Exogenous Silicon Applications Enhance Peach Seedling Response to Flooding-Induced Hypoxia Stress

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Abstract

Peach trees are highly susceptible to hypoxic conditions during flooding, which causes oxidative damage in plant cells, resulting in death. Silicon (Si) has been reported to improve plants' performance under abiotic stresses, such as flooding. This study aimed to evaluate the performance of peach rootstock ('MP-29') seedlings under hypoxic stress. Seedlings were foliar sprayed with two levels (1000 and 1500 ppm) of silicon dioxide nanoparticles (SiNPs) and silicon dioxide solution (SiSol) to determine their capacity to mitigate oxidative damage. Seedlings treated with SiNPs had significantly higher antioxidant activities (superoxide dismutase, peroxidase, and catalase), and accumulation of compatible solutes (proline and glycine betaine) compared to those treated with SiSol and control seedlings. The rate of lipid peroxidation and generation of reactive oxygen species (ROS) O_2 and H_2O_2 was higher in flooding stressed seedlings as compared to control seedlings; however, Si applications reduced those differences. SiNPs were more effective than SiSol in lowering the rate of lipid peroxidation and formation of ROS. In addition, Si applications to seedlings under non-hypoxic conditions aided to increase N, P, K, and Zn contents in leaves, compared to hypoxic-stressed and control seedlings, particularly when using 1500 ppm. Micronutrient content (Fe and Mn) was high in flooding-stressed seedlings, but SiNPs limited their concentration to levels lower than those in SiSol treated seedlings. In conclusion, Si applications improved the performance of peach seedlings exposed to flooding conditions. Additionally, SiNPs were more effective than SiSol in improving the adaptative stress response of peach seedlings to flooding stress.

1. Introduction

Abiotic stress factors such as radiation (Huang et al., 2010), high temperatures (Hatfield and Prueger, 2015), drought (Hasanuzzaman et al., 2018a), salinity (Kim et al., 2014), heavy metals toxicity (Farooq et al., 2013), nutrient deficiency (Zhang et al., 2019), and flooding (Lal et al., 2015a; Sayed and Gadallah, 2014) represent major challenges for food production. Flooding is a serious problem in subtropical areas such as Florida, where heavy rains, tropical storms, and hurricanes occur. Improper irrigation and/or poor soil drainage could also lead to flooding conditions (Insausti and Gorjón, 2013). For instance, peach orchards established in the flatwoods of the central region of Florida are prone to flooding. The dominant soil type in this region is the Aquods, a poorly drained suborder of Spodosols, characterized by a shallow and fluctuating water table (Mylavarapu et al., 2016; USDA, 2020; USDA Soil Survey Staff, 1999).

Peaches [*Prunus persica* (L.) Batsch] are highly susceptible to flooding (Drew, 1997; Gibbs and Greenway, 2003). During flooding, plants are exposed to hypoxic conditions due to the low gas diffusion rate and low solubility of oxygen in the water (Armstrong, 2002; Insausti and Gorjón, 2013). Under hypoxic conditions, lipids, proteins, and DNA are damaged due to the accumulation of reactive oxygen (ROS) and reactive nitrogen species (RNS) in the plant cells (Blokhina and Fagerstedt, 2010; Farmer and Mueller, 2013). Hypoxia limits plant's performance, eventually resulting in death (Voesenek et al., 2006). An essential role of oxygen in plant metabolism is as a terminal electron acceptor in the oxidative phosphorylation pathway, which generates most of the ATP required for cellular metabolism (Fukao and

Bailey-Serres, 2004). In the absence of oxygen, anaerobic glycolysis and ethanol fermentation are the main metabolic adaptations involved in the generation of ATP (Bui et al., 2019). However, the production of ATP molecules under those conditions is considerably lower compared to ATP production in oxidative phosphorylation (Drew, 1997). Oxygen is also essential for the haem, sterol, and fatty-acid biosynthesis pathways (Geigenberger, 2003).

Hypoxic conditions diminish photosynthetic activity (*Pn*), resulting in stomatal closure and reduction of rubisco enzyme activity, besides chlorophyll degradation (Sayed and Gadallah, 2014). Peaches exposed to short hypoxic periods exhibit poor fruit set, and lesser weight (Alvino et al., 1986; Insausti and Gorjón, 2013). Prolonged exposure to hypoxic conditions is lethal. Although root tolerance to the lack of oxygen is determined mainly by rootstock characteristics (Schaffer et al., 2006), there is no flood-tolerant rootstock available for commercial peach production until date. The development of management practices to overcome flooding-related issues is critical for growers.

Silicon is the second most abundant element in the soil (Yamaji et al., 2008). Although it is not considered essential for plants, it is recognized as a “beneficial substance” by the International Plant Nutrition Institute (IPNI) (Reynolds et al., 2016). Silicon has been observed to restore diminished functions to non-stressed levels (Ma, 2004), ameliorating plant’s performance under several stress types, such as radiation (Takahashi, 1966), drought (Hasanuzzaman et al., 2018a), salinity (Kim et al., 2014), heavy metals toxicity (Farooq et al., 2013), nutrient deficiency (Zhang et al., 2019), and flooding (Lal et al., 2015a; Sayed and Gadallah, 2014). Furthermore, the availability of Si in the form of nano-sized particles could increase its efficiency to decrease the adverse effects of abiotic stresses, as nanoparticles have some novel properties compared to regular size particles. Research focused on developing and improving nano-fertilizers, nano-herbicides, and nano-pesticides is in progress (Rastogi et al., 2019). Besides improving plant growth and yield (Rastogi et al., 2019), nanoparticles increase precision of agrochemical applications, enhance plant nutrient uptake, and increase nutrient bioavailability (Panpatte et al., 2016). However, plants’ response to different nanoparticles varies depending on the species (Siddiqui and Al-Whaibi, 2014).

Considering the high sensitivity of peaches to hypoxia stress, and stress mitigating action of Si, the current study aimed to evaluate the effect of SiNPs in hypoxia-stressed peach rootstock seedlings ‘MP-29’. The hypothesis was that exogenous applications of SiNPs are more efficient than SiSol in alleviating hypoxia-induced stress in peach seedlings.

2. Materials And Methods

2.1. Plant Material and Growth Conditions

The experiment was conducted under greenhouse conditions at the University of Florida, in Gainesville (Florida, USA: Latitude 29.6381, Longitude − 82.3609). The average ambient day and night air temperatures were 25°C and 19°C, respectively. The average relative humidity was 77% approximately.

Twenty-weeks old tissue-cultured ‘MP-29’ peach rootstock seedlings were obtained from a commercial nursery (AgriStarts, Apopka, FL, USA) and transferred to hydroponic systems. Each hydroponic system consisted of a 13.25 L bucket filled with a full-strength Hoagland nutrient solution (Hoagland and Arnon, 1950). Buckets were covered by a lid with a small hole for the aeration hose and two larger holes for the two seedlings placed in each system. Net pots (5-cm diameter) and cloning collar foam were used as a base for the seedlings. Two 1-meter-long bamboo sticks were attached to the sides of the buckets to serve as a trellis system. For non-hypoxic treatments, the solution was continuously aerated using an electric air pump (950 GPH 6 Outlet, Vivosun, City of Industry, CA, USA). To simulate hypoxic stress, the solution was not aerated. Solution pH was measured every two days with a portable pH meter (SI600, Spectrum Technologies Inc., Aurora, IL, USA) and maintained in a pH range of 6.1–6.5 using 2M HCl and 2M NaOH. The nutrient solution was replenished at two weeks interval. Seedlings were maintained in the greenhouse for 12 weeks for adaptation and canopy development before imposition of treatments.

2.2. Preparation of Si suspensions and treatments

An extra pure sodium silicate solution (7.5–8.5% Na_2O, 25.5–28.5% SiO_2; Millipore Sigma, Burlington, MA, USA) was used as a source of SiSol. The sodium silicate solution was diluted in deionized water and mixed using a magnetic stirrer (S131125, Thermo Scientific, Waltham, MA, USA) to prepare the SiO_2 solutions for the treatments. SiO_2 nano-powder (particle size: 20–30 nm diameter; surface area: 180–600 m^2g^{-1}; US Research Nanomaterials Inc., Houston, TX, USA) was used as a source of SiNPs. SiNPs were dispersed in deionized water using an ultrasonic bath (B-22-4 Ultrasonic Cleaner, Branson Cleaning Equipment Company, Shelton, CT, USA) for 10 min to obtain a homogeneous suspension of nanoparticles for the treatments. Solutions and suspensions were applied as foliar spray twice on the peach seedlings, 1) when the hypoxic treatments were initiated (12 weeks after plants were transferred to the hydroponic systems) and 2) seven days after the first application. Pure deionized water was sprayed on control seedlings. Seedlings were harvested for foliar and root samples collection two weeks after the treatment. The following treatments were applied: T1 [Control (only water, no Si)], T2 [Non-hypoxia (aerated) + SiSol (1000 ppm)], T3 [Non-hypoxia (aerated) + SiSol (1500 ppm)], T4 [Hypoxia + SiSol (1000 ppm)], T5 [Hypoxia + SiSol (1500 ppm)], T6 [Non-hypoxia (aerated) + SiNPs (1000 ppm)], T7 [Non-hypoxia (aerated) + SiNPs (1500 ppm)], T8 [Hypoxia + SiNPs (1000 ppm)], and T9 [Hypoxia + SiNPs (1500 ppm)].

2.3. Plant measurements

Thirteen days after treatment imposition, survey photosynthesis measurements were collected with an infrared gas analyzer (LI-6400; LI-COR, Lincoln, NB, USA). The newest fully expanded and mature leaf from the middle portion of each seedling in each hydroponic system was measured. Chamber temperature and relative humidity were adjusted to environmental conditions; CO_2 and airflow were set to 400 ppm and 500 µmol s^{-1}, respectively. Three of the newest fully expanded and mature leaves from each seedling in each hydroponic system were measured to obtain average Soil-Plant Analysis Development (SPAD) index values (SPAD-502; Konica Minolta Sensing Inc., Osaka, Japan) which were collected to assess leaf greenness. Shoots and roots of seedlings were harvested at the end of the

experiment. Shoot fresh weight was recorded using a digital scale; roots were dried with paper towels to remove Hoagland solution residuals and then fresh root weight was recorded. Fresh foliar samples were collected and stored in liquid nitrogen for determination of antioxidant activity, osmolytes concentration, and nutrient analysis. Shoot and root samples were oven-dried at 60°C for 72 h to determine dry weight.

2.4. Antioxidant enzymes activity

Leaves and roots (0.5 g) were ground in an ice-cooled tissue grinder with 5 mL of 50 mM cooled phosphate buffer (pH 7.8) to estimate the antioxidant enzymes activity, l. The homogenate was centrifuged at 15,000 × g for 20 min at 4°C. The supernatant was used to determine the activity of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). The SOD activity was analyzed by photoreduction of nitroblue tetrazolium (NBT) (Giannopolitis and Ries, 1977). The CAT and POD activities were measured according toMaehly and Chance (1954) with some modifications. The CAT reaction solution (3 mL) comprised of 50 mM phosphate buffer (pH 7.0), 5.9 mM H_2O_2, and 0.1 mL of enzyme extract. Changes in the absorbance of the reaction solution at 240 nm were recorded every 20 s. One unit of CAT activity was defined as an absorbance change of 0.01 units min^{-1}. The POD reaction mixture (3 mL) comprised of 50 mM phosphate buffer (pH 5.0), 20 mM guaiacol, 40 mM H_2O_2, and 0.1 mL of enzyme extract. Changes in the absorbance of the reaction solution at 470 nm were recorded every 20 s. One unit of POD activity was defined as an absorbance change of 0.01 units min^{-1}. The activity of each enzyme was expressed based on the protein content. The protocol described by Bradford (1976) for the estimation of protein was used to determine the ratios of protein in the extracts. However, bovine-serum-albumin was used as standard protein in this procedure.

2.5. Proline, Glycine betaine, Lipid peroxidation, and ROS

Both leaf and root tissues were used for the determination of reactive oxygen species, lipid peroxidation, and osmolytes. The superoxide generation (O_2^-) rate was calculated adopting the procedure of Elstner and Heupel (1976). A mixture of leaves or roots supplemented with 0.5 mL of phosphate buffer, 1 mL of xanthine oxidase, and 0.1 mL of hydroxyl-ammonium-chloride was incubated at 25°C for 20 min. After that, 0.5 mL of this mixture was mixed with 0.5 mL of sulfanilic acid and 0.5 mL of α-nepthylamine and shaken well. The mixture was allowed to stay at room temperature for 20 min and then the optical density was recorded at 530 nm in a spectrophotometer. The O_2^- values were calculated through a standard calibration curve plotted between standard vs absorbance at 530 nm. Hydrogen peroxide (H_2O_2) was measured following the protocol of Patterson et al. (1984), to which plant tissues (leaves and roots) were homogenized with acetone (1 g of tissue with 2 mL of acetone). Titanium reagent [$TiOSO_4$ (15% weight) diluted in H_2SO_4) was added to the supernatant and then a 17 M ammonia solution was added to the mixture. The precipitates were separated, washed with acetone, and dissolved in 3 mL of H_2SO_4 (2 N). The absorbance of the solution was read at 410 nm against the blank prepared by the same procedure but without plant tissue. The values were calculated through a standard calibration curve between concentration of H_2O_2 vs absorbance at 410 nm.

Lipid peroxidation was estimated by measuring the concentration of malondialdehyde (MDA), as described by Heath and Packer (1968). Equal volumes of leaf or root extract and 0.5% (w/v) thiobarbituric acid (TBA) solution containing 20% (w/v) trichloroacetic acid (TCA) were mixed. This mixture was kept at 95°C for 30 min and then rapidly cooled in a cooling bath filled with ice. The mixture was then centrifuged at 3000 × g for 10 min. The absorbance of the supernatant at 532 and 600 nm was measured. The MDA concentration was calculated from its molar extinction coefficient (155 mM^{-1} cm^{-1}) and expressed in µmol MDA mL^{-1} g^{-1} DW. MDA (nmol) = Δ (A 532nm-A 600 nm)/1.56×105. The free proline contents of leaves and roots were estimated using the method of Bates et al. (1973) and glycine betaine (GB) adopting the method described by Grieve and Grattan (1983).

2.6. Nutrient determination

Leaf and root samples were dried at 65°C for elemental analysis. The concentrations (mmol g^{-1}) of nitrogen (N), phosphorus (P), potassium (K), iron (Fe), manganese (Mn), and zinc (Zn) were determined for root and leaf tissues. Nitrogen was determined with a Perkin-Elmer PE 2400 CHN analyzer. Samples for the determination of P, K, Fe, Mn, and Zn were ashed at 500°C for 4 h, dissolved in 1 M HCl, and analyzed for P by an ammonium molybdate–ascorbic acid procedure (Murphy and Riley, 1962), for K by atomic emission spectrometry and Zn, Fe, and Mn by atomic absorption spectrometry and values were represented on dry weight basis. Silicon concentration was determined by following the procedure described by Hogendorp et al. (2012). Plant material was burned to ash in a muffle furnace at 550°C for 3.5 h followed by alkaline fusion and colorimetric analysis. The total Si content was determined using the reduced $H_2MoO_7Si_2$ at 820 nm using the spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.7. Statistical analysis

The experiment was designed as a randomized complete block design with five blocks consisting of nine treatments of hydroponic systems distributed in parallel to the greenhouse's cooling pad. Each hydroponic system had two peach seedlings. The response variables were analyzed with the software Statistix 10 © (Analytical Software; Tallahassee, FL). An analysis of variance (ANOVA) was performed to identify differences between individual treatments. The model structure of ANOVA was Yij = µ + Ti + €ij, where Yij is the observed response variable, µ is an overall mean, Ti is the explanatory variable and €ij is the error. The significant differences among treatment means were identified using Fisher's LSD at $P < 0.05$ (Steel et al., 1997).

3. Results

3.1. Photosynthesis net (Pn)

All Si treated seedlings, except for seedlings under non-hypoxic conditions treated with low (1000 ppm) and high dose (1500 ppm) dose of SiNPs, showed at least 10% lower *Pn* values compared to non-stressed, control seedlings (Fig. 1). All SiNPs-treated seedlings showed at least 7% higher *Pn* than SiSol-

treated seedlings, except for those treated with high dose (1500 ppm) of SiSol under hypoxic conditions, which had similar values to seedlings treated with both doses of SiNPs under hypoxic conditions. For seedlings exposed to hypoxic conditions, seedlings treated with low dose (1000 ppm) of SiSol showed the lowest *Pn* values, which were similar to those observed on seedlings under non-hypoxic conditions treated with both doses of SiSol.

3.2. Leaf greenness

Leaf greenness was similar for control seedlings, all seedlings sprayed with SiSol regardless oxygen availability, and seedlings under hypoxic conditions treated with high (1500 ppm) and low dose (1000 ppm) of SiNPs, although these last SiNPs-treated seedlings significantly differed between them. However, seedlings treated with both doses of SiNPs and under non-hypoxic conditions showed significantly higher values (35–37 SPAD Index) compared to control seedlings (32 SPAD Index). Moreover, they exhibited 17% higher leaf greenness values compared to all seedlings treated with SiSol, regardless of oxygen availability, although such values were not significantly different from those shown by seedlings under hypoxic conditions treated with high dose of SiNPs (Fig. 2).

3.3. Antioxidant enzymes activity

The activity of the antioxidant enzymes SOD, POD, and CAT was similar in leaves and roots. Seedlings under control treatment showed the lowest SOD, POD, and CAT values (Fig. 3). The highest SOD values in leaves were observed in seedlings exposed to hypoxic conditions and treated with a high dose (1500 ppm) of SiNPs, which were 27% and 29% higher, respectively, compared to seedlings exposed to hypoxic conditions and treated with low (1000 ppm) and high dose (1500 ppm) of SiSol. For roots, the highest SOD values were observed in all seedlings under hypoxic conditions treated with SiNPs, regardless of dose, which were 19% higher in average compared to seedlings under hypoxic conditions treated with both doses (1000 ppm and 1500 ppm) of SiSol. For leaves under non-hypoxic conditions, seedlings treated with high dose of SiNPs exhibited higher SOD values than seedlings treated with both doses of SiSol. POD activity was similar to SOD. CAT values in leaves and roots of seedlings under hypoxic conditions were higher compared to seedlings under non-hypoxic conditions, regardless of Si treatment. However, no significant differences in CAT were observed in leaves of seedlings treated with SiSol or SiNPs under non-hypoxic, although SiNPs-treated seedlings showed higher CAT values than SiSol-treated seedlings under hypoxic conditions. Contrary to those observed in leaves, CAT values for roots of seedlings treated with SiNPs tended to be higher compared to seedlings treated with SiSol.

3.4. Proline and glycine betaine content

The leaves of control seedlings exhibited the lowest proline and GB content, although it was not different from the content observed in seedlings under non-hypoxic conditions treated with low dose (1000 ppm) of SiSol (Fig. 4). Proline content in leaves and roots of seedlings under hypoxic conditions treated with high dose (1500 ppm) of SiNPs were 63% and 57% higher respectively, compared to leaves and roots of

seedlings treated with high dose (1500 ppm) of SiSol. Similarly, GB content in leaves and roots of seedlings under hypoxic conditions treated with high dose (1500 ppm) of SiNPs were 56% higher respectively, compared to leaves and roots of seedlings treated with high dose (1500 ppm) of SiSol. Furthermore, seedlings under hypoxic conditions treated with any dose of SiNPs showed the highest proline and GB content in leaves and roots. Overall, seedlings treated with SiNPs exhibited higher proline and GB content than those treated with SiSol regardless of dose, under non-hypoxic or hypoxic conditions, respectively.

3.5. Lipid peroxidation

Lipid peroxidation measured as MDA content, O_2^- content, and H_2O_2 content in leaves and roots were also similar (Fig. 5). Under non-hypoxic conditions, all seedlings showed similar responses to control seedlings, regardless of Si source or dose. Lipid peroxidation in seedlings under hypoxic conditions treated with high (1500 ppm) and low (1000 ppm) dose of SiNPs was 37% and 39% lower in leaves and 24% and 28% lower in roots compared to seedlings treated with high (1500 ppm) and low (1000 ppm) dose of SiSol, respectively. Similarly, under hypoxic conditions, O_2^- content in seedlings treated with high (1500 ppm) and low (1000 ppm) dose of SiNPs was 63% and 45% lower in leaves and 66% and 52% lower in roots compared to seedlings treated with high (1500 ppm) and low (1000 ppm) dose of SiSol, respectively. Following a similar trend, H_2O_2 content in seedlings under hypoxic conditions treated with high (1500 ppm) and low (1000 ppm) dose of SiNPs was 53% and 41% lower in leaves and 49% and 53% lower in roots compared to seedlings treated with high (1500 ppm) and low (1000 ppm) dose of SiSol, respectively. However, seedlings treated with high dose (1500) of SiSol showed a similar response to those treated with low dose (1000 ppm) of SiNPs for MDA content in roots and O_2 content in leaves.

3.6. Nutrient contents

The contents of N, P, K, and Zn in leaves from seedlings treated with SiNPs were higher compared to control, regardless of oxygen availability [except for Zn in seedlings treated with low dose (1000 ppm) of SiNPs under hypoxic conditions] (Fig. 6). The high dose (1500 ppm) of SiNPs stimulated higher N, P, K, and Zn accumulation in leaves compared to SiSol, under non-hypoxic and hypoxic conditions, respectively. The accumulation of Mn and Fe in seedlings under non-hypoxic conditions was similar to control seedlings, regardless of Si source or dose (Fig. 7). However, the highest Mn and Fe contents were observed in seedlings treated with SiSol under hypoxic conditions, regardless of dose. Control seedlings exhibited the lowest Si content in leaves and roots. Si content in leaves of seedlings treated with high (1500 ppm) and low (1000 ppm) dose of SiNPs was at least 25% higher under non-hypoxic conditions and least 25% higher under hypoxic conditions compared to seedlings treated with high (1500 ppm) and low (1000 ppm) dose of SiSol, respectively. Similarly, in roots, Si content of seedlings treated with high (1500 ppm) and low (1000 ppm) dose of SiNPs was at least 18% higher under non-hypoxic conditions and at least 41% higher under hypoxic conditions compared to seedlings treated with high (1500 ppm)

and low (1000 ppm) dose of SiSol, respectively. Additionally, Si applied either as SiSol or SiNPs significantly increased Si content in leaves and roots compared to control seedlings.

4. Discussion

Significant differences between SiNPs and SiSol-treated seedlings were observed. These differences could be attributed to higher efficiency of SiNPs in delivering Si to the seedlings. The solubility of Si is highly dependent on its surface area (Balakhnina et al., 2012; Iler, 1979) and Si nanoparticles, due to their lower particle size (20–30 nm diameter), have higher surface area than regular Si particles (5–200 nm diameter) (Corrin and Nicholson, 2011), making them easier to disperse in water, consequently facilitating Si uptake, and potentially increasing its biochemical activity (Dubchak et al., 2010).

Silicon is involved in different plant protective functions, such as epidermal thickening, protection of cellular membrane, reduction of toxic elements uptake by roots, and preservation of *Pn* under stress conditions (Balakhnina et al., 2012; Coskun et al., 2016; Hammond et al., 1995; Mostofa et al., 2021; Savant et al., 1996), allowing stressed plants to return to physiological non-stress levels (Ma, 2004). Ma and Takahashi (2002) reported that Si accumulation induced the formation of two types of silicified cells in rice: silica cells and silica bodies or bulliform silica cells. Those structures increased strength and rigidity of leaves, enabling them to counteract abiotic and biotic stresses (Ma and Takahashi, 2002; Yamaji et al., 2008). Preserving cell rigidity and structure is critical to conserve membrane functions, such as the transport of substances inside and outside the cell, thereby maintaining vital physiological.

Flooding limits availability of O_2 for roots, causing hypoxia stress and restricting roots' water uptake capacity, consequently stimulating stomatal closure, and reducing *Pn* (Herrera, 2013). Silicon was reported to mitigate detrimental effects unfavorable conditions on *Pn* and on the content of photosynthetic pigments (Al-aghabary et al., 2005; Avestan et al., 2019). This was reported for plant species exposed to varied stress factors, as in maize (Sayed and Gadallah, 2014) and rice (Lal et al., 2015) under oxygen deficiency; tomato (Al-aghabary et al., 2005), maize (Moussa, 2006), strawberry (Avestan et al., 2019), and soybean (Lee et al., 2010) under salinity stress; maize (Sayed and Gadallah, 2014) under drought; or tomato (Zhang et al., 2019) under phosphorus deficiency. However, Si applied either as SiSol and SiNPs on seedlings under hypoxic conditions did not restore *Pn* to the levels observed in control, non-stressed seedlings in the current study. Moreover, when Si was applied as SiSol on non-stressed seedlings, *Pn* was significantly reduced when compared to control seedlings. Similar Si detrimental effects were reported by Qin et al. (2016) on grapevines. Only seedlings treated with SiNPs under non-hypoxic conditions showed similar *Pn* values compared to control seedlings. Yet, SiNPs-treated seedlings under non-hypoxic conditions showed leaf greenness values significantly higher than control seedlings. However, control seedlings showed no leaf greenness differences when compared to SiSol-treated seedlings regardless oxygen availability, or SiNPs-treated seedlings under hypoxic conditions.

Silicon applications on hypoxia-stressed and non-stressed seedlings contributed to the increase of antioxidant enzymes activity (SOD, POD, and CAT) to levels above control, non-stressed seedlings. As

mentioned by Liang et al. (2007), the stimulation of antioxidant enzyme activity in plants is thought to be one of the most important Si-mediated mechanisms for mitigating abiotic stress. This enhanced antioxidant activity might be associated with changes in the expression level of genes related to antioxidant enzyme synthesis, probably induced by Si (Elsheery et al., 2020). In our study, the enhanced antioxidant enzyme activity in SiNPs-treated seedlings compared to SiSol-treated seedlings is also reflected in their lower MDA, O_2^-, and H_2O_2 content, in leaves and roots. The capacity of Si to stimulate antioxidant enzyme activity and reduce oxidative damage in plants has been reported under salinity, drought stress, heat stress, freeze, nutrient deficiency, and heavy metal toxicities (Ashraf et al., 2010). Silicon-induced antioxidant activity has been described for several crop species, such as mango (Elsheery et al., 2020), tomato (Zhang et al., 2019), strawberry (Muneer et al., 2017), rapeseed (Hasanuzzaman et al., 2018b), rice (Khan and Gupta, 2018; Kim et al., 2014), and wheat (Tripathi et al., 2017).

The present study showed that all Si treatments, except T2 (seedlings under non-hypoxic conditions and sprayed with SiSol at 1000 ppm) in leaves, increased proline content in leaves and roots of peach seedlings, compared to the control. Moreover, SiNPs-treated seedlings showed higher proline content than SiSol-treated seedlings, under hypoxic and non-hypoxic conditions, respectively. Under stress, proline was reported to act as an easy-to-access source of carbon and nitrogen, also participating in the stabilization of cell membranes, macromolecules, and other subcellular structures, besides serving as a quencher for free radicals (Jain et al., 2001). Results obtained in the current study are in line with the findings of researchers reporting increase in proline content in response to Si application (Elsheery et al., 2020; Fatemi et al., 2020; Zhang et al., 2019). Improvement of plant's performance under stress related to increase in proline content has been reported for peaches (Tuo et al., 2015; Yun et al., 2014), olives (Zouari et al., 2016), alemow (Gimeno et al., 2014), sunflowers (Jan and Hadi, 2015), and lentils (Misra and Saxena, 2009).

Similarly, an increase in GB was observed under hypoxia stress in the current study. Silicon applications further improved both proline and GB accumulation, in agreement with the reports of several researchers (Abbas et al., 2015; Ahmad et al., 2019; Al-Huqail et al., 2019). As proline, GB helps to sustain the activity of some macromolecules, contributes to preserving cell membrane integrity, and works as a quencher for ROS (Annunziata et al., 2019). Interestingly, GB can increase its own content in plants and stimulate the expression of genes related to the activity of antioxidant enzymes, further reducing oxidative damage (Annunziata et al., 2019).

Proline and GB also contribute to the osmotic regulation of plants (Lo Bianco et al., 2000; Sacala, 2009), which could have further contributed to sustain water uptake by peach seedlings under hypoxia stress. Additionally. Si was observed to stimulate the upregulation of aquaporin genes in sorghum roots, thereby improving root hydraulic conductance, consequently increasing water uptake (Liu et al., 2015). Improved root capacity for water uptake by Si applications might have helped peach seedlings to sustain nutrient uptake under hypoxic conditions. The benefits of Si applications for nutrient uptake have been reported for tomatoes (Zhang et al., 2019), rice (Cuong et al., 2017; Jang et al., 2018), and beans (Zuccarini, 2008).

It is known that Si promotes and inhibits uptake of some nutrients, although the mechanism controlling those responses remains unrevealed (Zhang et al., 2019). Results of the current study also showed that Mn and Fe content in non-stressed seedlings did not differ from the content in control seedlings. However, Mn and Fe contents were significantly higher in seedlings under hypoxic conditions, which suggests that stress may generate some nutritional imbalances. The contents of N, P, K, and Zn increased in peach seedlings treated with Si, regardless of the source (SiNPs or SiSol) or oxygen availability, although tended to be higher in seedlings treated with SiNPs.

5. Conclusion

The findings of this study indicated that application of Si enhanced antioxidant activity and accumulation of organic osmolytes, which were the main stress adaptative strategies adopted by seedlings to survive under hypoxic stress. Furthermore, SiNPs was more effective than SiSol in improving seedlings' capacity to alleviate oxidative damage. However, further research is required to elucidate the underlying mechanisms through which Si participates in the stress adaptive response of plants. Foliar applications of Si to peach plants exposed to flooding conditions might be an alternative management strategy that could help growers to protect their orchards from unexpected flooding events. However, further field research at commercial plantations is required to optimize the dose and validate this alternative. The current study supports the use of Si nanoparticles in agriculture, considering the better delivery capacity showed by SiNPs compared to regular size particles. Furthermore, research on different types of nanoparticles might help develop different management strategies to cope stress in plants. The impact of nanoparticles on the production of temperature fruit crops, particularly as a tool to ameliorate stress symptoms, is a promising area yet to explore.

Declarations

6.1. Conflict of interest

The authors declare that they have no conflicts of interest.

Ethics approval and consent to participate

All authors approved this manuscript and voluntarily participated in its elaboration.

Consent for publication

All authors consent the publication of this manuscript.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors contributed equally to the elaboration of this manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this manuscript.

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Figures

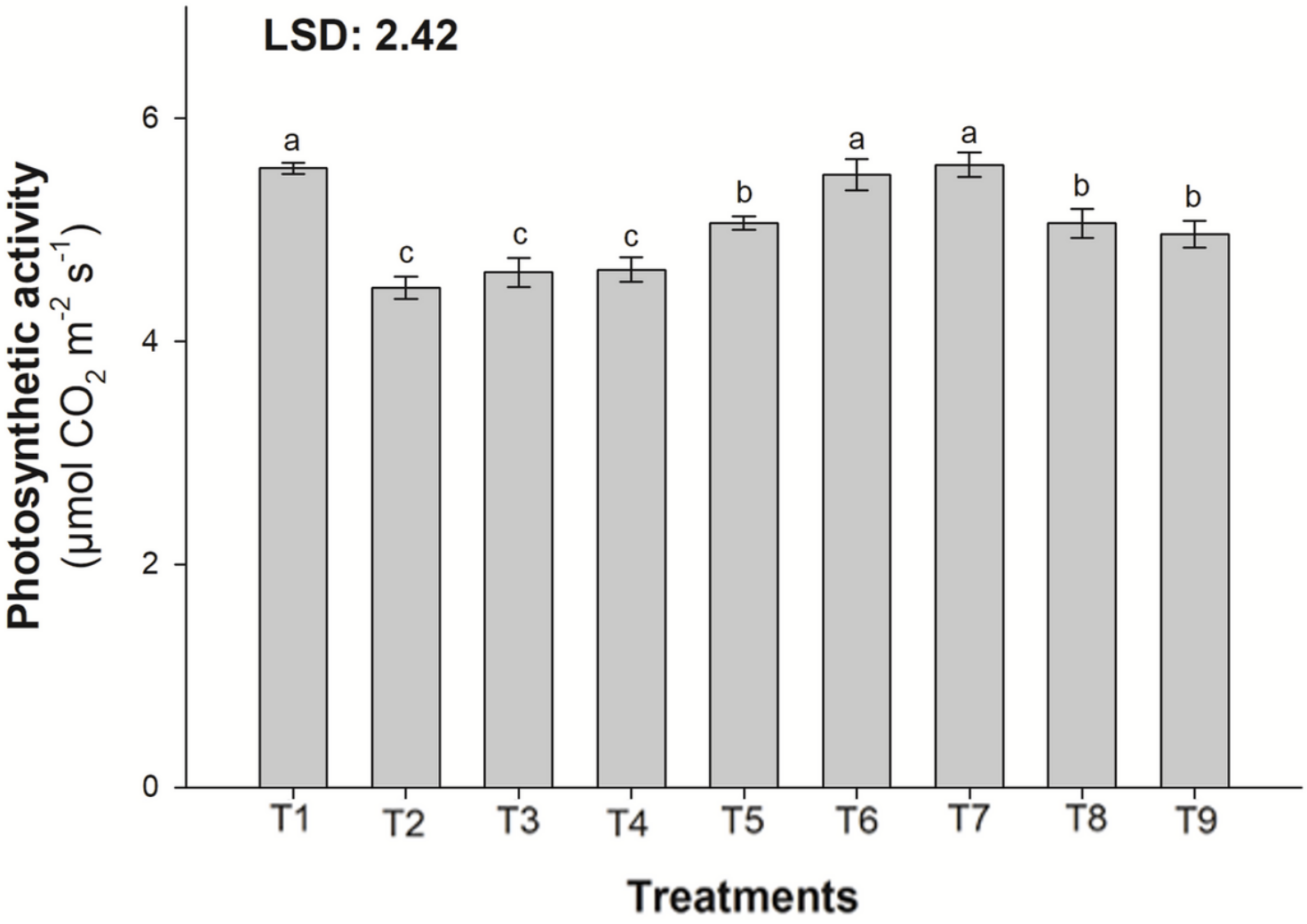

Figure 1

Photosynthetic activity of ‘MP-29’ peach seedlings exposed to hypoxic and non-hypoxic conditions, treated with foliar applications of a low (1000 ppm) and high dose (1500 ppm) of Si solution (SiSol) particles and Si nanoparticles (SiNPs). The treatments setting was the following: T1 (Control) – water only, no SiSol or SiNPs, non-hypoxic conditions; T2 – SiSol, 1000 ppm, non-hypoxic conditions; T3 - SiSol, 1500 ppm, non-hypoxic conditions; T4 - SiSol, 1000 ppm, hypoxic conditions; T5 - SiSol, 1500 ppm, hypoxic conditions; T6 – SiNPs, 1000 ppm, non-hypoxic conditions; T7 - SiNPs, 1500 ppm, non-hypoxic conditions; T8 - SiNPs, 1000 ppm, hypoxic conditions; T9 - SiNPs, 1500 ppm, hypoxic conditions. Means labeled with the same letters do not differ (Error bars represent Standard Error - SE, Least significant difference - LSD, $p<0.05$).

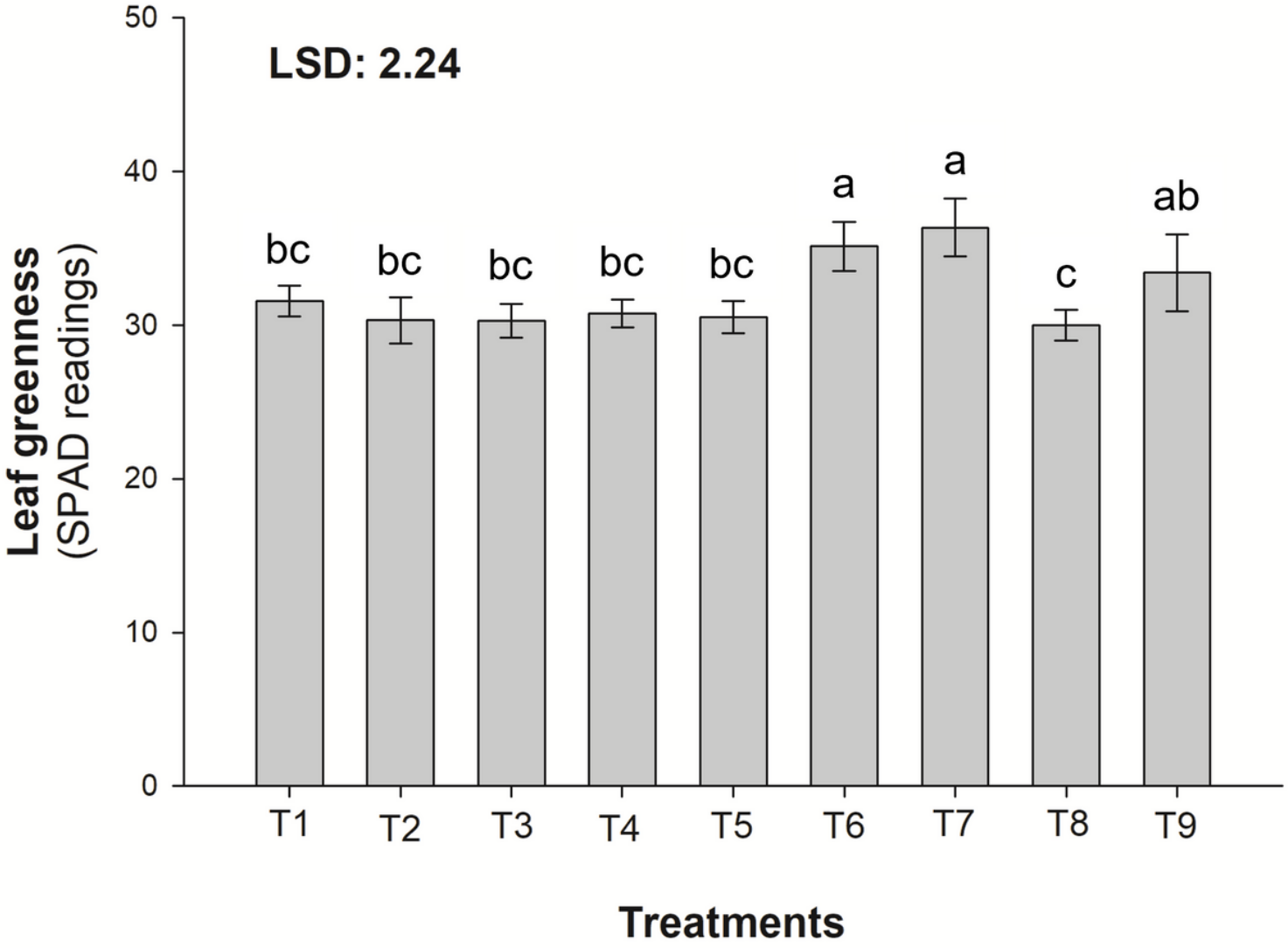

Figure 2

Leaf greenness of ‘MP-29’ peach seedlings exposed to hypoxic and non-hypoxic conditions, treated with foliar applications of a low (1000 ppm) and high dose (1500 ppm) of Si solution (SiSol) particles and Si nanoparticles (SiNPs). The treatments setting was the following: T1 (Control) – water only, no SiSol or SiNPs, non-hypoxic conditions; T2 – SiSol, 1000 ppm, non-hypoxic conditions; T3 - SiSol, 1500 ppm, non-hypoxic conditions; T4 - SiSol, 1000 ppm, hypoxic conditions; T5 - SiSol, 1500 ppm, hypoxic conditions; T6 – SiNPs, 1000 ppm, non-hypoxic conditions; T7 - SiNPs, 1500 ppm, non-hypoxic conditions; T8 - SiNPs, 1000 ppm, hypoxic conditions; T9 - SiNPs, 1500 ppm, hypoxic conditions. Means labeled with the same letters do not differ (Error bars represent Standard Error - SE, Least significant difference - LSD, $p<0.05$).

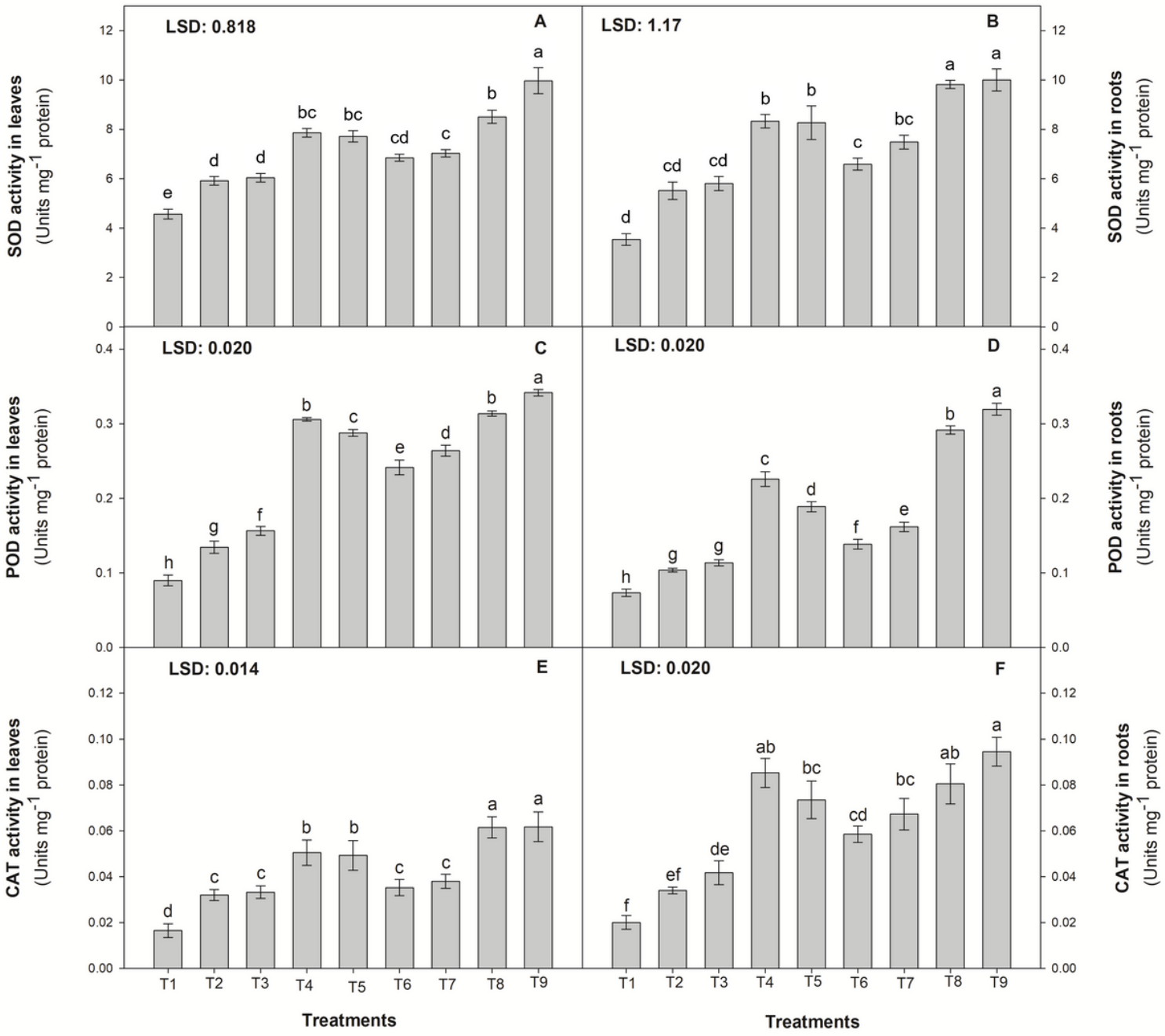

Figure 3

Superoxide dismutase (SOD, figures A and B), peroxidase (POD, figures C and D), and catalase activity (CAT, figures E and F) in leaves (left column) and roots (right column) of 'MP-29' peach seedlings exposed to hypoxic and non-hypoxic conditions, treated with foliar applications of a low (1000 ppm) and high dose (1500 ppm) of Si solution (SiSol) particles and Si nanoparticles (SiNPs). The treatments setting was the following: T1 (Control) – water only, no SiSol or SiNPs, non-hypoxic conditions; T2 – SiSol, 1000 ppm, non-hypoxic conditions; T3 - SiSol, 1500 ppm, non-hypoxic conditions; T4 - SiSol, 1000 ppm, hypoxic conditions; T5 - SiSol, 1500 ppm, hypoxic conditions; T6 – SiNPs, 1000 ppm, non-hypoxic conditions; T7 - SiNPs, 1500 ppm, non-hypoxic conditions; T8 - SiNPs, 1000 ppm, hypoxic conditions; T9 - SiNPs, 1500

ppm, hypoxic conditions. Means labeled with the same letters do not differ (Error bars represent Standard Error - SE, Least significant difference - LSD, p<0.05).

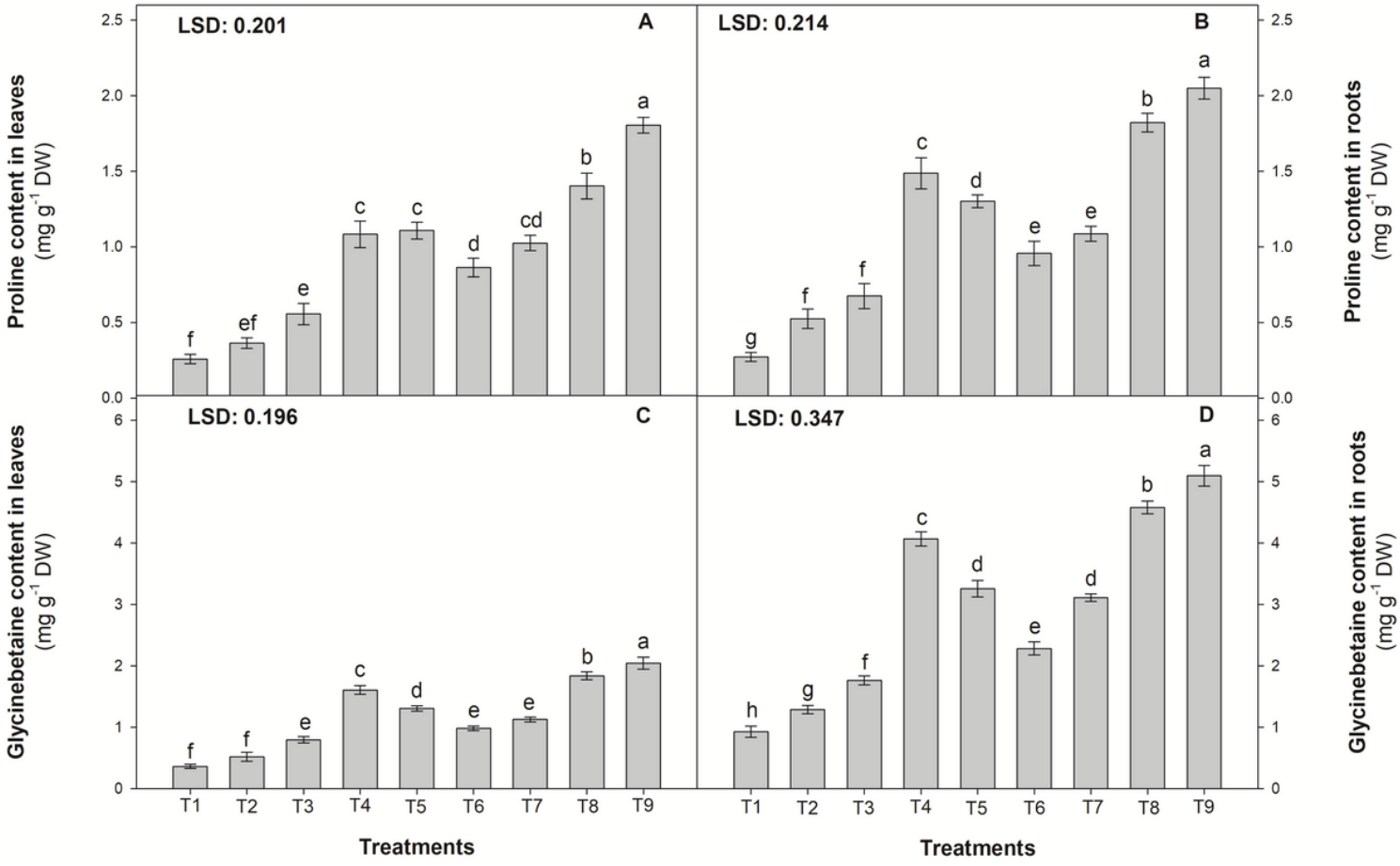

Figure 4

Proline (figures A and B) and glycinebetaine content (figures C and D) in leaves (left column) and roots (right column) of ‘MP-29’ peach seedlings exposed to hypoxic and non-hypoxic conditions, treated with foliar applications of a low (1000 ppm) and high dose (1500 ppm) of Si solution (SiSol) particles and Si nanoparticles (SiNPs). The treatments setting was the following: T1 (Control) – water only, no SiSol or SiNPs, non-hypoxic conditions; T2 – SiSol, 1000 ppm, non-hypoxic conditions; T3 - SiSol, 1500 ppm, non-hypoxic conditions; T4 - SiSol, 1000 ppm, hypoxic conditions; T5 - SiSol, 1500 ppm, hypoxic conditions; T6 – SiNPs, 1000 ppm, non-hypoxic conditions; T7 - SiNPs, 1500 ppm, non-hypoxic conditions; T8 - SiNPs, 1000 ppm, hypoxic conditions; T9 - SiNPs, 1500 ppm, hypoxic conditions. Means labeled with the same letters do not differ (Error bars represent Standard Error - SE, Least significant difference - LSD, p<0.05).

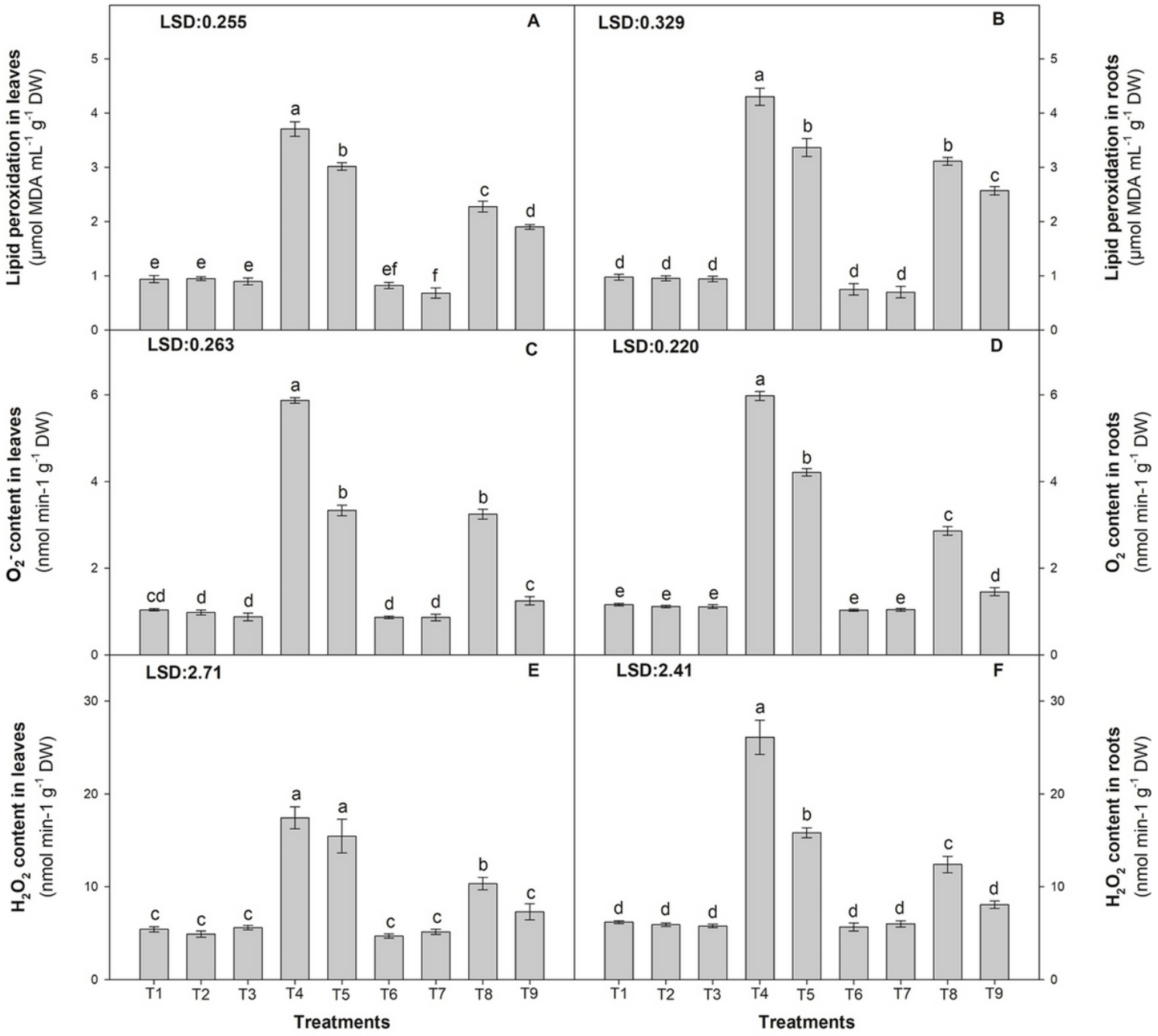

Figure 5

Lipid peroxidation (figures A and B), O2- content (figures C and D), and H2O2 content (nmol min-1 g-1dry weight; figures E and F) in leaves (left column) and roots (right column) of ‘MP-29’ peach seedlings exposed to hypoxic and non-hypoxic conditions, treated with foliar applications of a low (1000 ppm) and high dose (1500 ppm) of Si solution (SiSol) particles and Si nanoparticles (SiNPs). The treatments setting was the following: T1 (Control) – water only, no SiSol or SiNPs, non-hypoxic conditions; T2 – SiSol, 1000 ppm, non-hypoxic conditions; T3 - SiSol, 1500 ppm, non-hypoxic conditions; T4 - SiSol, 1000 ppm, hypoxic conditions; T5 - SiSol, 1500 ppm, hypoxic conditions; T6 – SiNPs, 1000 ppm, non-hypoxic

conditions; T7 - SiNPs, 1500 ppm, non-hypoxic conditions; T8 - SiNPs, 1000 ppm, hypoxic conditions; T9 - SiNPs, 1500 ppm, hypoxic conditions. Means labeled with the same letters do not differ (Error bars represent Standard Error - SE, Least significant difference - LSD, p<0.05).

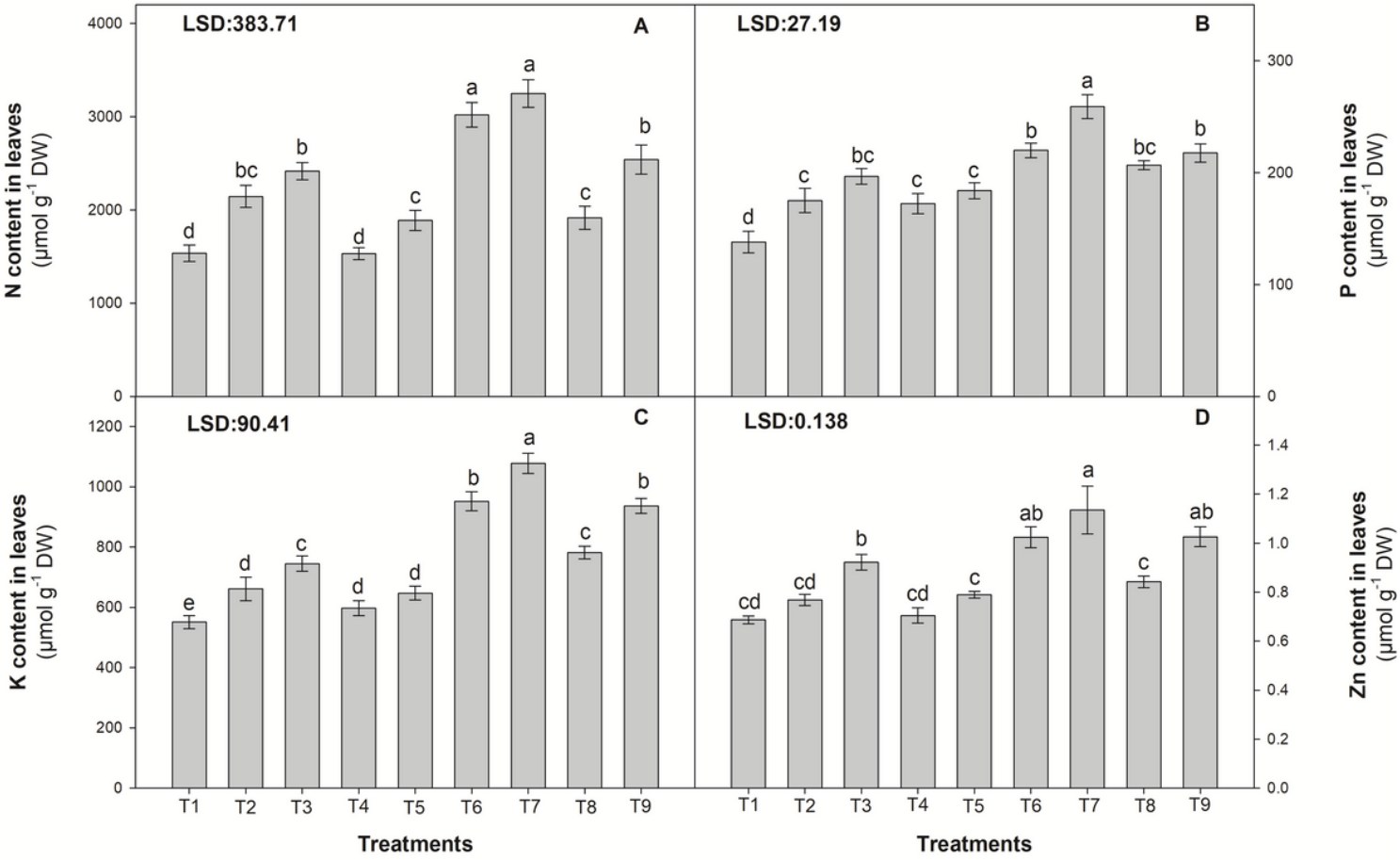

Figure 6

Nitrogen (A), phosphorus (B), potassium (C), and zinc (D) content in leaves of ‘MP-29’ peach seedlings exposed to hypoxic and non-hypoxic conditions, treated with foliar applications of a low (1000 ppm) and high dose of Si (1500 ppm) solution (SiSol) particles and Si nanoparticles (SiNPs). The treatments setting was the following: T1 (Control) – water only, no SiSol or SiNPs, non-hypoxic conditions; T2 – SiSol, 1000 ppm, non-hypoxic conditions; T3 - SiSol, 1500 ppm, non-hypoxic conditions; T4 - SiSol, 1000 ppm, hypoxic conditions; T5 - SiSol, 1500 ppm, hypoxic conditions; T6 – SiNPs, 1000 ppm, non-hypoxic conditions; T7 - SiNPs, 1500 ppm, non-hypoxic conditions; T8 - SiNPs, 1000 ppm, hypoxic conditions; T9 - SiNPs, 1500 ppm, hypoxic conditions. Means labeled with the same letters do not differ (Error bars represent Standard Error - SE, Least significant difference - LSD, p<0.05).

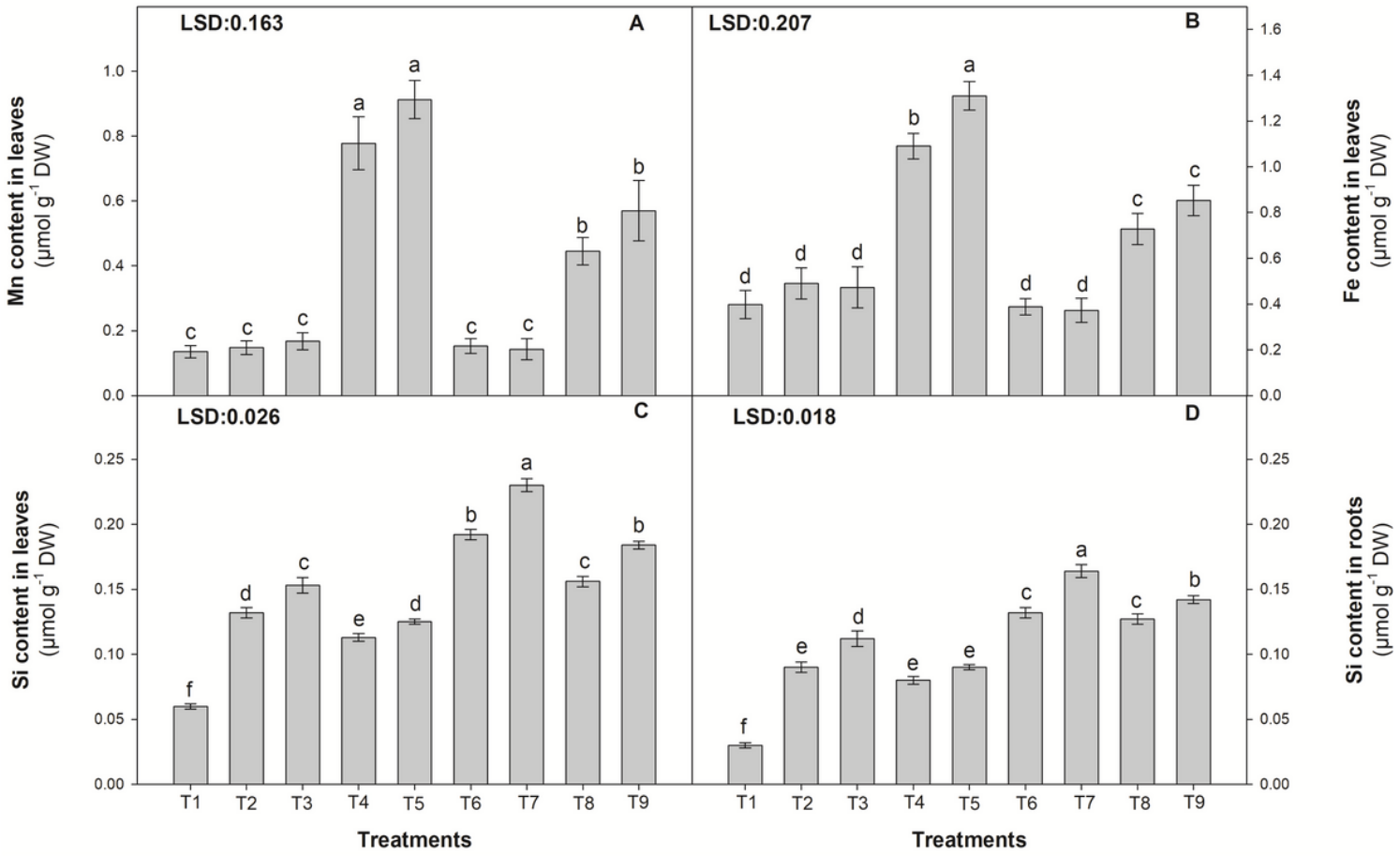

Figure 7

Manganese (A) and iron (B) content in leaves, and Si content (µmol g-1 dry weight) in leaves (C) and roots (D) of 'MP-29' peach seedlings exposed to hypoxic and non-hypoxic conditions, treated with foliar applications of a low (1000 ppm) and high dose (1500 ppm) of Si solution (SiSol) particles and Si nanoparticles (SiNPs). The treatments setting was the following: T1 (Control) – water only, no SiSol or SiNPs, non-hypoxic conditions; T2 – SiSol, 1000 ppm, non-hypoxic conditions; T3 - SiSol, 1500 ppm, non-hypoxic conditions; T4 - SiSol, 1000 ppm, hypoxic conditions; T5 - SiSol, 1500 ppm, hypoxic conditions; T6 – SiNPs, 1000 ppm, non-hypoxic conditions; T7 - SiNPs, 1500 ppm, non-hypoxic conditions; T8 - SiNPs, 1000 ppm, hypoxic conditions; T9 - SiNPs, 1500 ppm, hypoxic conditions. Means labeled with the same letters do not differ (Error bars represent Standard Error - SE, Least significant difference - LSD, $p<0.05$).