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Silicon Compensates Phosphorus Deficit-Induced Growth Inhibition by Improving Photosynthetic Capacity, Antioxidant Potential, and Nutrient Homeostasis in Tomato

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Abstract: Phosphorus (P) deficiency in soils is a major problem for sustainable crop production worldwide. Silicon (Si) is a beneficial element that can promote plant growth, development and responses to stresses. However, the effect of Si on tomato (*Solanum lycopersicum* L.) growth, photosynthesis and mineral uptake under P deficit conditions and underlying mechanisms remain unclear. Here, we showed that low P (LP) supply inhibited tomato growth as revealed by significantly decreased fresh and dry weights of shoots and impaired root morphological traits. LP-induced growth inhibition was associated with decreased photosynthetic pigment content, net photosynthetic rate (Pn), stomatal conductance, transpiration rate and water use efficiency. However, exogenous Si application alleviated LP-induced decreases in growth and physiological parameters. In particular, Si increased Pn by 65.2%, leading to a significantly increased biomass accumulation. Biochemical quantification and in situ visualization of reactive oxygen species (ROS) showed increased ROS ($O_2^{-}\cdot$ and H_2O_2) accumulation under LP stress, which eventually elevated lipid peroxidation. Interestingly, exogenous Si decreased ROS and malondialdehyde levels by substantially increasing the activity of antioxidant enzymes, including superoxide dismutase, peroxidase, and catalase. In addition, Si increased concentrations of osmoregulatory substances, such as proline, soluble sugar, soluble proteins, free amino acids, and organic acids under LP stress. Analysis of major element concentrations revealed that exogenous Si application under LP stress not only increased Si uptake but also enhanced the concentrations of most essential elements (K, Na, Ca, Mg, Fe, and Mn) in different tissues (roots, leaves, and stems). These results reveal that Si mitigates LP stress by improving photosynthetic capacity, antioxidant potential, and nutrient homeostasis and that it can be used for agronomic management of vegetable crops in P-deficient soils.

Keywords: antioxidants; nutrient homeostasis; oxidative stress; phosphorus deficiency; silicon

1. Introduction

Macronutrients are the elements required at relatively large amounts for growth and development of plants [1]. Phosphorus (P) is a key macronutrient that physically participates in multiple basic biological processes, such as photosynthesis, respiration, nucleic acid synthesis, enzyme

activation/inactivation, signaling, and redox reactions [2–4]. However, P concentration in most soils is very low, which necessitates P fertilization from synthetic sources [5]. On a global scale, about 5.7 billion hectares of lands (30–40% of arable lands) suffer from the P-deficiency problem. Low availability of inorganic P is due to its inherent binding tendency with cations (Fe^{3+} and Al^{3+}) and conversion to organic matter through soil-microbial degradation [6]. Therefore, it is important to enhance P uptake capacity of plants to sustain crop production under P-limited conditions.

Phosphorus deficiency reduces plant growth and biomass production [3]. Because of the important role of P in photosynthesis, P deficiency largely affects CO_2 assimilation capacity of plants. At the cellular level, P deficiency induces oxidative stress by triggering the production of reactive oxygen species (ROS), such as superoxide ($O_2^{-}\cdot$) and hydrogen peroxide (H_2O_2) [1]. ROS overload results in the oxidation of lipids, proteins, amino acids, and nucleic acids [7,8]. To minimize ROS-induced damage to biomolecules, a plant should efficiently utilize its antioxidant defense system composed of enzymatic and nonenzymatic antioxidants [9]. The components of antioxidant defense system function collaboratively to keep the levels of ROS at optimal levels by a series of scavenging and/or detoxification processes. In addition, plants have evolved multiple adaptive strategies to overcome limited P availability in soils [5]. In response to P deficiency, plants increase P uptake by altering root architecture, the expression of P-related genes, and overall metabolic and developmental processes [3,10].

Silicon (Si) ranks the second in terms of abundance of element in the earth's crust [11]. Almost all terrestrial plant species accumulate Si in different tissues, but the mechanism of Si accumulation still remains unclear [12]. Si is a beneficial element that promotes growth and development in a number of plant species [13]. However, benefits from Si largely depend on the presence of an efficient Si transport system. It is believed that the positive effects of Si on plants are due to its deposition in roots, leaves, stems, and so on, which strengthens those tissues and creates physical barriers [12]. Si fertilization as a means for nutrient management has shown to increase rice productivity in Japan [13]. Si also improves plant tolerance to a wide range of biotic and abiotic stresses, such as drought, salinity, heat, cold, metal toxicity, and nutrient stress [11,14–17]. Under heavy metal stress, Si enhances precipitation of toxic ion, reduces its transport to above ground plant parts, and promotes *in planta* sequestration; however, the phenomena are closely associated with Si-induced enhanced ROS scavenging in plants [14,18]. For example, seed priming with Si alleviates arsenic (As) stress in rice by improving antioxidant capacity and osmoregulatory substances (proline), which greatly reduced oxidative stress [16]. Not only As but also Si have been shown to increase plant growth in soils that are contaminated with multiple heavy metals, such as Cd, Zn, Cu, and Pb, wherein stimulation of enzymatic and nonenzymatic antioxidants and sequestration of metals into vacuoles and cell walls might function as important internal mechanisms [11].

Recently, the role of Si in mitigating nutrient imbalance has received great attention due to rising deficiency of essential elements in soils worldwide [19]. Studies revealed that Si plays a dual role in deficiency and abundance (toxicity) of an essential element. For instance, Si alleviates Fe toxicity by reducing Fe uptake and by improving antioxidant enzyme activity in rice, which result in reduced ROS accumulation in plants [14]. On the other hand, exogenous Si application mitigates Fe deficiency by increasing the root apoplastic Fe pool and by enhancing overall Fe acquisition in cucumber [20]. Similarly, under P-deficit conditions, Si increases P availability in soils and improves internal P use efficiency in plants; however, under high P conditions, Si decreases P uptake and its apoplastic flow and permeability [2,21].

The stress protective role of Si in various abiotic and biotic stresses has been well documented in higher plants [12]. However, the underlying mechanisms and changes in growth, photosynthesis, and mineral uptake under low phosphorus supply remain unclear. Moreover, the effects of exogenous Si on ROS, compatible solute accumulation, and antioxidant defense under limited P supply are not well described in tomato. Thus, the aim of this study was to elucidate the effects of exogenous Si on tomato growth, photosynthesis, ROS accumulation, enzymatic antioxidants, and some essential nutrient content under low P conditions in tomato plants.

2. Materials and Methods

2.1. Plant Materials, Growth Conditions, and Treatments

Tomato (*Solanum lycopersicum* cv. Zhong Za "No. 9") seeds were soaked in warm water (55 °C) for 15 min and then immersed in distilled water for 6 h at room temperature. The seeds were germinated on two layers of moist filter paper for 2 days in an incubator at 28 °C, and germinated seeds were placed in a 72-well tray filled with peat and vermiculite (1:2) in a climate chamber maintaining the following conditions: a relative humidity (RH) of 60–80%, temperature of 28/22 °C (day/night), and 12/12 h photoperiod at a light intensity of 600 μmol·m^{-2}s^{-1}. Four weeks after germination, tomato seedlings with two true leaves were hydroponically grown in $\frac{1}{2}$ strength Japanese Yamazaki nutrient solution for one week. At three-leaf stage, the seedlings were treated with different levels of P and Si. Based on preliminary dose trial, 2/3rd P supply was chosen for further experiment as it caused a significant increase in lipid peroxidation (Supplementary Figure S1). Thus, the experiment comprised of the following treatments: (1) CT = normal phosphorus supply (P 0.66 mM); (2) LP = low phosphorus supply (P 0.44 mM + Si 0 mM); and (3) LP + Si = combined treatment with low phosphorus and exogenous silicon (P 0.44 mM + Si 1.5 mM). There were three pots per treatment in the experiment. In each pot, eight seedlings with similar growth were transplanted. A total of twenty-four seedlings were used in each treatment. As a P source, NaH_2PO_4 was used to control P supply, and potassium silicate ($K_2SiO_3{\cdot}nH_2O$) was used as the Si source. The additional potassium from K_2SiO_3 was subtracted from potassium nitrate, and the resultant loss of nitrate ions was supplemented with dilute nitric acid. The solution pH was adjusted to 5.8–6.0 daily with HNO_3, and the nutrient solution was replaced each week. After about three weeks of treatment, samples were collected for different morphological, physiological and biochemical analyses. The composition of Japanese Yamazaki nutrient solution used in this study was as follows: the macronutrient stock was comprised of 4 mM KNO_3, 1.5 mM $Ca(NO_3)_2{\cdot}4H_2O$, 2 mM $MgSO_4{\cdot}7H_2O$, and 0.66 mM $NH_4H_2PO_4$, and the micronutrient stock was comprised of 30 mg/L iron (Na_2Fe-EDTA), 2.86 mg/L H_3BO_3, 2.13 mg/L $MnSO_6{\cdot}7H_2O$, 0.22 mg/L $ZnSO_4{\cdot}7H_2O$, 0.08 mg/L $CuSO_4{\cdot}5H_2O$, and 0.02 mg/L $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$.

2.2. Determination of Biomass and Root Morphology

After measuring the fresh weights of shoot and root, plant samples were kept in an oven at 75 °C for 72 h, and then, dry weights were measured with an electrical balance. For root morphology measurements, tomato roots were cleaned with tap water and the roots were gently spread on a root scanner (MRS-9600TFU2L, Shanghai Zhongjing Technology Co., Ltd., Shanghai, China). Root morphology-related parameters such as root volume, root length, average diameter, and surface area were analyzed from the scanned image of the roots with root analysis software [22]. Six biological replicates were carried out in each experiment.

2.3. Determination of Photosynthetic Pigment Content and Leaf Gas Exchange Parameters

Chlorophylls (Chl) such as Chl a, Chl b, and carotenoids were determined in the third fully expanded leaves [23]. About 0.1 g of leaf tissue was placed in a tube containing 96% ethanol. The tubes were kept in the dark for 24 h. The absorbance of the pigment extract was measured at 470 nm, 649 nm, and 665 nm wave length with a spectrophotometer (UV-2450, Shimadzu, Kyoto Prefecture, Japan). Gas exchange was measured fixing the conditions of CO_2 concentration at 400 μmol·mol^{-1}, the photosynthetically active radiation (PAR) at 800 μmol·m^{-2}·s^{-1}, and the speed of the circulating air flow inside the system at 500 μmol·s^{-1}. Gas exchange parameters such as net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr) were measured with a LI-6400 Portable Photosynthesis System (LI-6400; LICOR, Lincoln, NE, United States). Water use efficiency (WUE) = Pn/Tr. Three biological replicates were carried out in each experiment.

2.4. Determination of Membrane Lipid Peroxidation, Reactive Oxygen Species, and Antioxidant Enzyme Activity

Malondialdehyde (MDA) content was measured as an index of lipid peroxidation in leaves and roots according to Bailly et al. [24] and relied on the thiobarbituric acid reaction [22]. H_2O_2 concentrations in leaves and roots were analyzed spectrophotometrically by a peroxidase assay according to Willekens et al. [25]. The production rate of superoxide ($O_2^-\cdot$) was measured by the method of Elstner and Heupel [26] using the sulfanilamide method, and the reaction was measured at 530 nm. The formation rate of $O_2^-\cdot$ was assayed using a standard curve of $NaNO_2$ reagent. In situ accumulation of $O_2^-\cdot$ was visualized following the nitroblue tetrazolium (NBT) staining as described previously [7,27]. NBT-stained leaves and roots were photographed with a digital camera (Canon DS126201, Japan).

For extraction of antioxidant enzymes, 0.3 g of leaves and roots was homogenized using 50 mM K_2HPO_4–KH_2PO_4 buffer (pH7.8) containing 0.2 mM EDTA and 2% (w/v) insoluble polyvinylpyrrolidone (PVP) and centrifuged at 12,000× *g* at 4 °C for 20 min. The resulted supernatant was used for the analysis of enzyme activity. Superoxide dismutase (SOD) activity was assayed by measuring its ability to inhibit the photoreduction of nitro blue tetrazolium (NBT) using the method of Giannopolitis et al. [28]. Peroxidase (POD) as well as catalase (CAT) activity was measured according to the method described by Cakmak and Marschner [29]. For each experiment, nine reactions were performed including three biological replicates and three technical replicates.

2.5. Determination of Osmotic Potential and Osmotic Regulatory Substances

Osmotic potential ($\Psi\pi$) was measured both in leaves and roots with a vapor pressure osmometer (Model 5520, Wescor, Logan, UT, USA). The $\Psi\pi$ was calculated using following formula: $\Psi\pi = -RTC$, where R, T, and C were molar gas constant, thermodynamic temperature, and reading from the osmometer, respectively [30].

The content of soluble sugar was measured by anthrone colorimetry [31]. About 0.1 g of leaf and root tissues was placed in a tube containing 80% ethanol; the tubes were kept in boiling water for 15 min. After that, the homogenate was centrifuged at 5000× *g* for 20 min and the supernatant was mixed with 1 mL 9% phenol and 5 mL H_2SO_4. The content of soluble sugar was determined as OD 485 per gram fresh weight.

Soluble protein content was measured using Coomassie Brilliant Blue G-250 according to the method of Bradford [32]. The samples of fresh leaves and roots (0.1 g) were placed into a 2-mL tube, and 1 mL of water was added. After centrifugation at 12,000× *g* for 10 min at 4 °C, the supernatant was mixed with 5 mL of Coomassie Brilliant Blue solution for the determination of soluble protein content. The absorbance was spectrophotometrically determined at 520 nm.

The content of proline was measured by sulfosalicylic acid colorimetry [31]. Briefly, 0.5 g of leaves and roots was homogenized in a 10-mL centrifuge tube, and 5 mL of 3% aqueous sulfosalicylic acid was applied. The tubes were kept in boiling water for 10 min. A 2-mL aliquot of the supernatant was mixed for reaction with 2 mL of freshly prepared acidic ninhydrin and 2 mL of glacial acetic acid in a test tube for 30 min at 100 °C. The reaction was terminated in an ice bath, and the mixture was extracted with 4 mL toluene. The extract was vigorously stirred for 20 s using a test tube vortexer. The chromophore-containing toluene was then aspirated from the aqueous phase, and its absorbance was photometrically determined at 520 nm using toluene for a blank.

Free amino acid and organic acid contents were measured according to the method described previously by Zhang et al. [22]. About 0.2 g of leaves and roots was placed in a 10-mL centrifuge tube, and 5 mL of 10% acetic acid was applied. The homogenate was centrifuged at 5000× *g* for 10 min, and the supernatant was mixed with 1.5 mL of hydrated ninhydrin and 50 µL of 0.1% ascorbic acid for 15 min at 100 °C. The reaction was terminated in an ice bath, and the volume of the mixture measured 10 mL with 60% ethanol. Its absorbance was photometrically determined at 570 nm.

The content of organic acid was measured by acid–base titration. The samples of fresh leaves and roots (0.5 g) were placed into a 10-mL tube, and 10 mL of water was added. The supernatant was extracted after centrifuged at 5000× *g* for 10 min, after which the volume was adjusted to 50 mL by water; 25 mL extract was taken out mixed with 100 µL phenolphthalein and titrated with NaOH standard solution to reddish (for 30 s, the color did not fade), and the amount of NaOH was recorded. All experiments were conducted with three biological replicates.

2.6. Determination of Element Content

Silicon content was determined by a molybdenum blue colorimetric method according to Iwasaki et al. [33]. Phosphorus content was determined using the vanadium-molybdenum-blue photometric method [34]. For quantification of other elements, dry plant samples were wet-digested in the concentrated HNO_3/H_2O_2 at 90, 120, and 140 °C for 2 h and then again digested at 180 °C to make the digest clear as described previously [34]. The concentrations of K, Na, Ca, Mg, Fe, Mn, Zn, and Cu in the digests were determined with an ICP-MS (Inductively coupled plasma mass spectrometer, Agilent 7500a, Santa Clara, CA, USA) and expressed on a dry-weight (DW) basis. Each experiment was repeated with three biological replicates.

2.7. Statistical Analysis

All data were subjected to the analysis of variance (ANOVA) and analyzed with SPSS 20.0 statistical software package. To determine statistical significance, we employed Tukey's least significant difference (LSD) test. The difference was considered significant at $p \leq 0.05$ and indicated by different letters.

3. Results

3.1. Exogenous Silicon Improves Plant Growth Under Low Phosphorus Supply

Phosphorus (P) is an essential macronutrient for plants and its deficiency results in growth inhibition [35]. In the present study, deficit P supply (low phosphorus, LP) drastically decreased the fresh weight (FW) and dry weight (DW) of tomato plants compared to that of control (CT) (Table 1). The difference in biomass accumulation was also apparent from unaided eye observation (Supplementary Figure S2). For instance, LP stress decreased shoot FW, root FW, shoot DW, and root DW by 50.52%, 59.78%, 55.32%, and 58.73%, respectively, compared with CT that received adequate P supply. However, the addition of exogenous silicon under phosphorus deficiency (LP + Si) significantly increased those parameters by 83.10%, 100.75%, 94.29%, and 84.62%, respectively, compared with LP stress. We also evaluated different root morphological indexes and found that LP significantly decreased the total root length, total root surface area, total root volume, and average root diameter by 31.88%, 48.48%, 51.34%, and 15.49%, respectively (Table 1). Compared with LP stress, LP + Si treatment increased those root morphological indexes by 56.87%, 60.00%, 61.52%, and 10.00%, respectively. All these results clearly suggest that exogenous Si could efficiently alleviate deficit phosphorus supply-induced growth inhibition in tomato plants.

3.2. Exogenous Silicon Increases Chlorophyll Content and Photosynthetic Capacity in Tomato Leaves Under Low Phosphorus Stress

Compared to the control (CT), the chlorophyll a (Chla), Chlb, carotenoids, and total chlorophyll content of tomato leaves decreased under phosphorus deficiency treatment. More precisely, low phosphorus treatment (LP) reduced Chla, Chlb, Chla + Chlb, and carotenoid content by 5.67%, 10.71%, 6.31%, and 9.80%, respectively, compared with CT (Table 2). However, exogenous Si treatment reversed LP effects on photosynthetic pigments, while LP significantly decreased net photosynthetic rate, stomatal conductance, transpiration rate, and water use efficiency by 42.74%, 15.38%, 44.81%, and 16.50%, respectively; it increased the intercellular CO_2 concentration (Ci) by 19.11%. When exogenous Si was combined with LP treatment (LP + Si), Ci significantly decreased, whereas net photosynthetic

rate, stomatal conductance, transpiration rate, and water use efficiency remarkably increased compared with that of only LP treatment. More precisely, compared with LP stress, LP + Si treatment increased net photosynthetic rate, stomatal conductance, transpiration rate, and water use efficiency by 65.94%, 9.09%, 25.88%, and 10.08%, respectively.

3.3. Effects of Exogenous Silicon and Low Phosphorus on Lipid Peroxidation and ROS Accumulation

Stress conditions trigger ROS production and associated lipid peroxidation [36]. The degree of lipid peroxidation can be evaluated by estimating the levels of MDA. As shown in Figure 1A, the exposure of plants to LP caused a significant increase in MDA content. For instance, the LP treatment increased MDA content by 62.35% and 52.60% in leaves and roots of tomato plants, respectively. However, exogenous Si treatment drastically decreased the LP-induced lipid peroxidation as evidenced by a reduced MDA content at LP + Si which was statistically similar to that of CT. Since lipid peroxidation is attributed to excessive accumulation of ROS, we determined ROS accumulation both quantitatively and qualitatively. As shown in Figure 1B, the LP treatment significantly increased the leaf and root H_2O_2 contents by 30.34% and 33.54%, respectively. The LP treatment also increased $O_2^{-}\cdot$ accumulation in leaves and root as evidenced by 52.54% and 55.77% increases in $O_2^{-}\cdot$ under LP treatment compared with CT. Histochemical staining with NBT that visualized $O_2^{-}\cdot$ accumulation in situ was in agreement with biochemical results (Figure 2). However, exogenous Si treatment drastically decreased the LP-induced ROS accumulation as evidenced by a reduced H_2O_2 and $O_2^{-}\cdot$ content at LP + Si. This implies that exogenous Si decreased ROS accumulation in tomato plants under LP stress which potentially contributed to reduced lipid peroxidation.

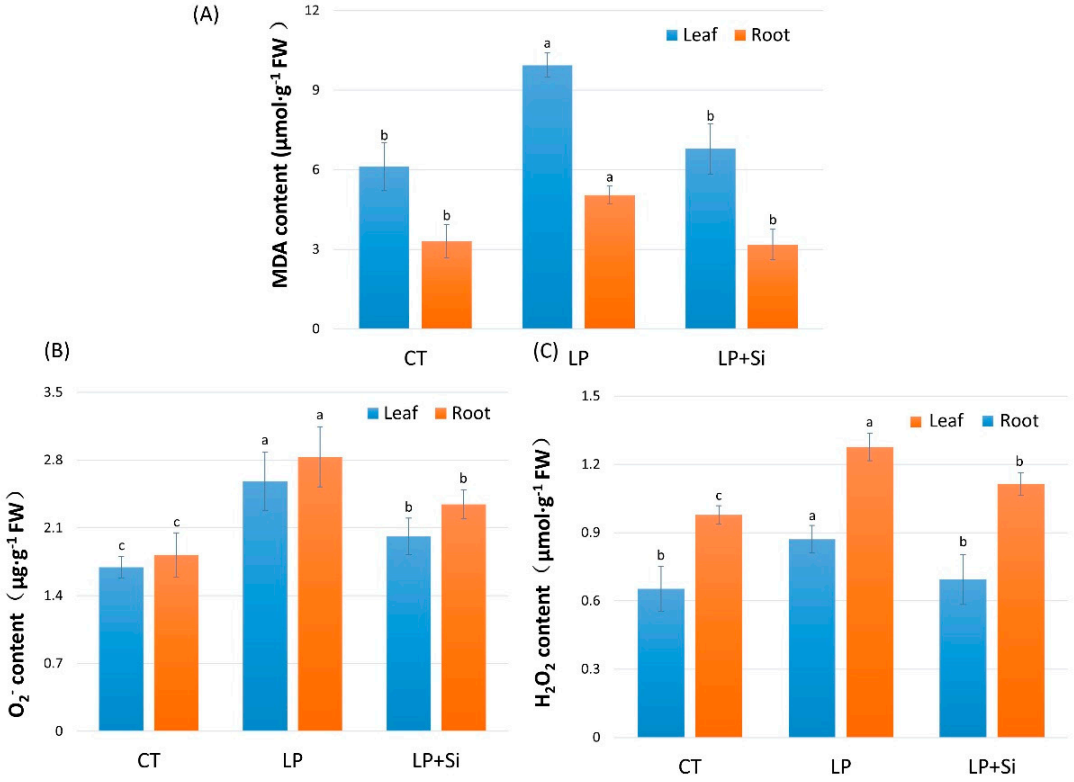

Figure 1. Effect of low phosphorus (LP) and silicon (Si) on lipid peroxidation and reactive oxygen species content in tomato plants: (**A**) Malondialdehyde (MDA) content, (**B**) $O_2^{-}\cdot$ content, and (**C**) H_2O_2 content in tomato leaves and roots. Data are shown as means ± standard deviation (SD) of three replicates. Bars with the same color but different letters indicate statistically significant differences at $p \leq 0.05$. CT = normal phosphorus supply (P 0.66 mM); LP = low phosphorus supply (2/3rd of normal P); Si = 1.5 mM Si.

Table 1. Effect of exogenous silicon on plant biomass and root morphological traits of tomato plants under deficit phosphorus supply.

Treatment	Shoot Fresh Weight (g)	Root Fresh Weight (g)	Shoot Dry Weight (g)	Root Dry Weight (g)	Total Root Length (cm)	Total Root Surface Area (cm^2)	Total Root Volume (cm^3)	Average Root Diameter (mm)
CT	28.7 ± 1.55a	9.97 ± 1.70a	2.35 ± 0.12a	0.63 ± 0.06a	2158 ± 173a	495 ± 23a	9.72 ± 1.02a	0.71 ± 0.04a
LP	14.2 ± 3.37b	4.01 ± 1.44b	1.05 ± 0.22b	0.26 ± 0.09c	1469 ± 305b	255 ± 36c	4.73 ± 0.12c	0.60 ± 0.02b
LP + Si	26.0 ± 1.43a	8.05 ± 0.36a	2.04 ± 0.11a	0.48 ± 0.02b	2306 ± 65a	408 ± 45b	7.64 ± 0.47b	0.66 ± 0.03a

Data are shown as means ± SD, n = 6. Within each column followed by the same lowercase letters are not significantly different according to Tukey's test at $p \leq 0.05$. CT, control; LP, low phosphorus.

Table 2. Effect of exogenous silicon on chlorophyll content and gas exchange parameters in tomato leaves under deficit phosphorus supply.

Treatment	Chl a	Chl b	Carotenoids	Chl (a + b)	Net Photosynthetic Rate (μmol $CO_2 \cdot m^{-2} \cdot S^{-1}$)	Stomatal Conductance ($molH_2O \cdot m^{-2} \cdot s^{-1}$)	Intercellular CO_2 Concentration (μmol $CO_2 \cdot mol^{-1}$ air)	Transpiration Rate ($mmolH_2O \cdot m^{-2} \cdot s^{-1}$)	Water Use Efficiency (mmol $CO_2 \cdot mol^{-1} \cdot H_2O$)
CT	1.94 ± 0.04a	0.28 ± 0.01a	0.51 ± 0.01a	2.22 ± 0.04a	24.1 ± 1.60a	0.13 ± 0.04a	246 ± 24.7c	4.62 ± 0.32a	2.97 ± 0.10a
LP	1.83 ± 0.01b	0.25 ± 0.01b	0.46 ± 0.01b	2.08 ± 0.03b	13.8 ± 0.89b	0.11 ± 0.03c	293 ± 14.3a	2.55 ± 0.34c	2.48 ± 0.05c
LP + Si	1.91 ± 0.03a	0.30 ± 0.03a	0.50 ± 0.02a	2.21 ± 0.04a	22.9 ± 1.72a	0.12 ± 0.07b	273 ± 14.9b	3.21 ± 0.27b	2.73 ± 0.06b

Data are shown as means ± SD, n = 3. Within each column followed by the same lowercase letters are not significantly different according to Tukey's test at $p \leq 0.05$. CT, control; LP, low phosphorus; Chl a, chlorophyll a; Chl b, chlorophyll b.

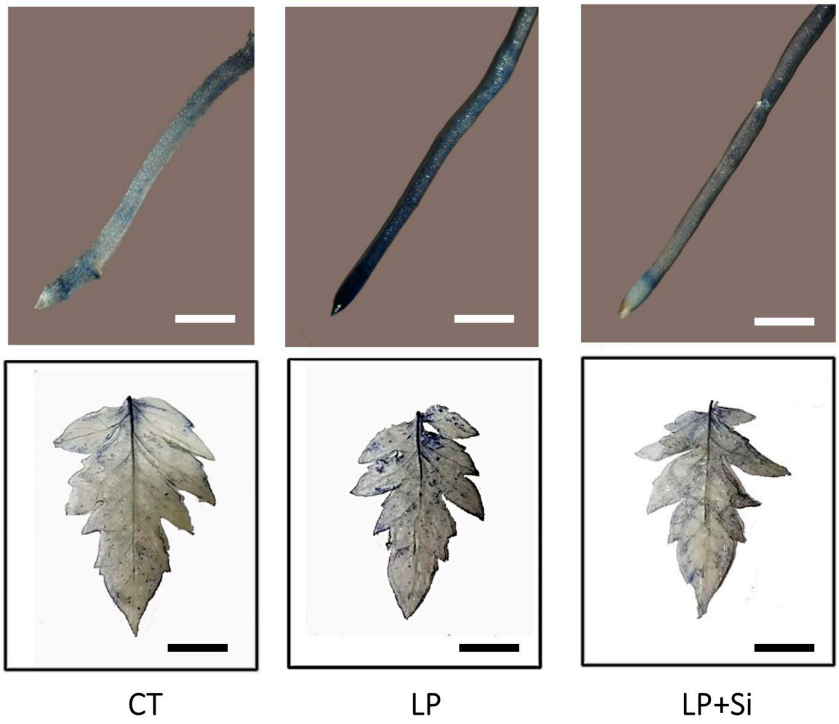

Figure 2. In situ superoxide ($O_2^{-}\cdot$) accumulation in tomato roots and leaves as influenced by low phosphorus (LP) and exogenous silicon (Si): $O_2^{-}\cdot$ accumulation was visualized following nitroblue tetrazolium chloride (NBT) staining. NBT-stained roots (upper panels) and leaves (lower panels) show localized $O_2^{-}\cdot$ accumulation as dark-blue spots under different treatments. Scale bars = 2 mm (upper panels) and 2 cm (lower panels).

3.4. Exogenous Silicon Alleviated Low Phosphorus-Induced Oxidative Stress by Enhancing Antioxidant Enzyme Activity

Antioxidant enzymes play an important role in ROS scavenging [9]. Since we found a significant reduction in ROS accumulation following exogenous Si treatment, we measured the activity of key antioxidant enzymes such as SOD, POD, and CAT in tomato leaves and roots under different treatments. Table 3 showed that LP treatment significantly decreased activity of all tested antioxidant enzymes in both leaves and roots. For instance, SOD activity in leaves and roots decreased by 19.44% and 19.59%, whereas POD activity decreased by 10.66% and 34.20%, respectively. Meanwhile, CAT activity in leaves and roots also decreased significantly by 25.43% and 35.16%, respectively. However, exogenous Si supply remarkably attenuated the LP-induced inhibition on antioxidant enzyme activity both in leaves and in roots. Compared with only LP, the combined treatment (LP + Si) increased SOD, POD, and CAT activity by 27.97%, 6.46%, and 37.14% in leaves and by 28.15%, 38.65%, and 56.74% in roots, respectively. Except for leaf POD activity, the activities of SOD, POD, and CAT between LP and LP + Si treatments were significantly different (Table 3).

Table 3. Effect of exogenous silicon on activity of antioxidant enzymes in tomato plants under deficit phosphorus supply.

Antioxidant Enzymes	Tissue	CT	LP	LP + Si
SOD activity ($U\cdot g^{-1}\cdot FW\cdot h^{-1}$)	Leaf	35.5 ± 2.37a	28.6 ± 1.79b	36.6 ± 2.20a
	Root	14.8 ± 1.68a	11.9 ± 1.09b	15.25 ± 1.61a
POD activity ($U\cdot g^{-1}$ FW)	Leaf	2617 ± 85.0a	2338 ± 78.4b	2489 ± 83.2b
	Root	21,964 ± 311a	14,452 ± 457c	20,037 ± 731b
CAT activity ($U\cdot g^{-1}$ FW)	Leaf	88.1 ± 7.42a	65.7 ± 5.16b	90.1 ± 8.51a
	Root	98.4 ± 6.67a	63.8 ± 7.74b	100 ± 5.59a

Data are shown as means ± SD, n = 3. Within each row (same tissue) followed by the same lowercase letters are not significantly different according to Tukey's test at $p \leq 0.05$. CT, control; LP, low phosphorus.

3.5. Effects of Exogenous Silicon on Osmotic Adjustment under Deficit Phosphorus Supply

As shown in Figure 3, leaf and root osmotic potential of tomato plants significantly decreased under LP stress compared with that of CT. However, exogenous Si increased osmotic potential in LP-stressed plants. Since osmotic potential was altered by LP stress, we analyzed some osmotic adjustment substances in leaves and roots. As shown in Figures 3 and 4, LP stress significantly increased proline content in both leaves and roots. Surprisingly, exogenous Si treatment further increased the proline content in leaves; however, proline content in root was statistically similar between LP and LP + Si. While the LP treatment significantly increased soluble sugar content in leaves, exogenous Si had no further effect. However, exogenous Si significantly elevated soluble sugar content in LP-stressed roots as compared with that in CT and LP alone. Unlike soluble sugar content, soluble protein concentrations in leaves and roots were significantly reduced by LP treatment. In contrast, exogenous Si treatment alleviated the LP-induced reduction in soluble protein content (Figure 4). Although LP treatment had no effect on leaf and root free amino acid content, exogenous Si significantly increased free amino acid content compared with that in CT and LP alone. Compared with CT, LP treatment remarkably increased organic acid content in leaves and roots, whereas exogenous Si treatment caused a further increase in both leaf and root organic acid content. Taken together, these results suggest that exogenous Si treatment improves accumulation of osmolyte or osmotic adjustment substances which potentially minimized LP-induced osmotic stress.

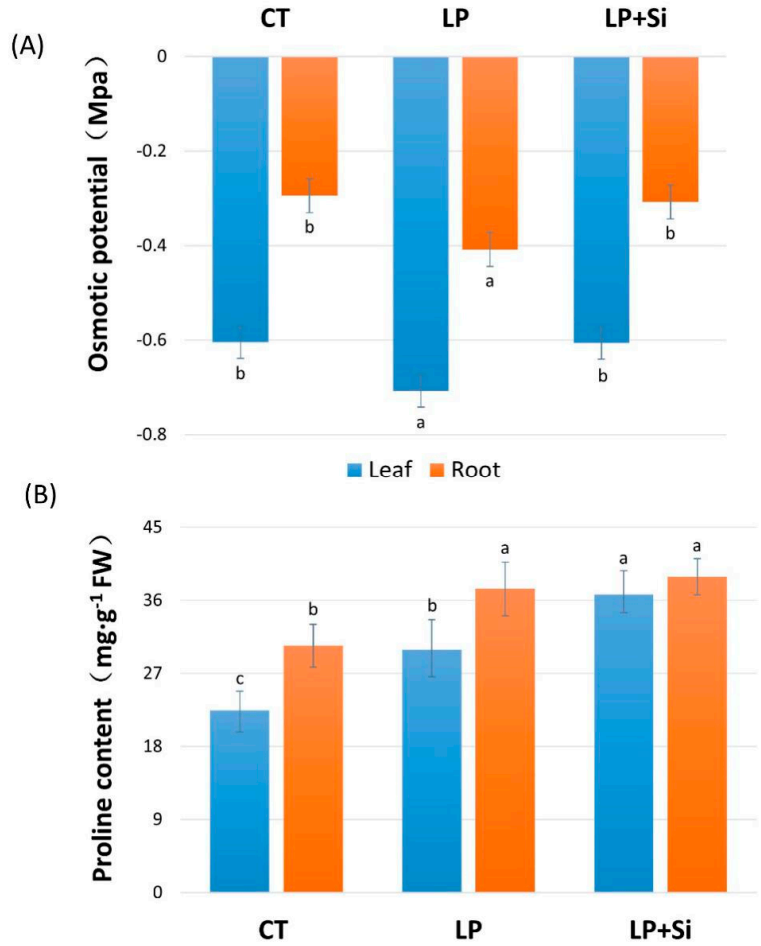

Figure 3. Effect of low phosphorus (LP) and silicon (Si) on osmotic potential and proline content in tomato plants: (**A**) Osmotic potential and (**B**) proline content in tomato leaves and roots. Data are shown as means ± standard deviation (SD) of three replicates. Bars with the same color but different letters indicate statistically significant differences at $p \leq 0.05$.

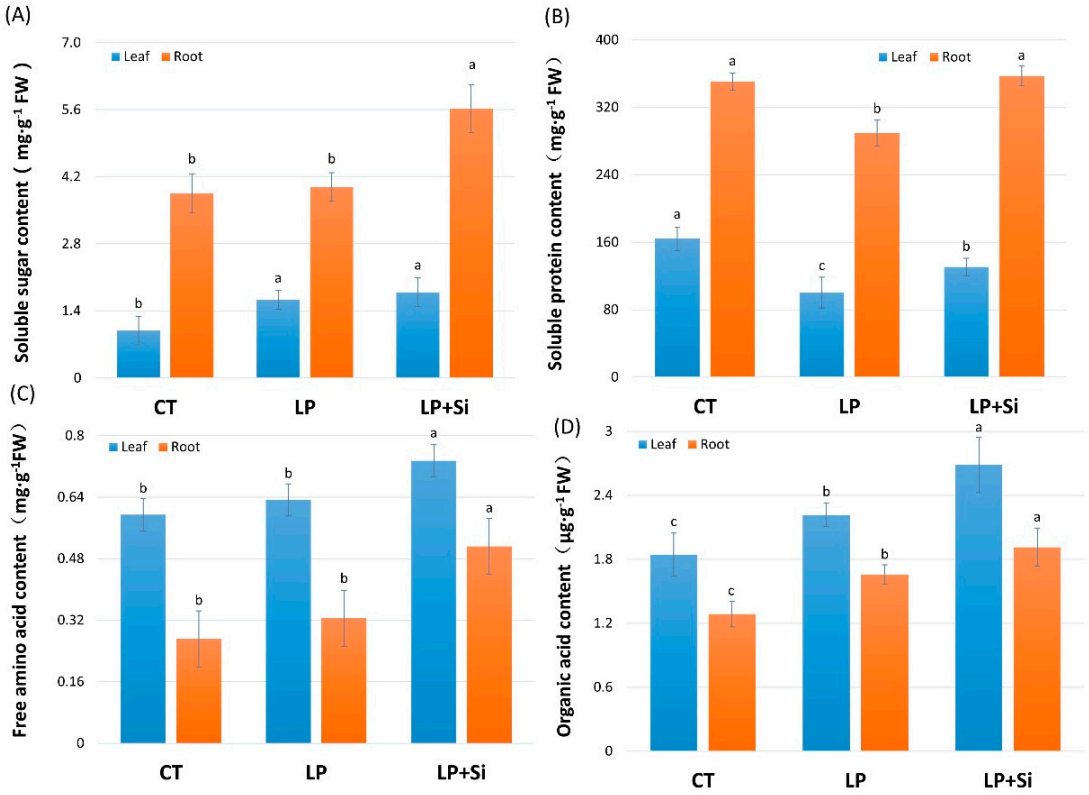

Figure 4. Changes in osmoregulatory substances as influenced by low phosphorus (LP) and exogenous silicon (Si) supply in tomato plants: (**A**) Soluble sugar content, (**B**) soluble protein content, (**C**) free amino acid content, and (**D**) organic acid content in tomato leaves and roots. Data are shown as means ± standard deviation (SD) of three replicates. Bars with the same color but different letters indicate statistically significant differences at $p \leq 0.05$.

3.6. Effects of Exogenous Silicon and Low Phosphorus on Different Element Content in Tomato Leaves and Roots

To explore whether phosphorus deficiency and exogenous Si could alter accumulation of other elements, we determined the content of a range of elements in different tissues such as leaf, stem, and root (Figure 5). While LP treatment significantly decreased leaf and root P content by 13.31% and 29.72%, respectively, LP did not affect P content in stem. Interestingly exogenous Si treatment significantly increased P content by 14.23%, 14.23%, and 57.52% in leaf, stem, and root respectively, compared with that of only LP treatment. Meanwhile, LP did not affect Si content in leaf, stem, and root of tomato plants, but Si content significantly increased in (LP + Si)-treated plants compared with that of CT and only LP-treated plants. The contents of other important elements were presented in Table 4. With only an exception for Cu, the contents of K, Na, Ca, Mg, Fe, Mn, and Zn more or less decreased in all tested tissues under LP stress. Cu content was not affected by either LP or LP + Si in leaves and roots of tomato plants. Meanwhile, compared with only LP treatment, exogenous Si treatment combined with LP improved accumulation of the K, Na, Ca, Mg, Fe, Mn, and Zn in leaf, stem. And root with a few exceptions. For example, leaf Zn content decreased in LP + Si treatment compared with LP. Moreover, exogenous Si did not cause significant increases in leaf K, Ca, and Mg content compared with only LP treatment. All these results clearly suggest that inadequate supply of P severely affects overall nutrient acquisition, whereas exogenous Si could improve nutrient homeostasis under LP stress.

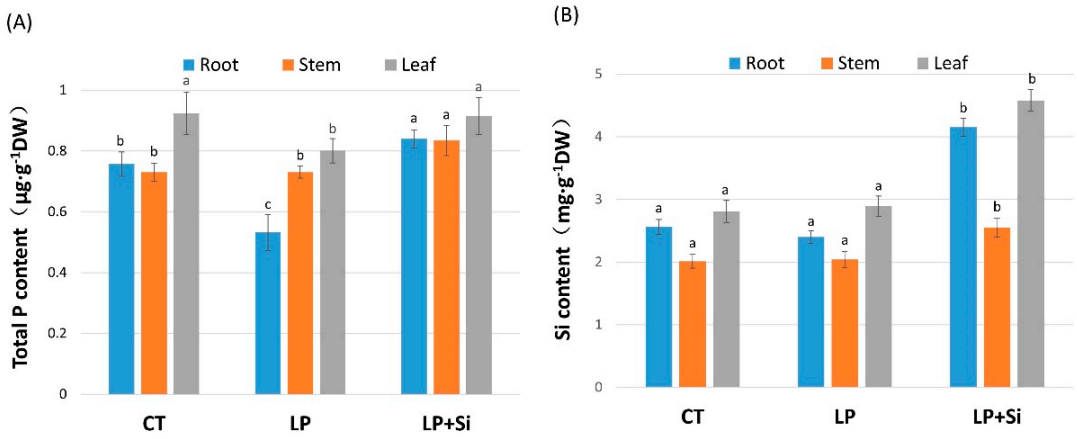

Figure 5. Accumulation of phosphorus and silicon in different tissues of tomato plants as influenced by low phosphorus (LP) and silicon (Si) supply: (**A**) Total P content and (**B**) Si content in tomato roots, stems, and leaves. Data are shown as means ± standard deviation (SD) of three replicates. Bars with the same color but different letters indicate statistically significant differences at $p \leq 0.05$.

Table 4. Effects of exogenous silicon on the concentrations of minerals in tomato under deficit phosphorus supply.

Element	Tissue	CT	LP	LP + Si
K content (g·kg^{-1} DW)	Root	36.0 ± 0.9a	29.8 ± 1.0c	32.9 ± 0.1b
	Stem	125 ± 6.4a	113 ± 1.8b	123 ± 3.4a
	Leaf	39.4 ± 0.5a	32.3 ± 1.2b	32.5 ± 1.8b
Na content (g·kg^{-1} DW)	Root	17.1 ± 0.5a	5.4 ± 0.3c	7.1 ± 1.3b
	Stem	11.6 ± 0.7a	3.7 ± 0.4c	7.6 ± 0.3b
	Leaf	2.1 ± 0.4a	1.4 ± 0.1b	2.2 ± 0.4a
Ca content (g·kg^{-1} DW)	Root	12.2 ± 2.3a	4.0 ± 0.1c	5.0 ± 0.7b
	Stem	11.1 ± 2.6a	3.5 ± 0.6b	14.0 ± 0.7a
	Leaf	15.8 ± 0.9a	11.7 ± 0.8b	12.8 ± 2.5ab
Mg content (g·kg^{-1} DW)	Root	5.9 ± 0.6a	4.4 ± 0.4c	5.1 ± 0.1b
	Stem	7.2 ± 1.3a	5.6 ± 1.1b	6.5 ± 0.1b
	Leaf	8.7 ± 0.9a	6.4 ± 0.3c	7.2 ± 0.3b
Fe content (mg·kg^{-1} DW)	Root	4562 ± 3571a	3171 ± 206b	4292 ± 355a
	Stem	632 ± 53.4a	231 ± 60.4b	584 ± 72.5a
	Leaf	459 ± 18.6a	381 ± 19.0b	434 ± 18.8a
Mn content (mg·kg^{-1} DW)	Root	936 ± 17.2a	615 ± 42.8b	862 ± 13.3a
	Stem	63.2 ± 2.7a	55.0 ± 3.0c	58.7 ± 2.1b
	Leaf	73.6 ± 2.5a	58.3 ± 3.9b	60.4 ± 1.6b
Zn content (mg·kg^{-1} DW)	Root	87.2 ± 1.4a	71.8 ± 6.4b	76.5 ± 3.3b
	Stem	34.5 ± 2.1a	21.3 ± 0.8b	32.8 ± 3.1a
	Leaf	21.9 ± 4.5a	15.5 ± 0.5b	13.3 ± 0.5c
Cu content (mg·kg^{-1} DW)	Root	87.9 ± 2.4a	79.9 ± 3.6a	82.6 ± 6.2a
	Stem	31.2 ± 7.5a	16.7 ± 1.8b	26.7 ± 7.4a
	Leaf	27.9 ± 3.0a	25.3 ± 0.01a	26.1 ± 2.9a

Data are shown as means ± SD, n = 3. Within each row (same tissue) followed by the same lowercase letters are not significantly different according to Tukey's test at $p \leq 0.05$. CT, control; LP, low phosphorus.

4. Discussion

Phosphorus is an essential macronutrient for plant growth and development, but its deficiency in most soils has been a major handicap for sustainable crop production worldwide [4]. In addition to the use of chemical fertilizers, a number of other approaches have been suggested to mitigate P deficiency-induced hazards in plants [2,3,5,37,38]. In particular, the applications of exogenous growth regulators/elements as a means for agronomic management have shown promising results in nutrient-deficit soils. Silicon (Si) is the second most abundant element in earth crust, which can be taken up by higher plants from the rhizospheric soil solution, mostly, in the form of silicic acid (H_4SiO_4) [12]. Over the last several decades, Si has been a focal point for plant scientists for its remarkable role in

regulating plant growth and stress responses [13]. However, the role of exogenous Si in photosynthesis, ROS metabolism, and nutrient homeostasis in tomato plants has been little understood. In the present study, we found that the application of exogenous Si not only induced Si uptake but also improved the total P content in different tissues (roots, stems, and leaves) of tomato plants under P-deficit (LP) conditions. Si uptake and increased P acquisition led to massive physiological changes as manifested by increased photosynthesis, decreased oxidative stress, and improved nutrient homeostasis under LP conditions (Figure 5; Table 2). Our results suggest that exogenous Si can mitigate LP-induced stress in dicotyledonous plants, such as tomatoes, and can be considered as an effective approach to manage such crops in P-limited soils.

Upon absorption by the plants, Si produces silica bodies that eventually confer tolerance to a range of environmental stressors [13]. It is believed that Si-induced tolerance to environmental stresses is mediated through the modulation of endogenous hormones that directly or indirectly regulate different physiological responses, such as photosynthesis [13]. For instance, Si application increases photosynthetic efficiency in rice, which directly contributes to the biomass accumulation as revealed by increased plant height and culm length, suggesting a profound effect of Si on yield components [13,39–41]. In the present study, LP stress decreased net photosynthetic rate, which was attributed to reduced stomatal conductance in tomato leaves (Table 2). Notably, P deficit conditions lead to breakdown of phospholipids in the chloroplast membranes to release Pi, which could be a potential reason for reduced photosynthetic rate [42]. In addition, enhanced carbohydrate (starch and sugars) accumulation in chloroplasts under Pi-depleted conditions may also result in attenuated photosynthetic activity [42,43]. However, exogenous Si application significantly increased photosynthetic pigment contents as well as net photosynthetic rate under LP stress compared with that under LP stress alone. Consistent with this, exogenous Si (K_2SiO_3) either applied on leaves or roots increases the expression levels of photosynthetic proteins, such as the PsaA (a core protein of photosystem I) and the PsbA (D1 protein of photosystem II) in strawberry, under high temperatures, which largely contribute to maintain photosynthesis under stressful conditions [17].

ROS production is a spontaneous and unavoidable incidence in plant cells [9]. However, excessive ROS accumulation is considered as a common stress marker under different environmental stresses as ROS can oxidize vital biomolecules, such as lipids, proteins, and DNA [36]. Oxidation of lipids, which can be measured by quantification of malondialdehyde (MDA) content, is a "hallmark" of oxidative stress. Generally, deficit macronutrient supply leads to oxidative stress through over production of ROS [1]. In our study, low P supply induced oxidative stress as revealed by increased MDA and ROS levels (Figure 1). Therefore, it is imperative to control ROS by modulating antioxidant defense, leading to reduced oxidative stress and increased nutrient use efficiency. Notably, enzymatic antioxidants play a pivotal role in mitigating oxidative stress induced by environmental stressors. Exogenous Si application can alleviate oxidative stress in a number of plant species, including tomatoes [44], cucumber [20], strawberry [17], rapeseed [15], and rice [16]. In agreement with this, we found that exogenous Si application remarkably alleviated LP-induced oxidative stress as evidenced by attenuated ROS accumulation and MDA content (Figures 1 and 2). This phenomenon was closely associated with the Si-induced enhancement in the activities of antioxidant enzymes, such as SOD, POD, and CAT (Table 3). In wheat, exogenous Si application confers tolerance to UV stress by regulating the activities of antioxidant enzymes, such as superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) [45]. Similarly, exogenous Si application (foliar or root treatment) increases the abundance of antioxidant proteins (SOD, CAT, and APX) which potentially alleviates oxidative stress and improves thermotolerance in strawberry plants [17]. All these results suggest that Si has the potential to stimulate antioxidant potential that can function to quench ROS in tomato plants.

Phosphorus is an essential macroelement that not only stimulates plant growth but also affects plant responses due to its direct involvement in energy production (ATP) and signaling cascades by mediating phosphorylation of various proteins [4,6,38]. Thus, P deficiency impairs nutrient homeostasis, leading to massive physiological and metabolic changes in plants [37,46]. Despite a large number

of studies in Si-mediated tolerance to abiotic stresses, the underlying mechanisms remain largely unknown [12]. In particular, how silicon promotes the uptake of some elements and restricts absorption and/or translocation of some other elements are little understood. For instance, Si significantly reduces uptake of Ca, but it increases accumulation of N and P in plants [13]. Consistent with this, we found that exogenous Si application not only increased Si uptake but also improved P content in tomato plants. In monocots, such as in rice and barley, low-silicon genes located along the Casparian strips mediate Si absorption and transport to the shoot via xylem [47]. In dicotyledonous plants, a Si uptake gene located in aquaporin mediates Si uptake through water [48], suggesting a potential mechanism of Si uptake in tomato plants. While deficit P supply decreased concentrations of different essential elements, such as K, Na, Ca, Mg, Fe, Mn, and Zn (Table 4), exogenous Si supply increased their concentration under low P conditions with a few exceptions. Among essential micronutrients, Fe is a key component of numerous proteins and enzymes and its deficiency impairs a number of metabolic processes in plants [19,20]. In cucumber, exogenous Si application alleviates Fe deficiency by regulating the expression of genes involved in Fe acquisition [20]. Similar sorts of mechanisms might also occur to mediate Si-induced Fe uptake in other strategy 1 plant species (all dicots and most monocots), including tomato plants. Although Si did not alter the content of leaf K, Ca, and Mn under low P supply, it did further reduce the content of leaf Zn in LP-stressed plants (Table 4). This implies that nutrient quality of vegetables in terms of Zn may not be compensated by exogenous Si application in P deficient soils.

Despite a large number of reports on the role of exogenous Si in relieving oxidative stress under abiotic stresses, such as drought, salinity, and high temperature, few reports have described the potential association between Si-induced amelioration of oxidative stress and improvement of macronutrient utilization efficiency [19]. In the present study, we showed that exogenous Si application not only alleviated LP-induced oxidative stress but also increased acquisition of most essential nutrients (Figure 6), leading to potential improvement in nutrient utilization efficiency under low P supply. Moreover, Si-induced improvements in plant growth, biomass accumulation, and root morphological traits were associated with the enhancement in photosynthesis, osmolyte accumulation, ROS scavenging, and antioxidant enzyme activity under LP stress. These findings may have practical importance for agronomic management of crops in P-deficient soils using Si as an amendment for sustainable crop production.

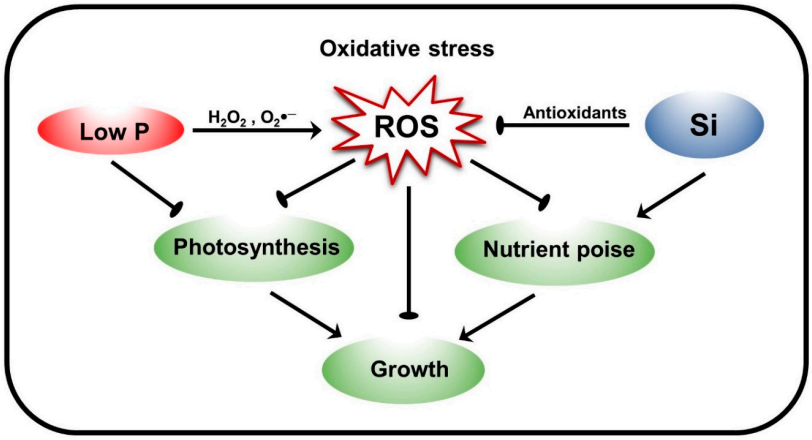

Figure 6. A working model showing potential mechanisms of Si-induced alleviation of low P stress in tomato: Low P stress induces oxidative stress by triggering reactive oxygen species (ROS) accumulation, leading to impairment in photosynthesis and nutrient homeostasis. Suppression in photosynthesis and nutrient acquisition due to deficient P supply results in growth inhibition; however, exogenous Si application reduces ROS accumulation and subsequent oxidative stress by enhancing antioxidant potential. Moreover, Si improves photosynthesis and essential nutrient acquisition, which eventually stimulate plant growth and biomass accumulation.

Supplementary Materials: The following is available online at http://www.mdpi.com/2073-4395/9/11/733/s1. Supplementary Figure S1. Effect of different concentrations of phosphorus (P) and silicon (Si) on lipid peroxidation measured as malondialdehyde (MDA) content in tomato leaves and roots. Supplementary Figure S2. Shoot phenotype of tomato plants as influenced by low phosphorus (LP) and silicon (Si) supply.

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