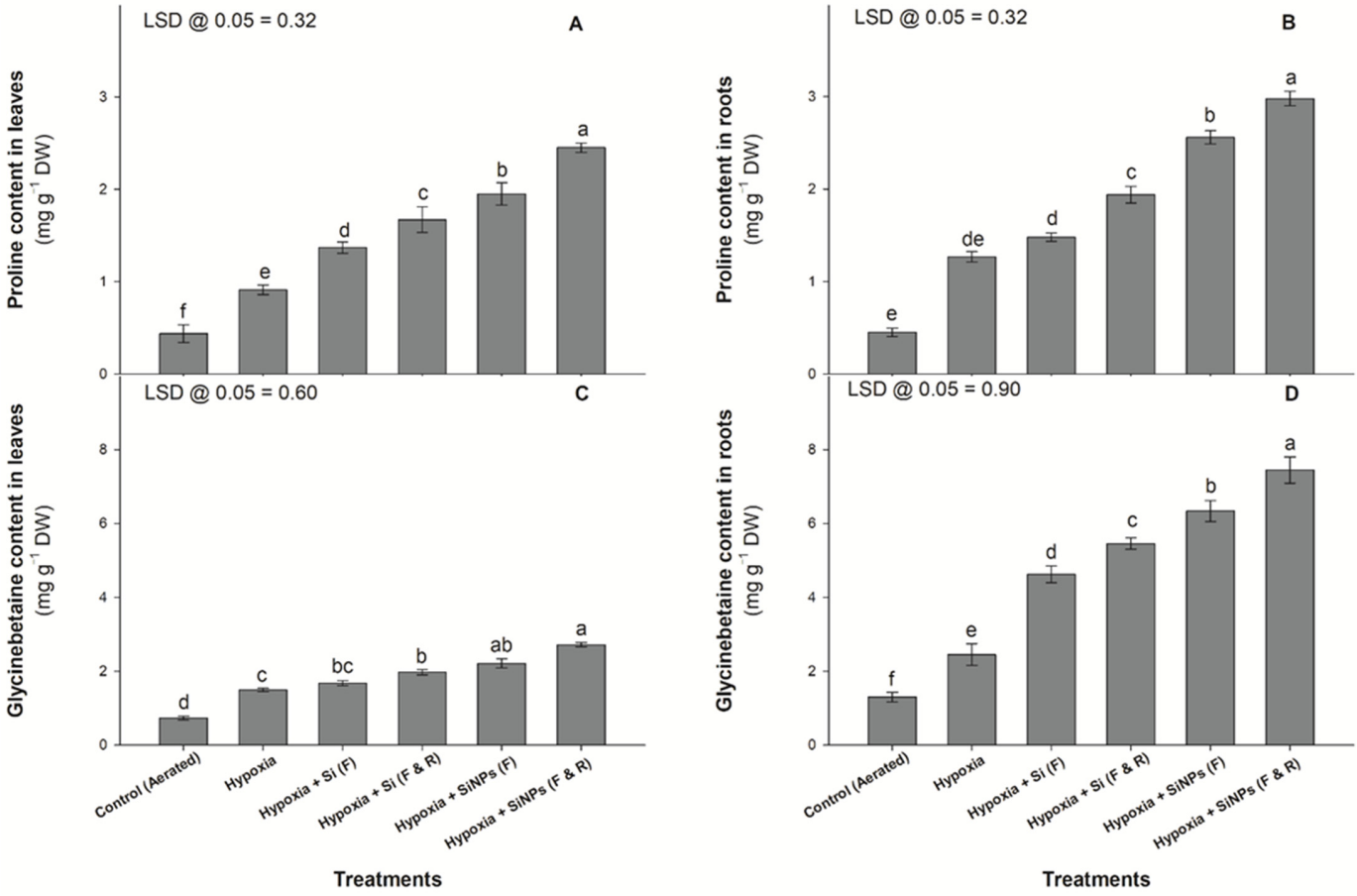

Figure 6. Proline (**A**,**B**), and glycinebetaine (**C**,**D**) content in leaves and roots of blueberry plants in response to six treatments i.e., (i) control (aerated), (ii) hypoxia, (iii) hypoxia + Si (F), (iv) hypoxia + Si (F & R), (v) hypoxia + SiNP (F), (vi) hypoxia + SiNPs (F & R). Values are mean of four independent replicates (n = 4) ± SE (shown in vertical bars), and values denoted by different letters (in small alphabets) differ significantly at $p < 0.05$, using the LSD test. The abbreviations used are F: foliar application of Si or SiNP; F & R: combined foliar and root zone application of Si; R: root zone application of Si or SiNP; Si: conventional silicon; SiNPs: silicon nanoparticles.

3.4. Oxygen, H_2O_2, and MDA Concentration in Plant Tissue

The flooding-induced hypoxia stress significantly increased the levels of O_2, H_2O_2, and lipid peroxidation in both the root and the leaf tissues compared to that of the control plants (aerated) (Figure 7). However, supplemented Si, either Si-C or SiNPs, reduced the level of O_2, H_2O_2, and lipid peroxidation in the root and leaf tissues. The reduction was in the order of Si-C (F) < Si-C (F & R) < SiNPs (F) < SiNPs (F & R). This indicates that applying the SiNPs via the F & R method was more effective at mitigating hypoxia-induced oxidative damage by the lowering of the the formation rate of O_2 and H_2O_2.

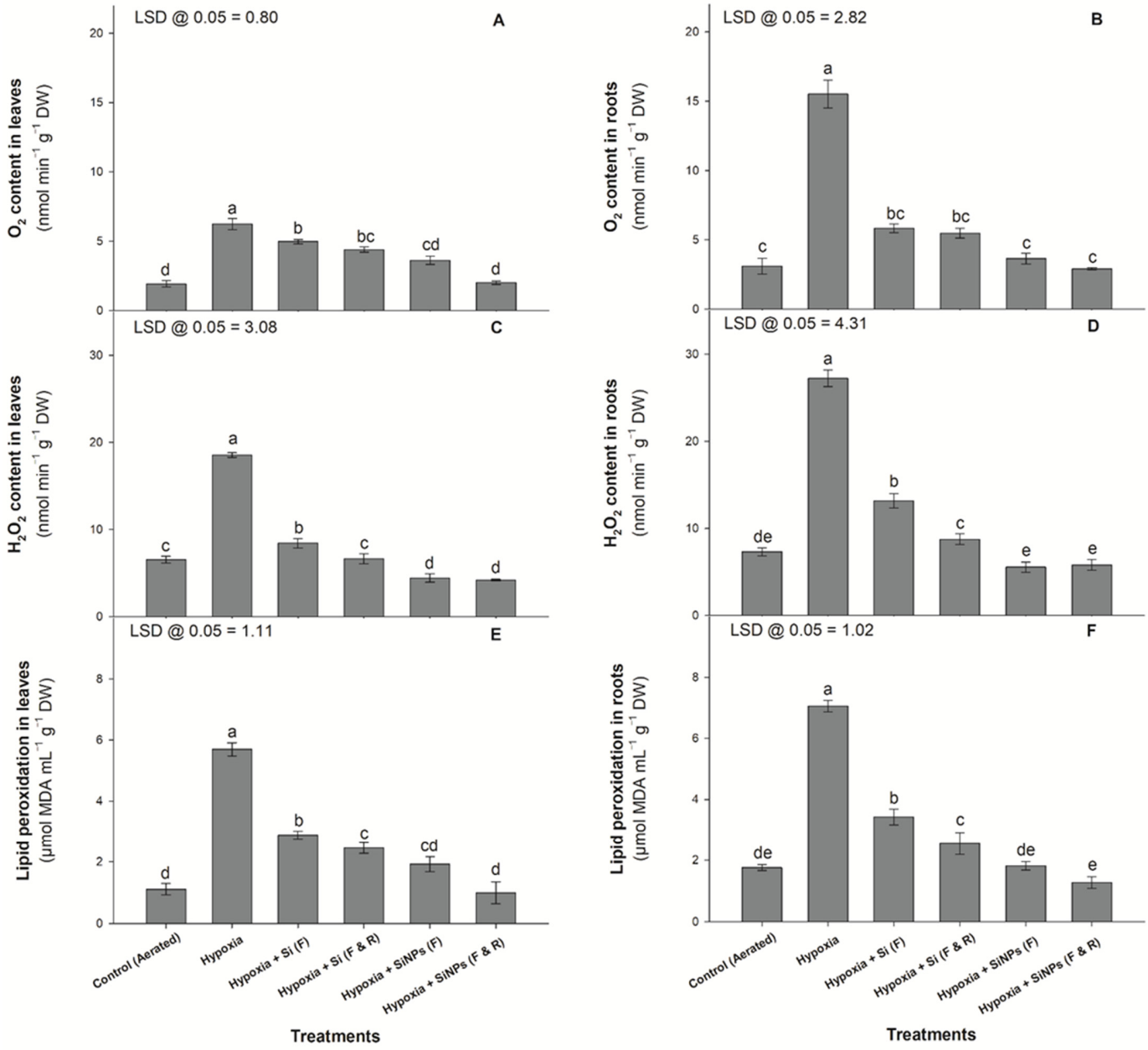

Figure 7. Lipid peroxidation rate (**A**,**B**), O_{2}- content (**C**,**D**), and H_2O_2 content (**E**,**F**) in leaves and roots of blueberry plants in response to six treatments i.e., (i) control (aerated), (ii) hypoxia, (iii) hypoxia + Si (F), (iv) hypoxia + Si (F & R), (v) hypoxia + SiNP (F), (vi) hypoxia + SiNPs (F & R). Values are mean of four independent replicates ($n = 4$) ± SE (shown in vertical bars), and values denoted by different letters (in small alphabets) differ significantly at $p < 0.05$, using the LSD test. The abbreviations used are F: foliar application of Si or SiNP; F & R: combined foliar and root zone application of Si; H_2O_2: hydrogen peroxide; O_2-: free radicals of oxygen oxide; R: root zone application of Si or SiNP; Si: conventional silicon; SiNPs: silicon nanoparticles.

3.5. Mineral Nutrients Concentration in Plant Tissue

The hypoxia stress caused a reduction in the concentration of leaf nitrogen (N), phosphorus (P), and potassium (K) relative to the control plants. However, this effect was reversed by applying Si because the Si-supplemented plants had improved values for N, P, and K compared to those grown without Si under hypoxia stress (Figure 8). The plants under hypoxia stress had either similar or higher leaf macronutrient concentrations relative to those of the control plants. Overall, the plants treated with the SiNP had higher N, P,

and K values than those treated with the Si-C. Likewise, it was also observed that the F & R method of Si application was more effective than the F. So, the plants treated with the SiNP (F & R) had higher leaf N and P concentrations than those treated with the SiNPs (F).

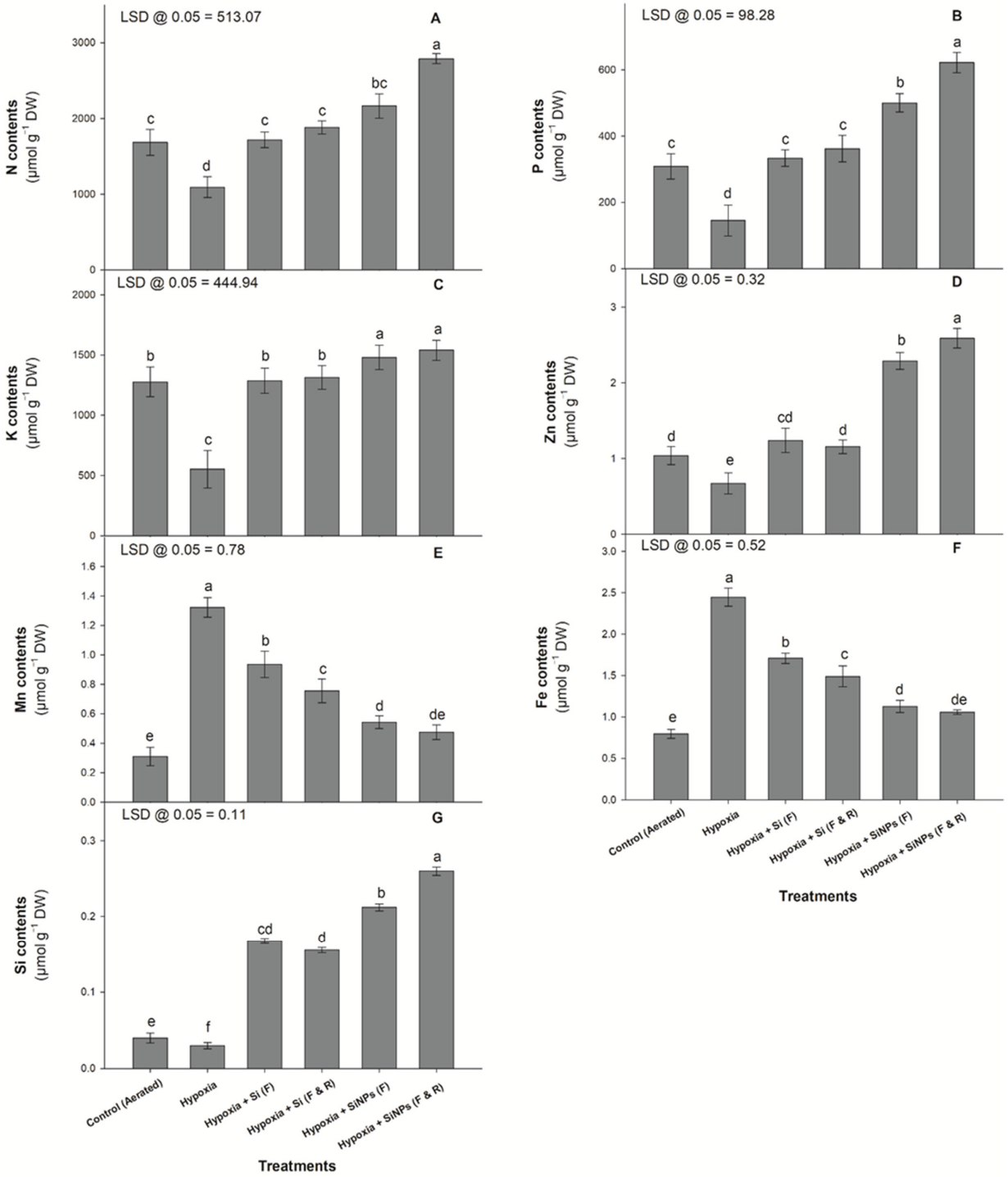

Figure 8. N (**A**), P (**B**), K (**C**), Zn (**D**), Mn (**E**), Fe (**F**), and Si (**G**) contents in leaves of blueberry plants grown in response to six treatments i.e., (i) control (aerated), (ii) hypoxia, (iii) hypoxia + Si (F), (iv) hypoxia + Si (F & R), (v) hypoxia + SiNP (F), (vi) hypoxia + SiNPs (F & R). Values are mean of four independent replicates (n = 4) ± SE (shown in vertical bars), and values denoted by different letters (in small alphabets) differ significantly at $p < 0.05$, using the LSD test. The abbreviations used are F: foliar application of Si or SiNP; F & R: combined foliar and root zone application of Si; Fe: iron; K: potassium; Mn: manganese; N: nitrogen; P: phosphorus; R: root zone application of Si or SiNP; Si: conventional silicon; SiNPs: silicon nanoparticles; Zn: zinc.

In the case of the leaf micronutrients, the plants grown under hypoxia in the absence of Si had highly increased Mn and Fe levels but reduced Zn levels. The Si application reduced the accumulation of Mn and Fe in the following order Si-C (F) > Si-C (F & R) > SiNPs (F) > SiNPs (F & R). There were no significant differences between the plants treated with the SiNPs (F & R) and the control plants (aerated). The leaf Si concentration was also increased in response to all of the Si treatments relative to the control treatment. The highest leaf Si concentration was observed in the plants grown under the SiNPs (F & R) followed by the SiNP (F) treated plants.

4. Discussion

In the United States, Florida commonly experiences a significant impact of flooding. Due to its subtropical climate, the high frequency of hurricanes and the tropical system, and the large extension of its coastline, the sea frequently invades the land surface [43]. As the high frequency of flooding events negatively affects crop growth and productivity, it is very important and essential to identify flood-tolerant genotypes and develop crop management strategies to alleviate the growth and yield-limiting effects of flooding in commercially important horticultural crops. Blueberries are one of the most widespread crops in Florida. However, no report indicates its physiological and biochemical response to hypoxia stress, especially in relation to antioxidant capacity, which is the most important mechanism describing the crop's tolerance potential to different abiotic stresses.

The findings of this study indicate that blueberry plants under flooding conditions without the Si application modulated their antioxidant defense system by increasing the enzymatic activities of SOD, POD, CAT and the Ascorbate-Glutathione Cycle in response to hypoxia stress (Figures 2–4). Higher antioxidant activities aid plants in scavenging excessive amounts of O_2- and H_2O_2, consequently limiting the rate of lipid peroxidation in plant cells [44]. The proline and GB concentration was another beneficial mechanism observed in hypoxia-stressed blueberry plants in this investigation (Figure 6). Under stressed conditions, the high accumulation of proline mitigates stress-induced cellular acidification, promotes the scavenging of ROS, facilitates osmotic adjustment in cells, and acts as a reservoir for nitrogen and carbon once the normal conditions are restored [45]. In our investigation, however, under prolonged stressed conditions the increased enzymatic activities of the antioxidants and the accumulation of osmolytes in the blueberry plants under flooding conditions were not sufficient to scavenge the O_2 and H_2O_2, which resulted in cellular damage due to the high production of MDA in the plant tissues (Figure 7).

A high concentration of MDA could have also been the contributing factor in the reduction in the Pn, gs, and SPAD under flooding (Figure 1). The hypoxia stress also caused a nutritional imbalance in the blueberry plants by abnormally increasing the Fe and Mn concentration and decreasing N, P, and K in leaves, which could have favored physiological disorders coupled with leaf chlorosis and reduced vegetative growth [39]. All this indicates that alterations in the antioxidant activities under flooding-induced hypoxia stress are species-dependent. While blueberry plants respond by increasing the antioxidant activities, other plant species such as Welsh onion, grown under flooding stress for 10 days, show a significant reduction in the activities of SOD, CAT, APX, and GR [46].

In addition to determining the antioxidant activities of the blueberry plants under flooding conditions, the present study was planned in order to ameliorate the deleterious effects of hypoxia stress in these plants by using two different forms of Si (Si-C and SiNPs) applied through two different methods (foliar, F; and foliar + root F & R). The results indicate that the combined application method of the foliar and rootzone supplementation was more effective than the foliar treatment alone. Regarding the efficacy of the two Si forms, the SiNPs were more efficacious than the Si-C. Thus, the SiNPs applied by the foliar and rootzone supplemented in nutrient solution (F & R treatment) successfully mitigated the growth-limiting effects of hypoxia stress by maintaining the high values of the leaf gas exchange traits and the chlorophyll contents as well as O_2, H_2O_2, and MDA contents very similar to those in the control treatment (aerated plants; Figure 6). The present study found

that hypoxia decreased leaf greenness and photosynthetic activity in blueberry plants, thus corroborating with other studies on plants [47–49]. The reduced chlorophyll content is considered a protective mechanism for photosynthetic structures in hypoxic plants, as it decreases sunlight absorption and prevents photo-oxidation [47]. The increased reduction in Chl a and Chl (a + b) indicates greater injury to PSII as a result of photosynthetic inhibition, as well as a more limited ability to absorb light energy and convert it to chemical energy; thus, the Pn was more suppressed in the blueberry plants under hypoxia. The SiNPs were highly effective in improving leaf greenness and photosynthetic activity. In general, Si is very effective in alleviating the oxidative damage in plants, particularly when they are under any kind of abiotic stress, because it regulates the moisture status in the tissues and facilitates the discharge of protons which neutralize hydroxyl ions, thereby decreasing the rate of lipid peroxidation [50]. Furthermore, the findings of this study depict that the Si modulated antioxidant activities by accelerating the enzymatic activities of the SOD, POD, and CAT and the enzymes of the ascorbate-glutathione cycle by improving the formation of antioxidant substrates, such as ascorbic acid and GSH, in the hypoxia-stressed blueberry plants. In addition, Si also improved the osmotic adjustment process by enhancing the accumulation of osmoprotectants, such as proline and GB, in the leaves and roots under hypoxia conditions. Therefore, it is suggested that Si significantly contributes to the regulating and coordinating of antioxidant responses in blueberry plants to reduce ROS generation under stressed conditions.

Furthermore, it was also observed that the most efficient and efficacious source of Si is in the form of the SiNP, mainly when applied by the combination of foliar and supplementation in nutrient solution (F & R). Our data indicate that this could have been due to several reasons: (i) the SiNPs, due to their unique size, are very reactive and get absorbed more quickly in plant tissues than the Si-C particles; therefore, plants treated with SiNPs, especially those applied with SiNP (F & R) had a higher concentration of Si in their leaves than the rest of the treatments. The smaller size and specific surface of the SiNPs (20–30 nm) allow them to penetrate into the cells more quickly and in a greater quantity [51]; (ii) Si applied in the form of nanoparticles significantly improved the photosynthetic activity in the leaves (Figure 1). Similar findings were reported in Cucurbita plants grown under saline conditions [52]; (iii) the SiNPs strengthened the antioxidant defense systems by accelerating the activities of enzymatic and non-enzymatic antioxidants; (iv) they enhanced the osmotic adjustment capacity of blueberry plants by increasing the accumulation of osmolytes such as proline and GB; and (v) the SiNPs maintained and regulated the blueberry plant's nutritional status by improving the N, P, K, and Zn levels while lowering the Fe and Mn concentration to normal levels, consequently improving plant growth and development in the blueberry plants growing under the oxygen deficient conditions. In addition, it has been reported in other publications that SiNPs can inhibit ethylene biosynthesis and regulate energy metabolism and cell death [46]. It is well documented that SiNPs are more effective than Si-C in alleviating different abiotic stresses, as indicated in other publications. Likewise, the Si-C or SiNP application in maize plants showed that both forms of silicon positively ameliorated the drastic impact of arsenate toxicity. However, the SiNPs were more effective and efficacious than the Si-C by their limiting of the accumulation of arsenate in plant tissues and combating the oxidative damage by triggering the glutathione cycle [23]. In another study, UV-B treatment caused adverse effects on wheat seedling by deteriorating the Pn, gs, and E due to increased lipid peroxidation and electrolyte leakage, but the application of Si and SiNP neutralized the UV-B negative effect by activating the antioxidant enzyme system through nitric oxide-mediated pathways [47]. In rice, Si pulsing removed the fluoride-induced inhibition of glutathione synthesis by activating glutathione reductase [52]. In rice plants grown under fluoride stress, the SiNP application regulated the osmotic potential and enhanced the uptake of the important co-factors of antioxidant enzyme systems, such as copper, zinc, and iron [48]. Plants are very plastic in terms of growth and development in changing oxygen conditions. Plants respond to hypoxia stress at the physiological, biochemical, and

molecular levels. Phenotypic changes during recovery are the result of increased oxidative stress caused by concurrent reoxygenation and reillumination. Apart from O_2 sensing via the N-degron proteolytic pathway or metabolic changes mediated by mitochondria, other molecular events regulating gene expression have recently been proposed as critical regulators of hypoxia and reoxygenation. Recent studies have elucidated the molecular mechanisms deployed to alleviate the hypoxia stress, such as RNA regulation, chromatin remodeling, protein synthesis, and post-translational modifications [49–52].

The findings of the present study complement all these reports and confirm the stress-mitigating potential of SiNPs in regulating different physiological processes and biochemical metabolism in plant tissues.

5. Conclusions

The current study significantly adds new information about the regulation of various physiological and biochemical characteristics in blueberry plants under hypoxia stress, and how the use of Si-C and SiNPs helps the plants in mitigating hypoxia stress. The application of SiNP through F & R has great potential to ameliorate the impact of flooding-induced hypoxia stress in blueberry plants. The combined application of SiNP (F & R) triggered the antioxidant enzyme system in blueberries, which detoxified the harmful oxygen species and protected the leaf pigments from the lipid peroxidation caused by the excessive formation of ROS, and restored the leaf gas exchange traits, i.e., the Pn and gs. Moreover, SiNP increased the accumulation of compatible solutes (proline and GB), which resulted in a greater osmotic adjustment potential of blueberry plants under hypoxia stress. However, elucidating the molecular mechanisms underlying these SiNP-mediated regulatory mechanisms requires further investigation and may be the subject of future studies. Another study on the impact of SiNPs on carbohydrate, nitrogen, and polyamine metabolism will further explore the stress mitigating action of SiNPs in hypoxia-stressed blueberry plants.

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