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The effect of silicon on the antioxidant system of tomato seedlings exposed to individual and combined nitrogen and water deficit

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Abstract

Exploring sustainable strategies for improving crop water and nitrogen use efficiency is essential. Silicon (Si) has been reported as a beneficial metalloid for plants since it alleviates several abiotic stresses (including drought) by triggering the plants´ antioxidant system. However, its role in mitigating the negative impact of nitrogen (N) deficit alone or when combined with water (W) deficit is not well studied. This study applied 0 or 2 mM of Na_2SiO_3 to 3-week-old tomato cv. Micro-Tom seedlings that were grown under the following conditions: control (CTR; 100%N+100% Field Capacity), N deficit (N;

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50% N + 100% Field Capacity), water deficit (W; 100% N + 50% Field Capacity) or combined stress (N+W; 50% N + 50% Field Capacity. The Si effect on tomato plant growth depended on the type of stress. Si could only alleviate stress caused by N+W deficit resulting in a higher root dry weight (by 28%), total dry weight (by 23%) and root length (by 37%). Alongside this, there was an increase in the antioxidant (AOX) system activity with the root activity of the studied AOX enzymes APX and CAT being enhanced by 48% and by 263%, respectively. Si application also enhanced AOX enzyme activity when tomato plants were subjected to individual deficits but to a lesser extent. In conclusion, Si-treated tomato plants could efficiently modulate their AOX networks in a situation of combined N and water limitation, thus mitigating some of the adverse effects of this combined stress.

Keywords

Abiotic stress, antioxidant system, cv Micro-Tom, phenolics, plant growth, silicon.

1 |Introduction

Considering the increasing demand for food and the need for more environmentally friendly agriculture (Jhariya et al., 2019), finding alternative and sustainable solutions to ensure efficient use of water and nitrogen (N) is a great priority, as these two resources have a huge impact on crop yield (Fernandes et al., 2022). Besides the direct consequences of climate change on water availability, this also brings an additional threat to food security as several studies have demonstrated that the continuous increase of the atmospheric CO_2 concentration negatively impacts the N status of most C3 plants, leading to lower yield and nutritional crop value (Gojon, 2017).

Numerous morphological, physiological, and metabolic changes are common in plants subjected to N or drought stress (reviewed by Machado et al., 2022). Commercial tomato cultivars (*Solanum lycopersicum* Mill.) are quite sensitive to these deficits at all stages of plant development (Du et al., 2018; Heuvelink, 2020) meaning that large amounts of fertilizers and irrigation must be used during their production cycle (Sandhu et al., 2021). Moreover, considering the increased consumption of fresh tomato and tomato-derived products and the economic importance of this crop worldwide (Cicco, 2019), it is of high importance to study its responses to these abiotic stresses.

A sustainable method that is receiving great attention in recent years, due to its role on alleviating the negative effects of several abiotic stresses, is the application of silicon (Si) (Bokor et al., 2021; Nunes da Silva et al., 2021). For instance, supplementation with Si has been adopted as an effective strategy for relieving drought stress and improving the drought resistance of several crops (recently reviewed by Thorne et al., 2020, Bokor et al., 2021, Malik et al., 2021, Wang et al., 2021, Verma et al., 2022). In contrast, knowledge about the role of Si in alleviating nutrient deficiencies is far scarcer, as pointed out by Ali et al. (2020) and Pavlovic et al. (2021). In recent studies, it was shown that the application of Si had a positive impact on N uptake, assimilation, utilization, and remobilization, which in turn can contribute to improving N utilization within plant tissues (Minden et al., 2020; Pavlovic et al., 2021). Nevertheless, most of this research was conducted on Si-accumulating species such as rice and wheat. Concerning Si non-accumulating species (Si excluders), such as tomato, which exhibit passive absorption and much less accumulation of Si (Liang et al., 2007; Hoffmann et al., 2020), the literature is limited.

One of the key mechanisms proposed by Liang et al. (2007) for the alleviation of abiotic stress in higher plants using Si has to do with the stimulation of the antioxidant

system (AOX). Previous reports demonstrated that, exogenously applied, Si can enhance the AOX system under drought stress, including in tomato plants, where a Si-mediated significant decrease of reactive oxygen species (ROS) (Shi et al., 2016) was observed. In the same study, the authors reported an increase in the activity of the AOX enzymes superoxide dismutase (SOD) and catalase (CAT) as well as in the concentration of the non-enzymatic antioxidant molecules ascorbic acid and glutathione. For wheat and maize, under drought stress, it has also been shown that Si decreases oxidative damage, ROS, phenolics concentration, and ascorbate peroxidase (APX) activity, but increases SOD and CAT (Gong et al., 2005; Xu et al., 2017; Parveen et al., 2019; Sattar et al., 2020). Peroxidase (POD) showed some variability in responses: while some authors found no significant differences (Gong et al., 2005), others noticed a decrease (Xu et al., 2017) or reported an increase in the activity of this enzyme (Parveen et al., 2019; Sattar et al., 2020). Concerning the effects of Si on crops grown at N deficit, most studies focus on the role of Si on N uptake and transport directly and less on the AOX system, which needs further investigation (Ali et al., 2020). Additionally, to the best of our knowledge, so far there are no studies on the effects of Si on combined N and water deficit, which is surprising given that often plants do not face only one individual abiotic stress, but rather a combination of them. This requires that more research explores the complex acclimation mechanisms, which cannot be predicted from the responses obtained when stressors are applied individually (Pandey et al., 2015; Hussain et al., 2018; García-Martí et al., 2019). Thus, we hypothesize that tomato responses to Si supplementation under combined N and W deficit cannot be directly predicted from the responses obtained for the individual stresses and that the AOX system plays an important role in the defence mechanism employed by tomato.

This study aimed to: (i) evaluate if Si supplementation can be used as a strategy to help tomato plants to cope with N deficit alone and/or when combined with water deficit; (ii) understand the impact of Si on the AOX responses (antioxidant activity, secondary metabolism responses, and enzymatic activity) in plants subjected to single or combined N and W deficit.

2 | Materials and methods

2.1 | Plant material and growth conditions

The experiment was carried out in a growth chamber under controlled environmental conditions (photoperiod: 16 h light; temperature: 25ºC day/ 23ºC night; photosynthetic photon flux density (PPFD): 150 µmol m^{-2} s^{-1}; relative humidity: 70%) according to a complete randomized design and using tomato plants cv. 'Micro-Tom'.

After surface sterilization with 70% (v/v) ethanol (10 min) and 20% (v/v) sodium hypochlorite (5% v/v active chloride) (6 min), and following multiple washes with deionized water, seeds were sown in potting soil and allowed to germinate. At the stage of third leaf appearance (approximately three weeks after sowing), seedlings were selected for uniformity and transplanted to single-plant pots (10 cm high, 10 cm diameter) filled with 0.1 to 1.5 mm-grade vermiculite (50 g). At this stage plants were divided into eight experimental groups, resulting from the combination of four levels of deficit conditions [control (CTR; 100% N requirements; 100% field capacity), N deficit (N; 50% N requirement), water deficit (W; 50% field capacity) and combined stress (N+W; 50% N requirement; 50% field capacity)] and two levels of Si supply [0mM or 2 mM of sodium silicate (Na_2SiO_3) solution (27% SiO_2 dissolved in 14% NaOH; Sigma). Immediately before transplanting, all pots were irrigated to 100% field capacity (FC) (determined

using the soil gravimetric water content method; Joshi et al., 2021), by adding ~230 mL of the nutrient solution supplied with 0 or 2 mM of Na_2SiO_3, depending on the treatment. Two types of nutrient solutions were used. A full nutrient solution was applied to the CTR and W deficit plants [10 mM NO_3^-; 0.5 mM NH_4^+; 1.9 mM $H_2PO_4^-$; 6.1 mM K+; 3.6 mM Ca^{2+}; 1.6 mM SO_4^{2-}; 2.5 mM Mg^{2+}; 2.6 mM Cl^-; 0.5 mM HCO_3- (pH 5.8 and electrical conductivity – EC – of 2.0 dSm^{-1})]. Plants subjected to single N deficit or combined N+W deficit were irrigated with a nutrient solution containing half the amount of N [5 mM NO_3^-; 0.3 mM NH_4^+; 1.9 mM $H_2PO_4^-$; 6.1 mM K+; 3.6 mM Ca^{2+}; 4.8 mM $SO_4$2-; 2.8 mM Mg^{2+}; 5.5 mM Cl^-; 0.5 mM HCO_3^- (pH 5.8 and EC of 2.1 dSm^{-1})]. Both nutrient solutions had the same composition in terms of micronutrients (35 μM Fe-EDDHA; 10 μM Mn^{2+}; 20.1 μM B^{3+}; 0.9 μM Cu^{2+}; 5.0 μM Zn^{2+}; 0.5 μM MoO_4^2). Following seedling transplanting, all the pots' surface was covered with black plastic to prevent evaporation.

During the experimental period each pot from the CTR and N treatment (with or without Si supplementation), was weighted and re-watered (with distilled water) on a daily basis, to maintain water content at 100% FC. In contrast, for plants under single or combined water deficit (W and N+W) with 0 or 2 mM of Na_2SiO_3, no additional irrigation was supplied, resulting in a progressive decrease of water content to 50% of FC (day 10) which was then kept at this point (with distilled water for an additional 17 days). At day 27 post-transplantation, all the plants were harvested and used for the analysis of morphological and physiological parameters (n=6). For the biochemical analysis, roots were carefully washed, and plants were quickly separated into roots and leaves being immediately frozen in liquid nitrogen and stored at -80ºC until analyses. For the analyses (n = 3), plant organs were ground and homogenized in liquid nitrogen

using a mortar and pestle. Each biological replicate resulted from a pool of three plants that were ground together.

2.2 | Biometric traits

At the end of the experimental period, plant height, as well as the maximum root length, were recorded. Total leaf number was obtained by counting leaves larger than 1 cm and total leaf area was measured using a LI-3100C area meter (LI-COR, Lincoln, Nebraska, USA). The dry weight (48 h at 105°C in a ventilated oven) of leaves, stems and roots were determined. Shoot-root ratio (S/R, ratio of the shoot to root dry weight; g g^{-1} DW) was calculated.

2.3 | Total antioxidant capacity and non-enzymatic components

2.3.1 | Preparation of the extracts

For the analysis of the total antioxidant activity (ABTS [2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)] free radical scavenging assay) and non-enzymatic components (total soluble polyphenols and flavonoids), 300 or 250 mg of freeze-dried samples of roots and leaves, respectively, were extracted with 1.2 or 1.5 mL of 80 % (v/v) methanol, respectively, under cold conditions. Samples were centrifuged at 15 000 ***g*** for 15 min and the resulting supernatant was used for all the analyses.

2.3.2 | ABTS Free Radical Scavenging Assay

ABTS [2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)] assay was performed following the protocol described by Gonçalves et al. (2009), with slight modifications for application using a 96-well microplate. Briefly, $ABTS^{\bullet+}$ solution was generated through a chemical oxidation reaction with potassium persulfate, and its concentration adjusted

with methanol to an initial absorbance of 0.700 (± 0.020) at 734 nm (Multiskan GO Microplate Spectrophotometer, Thermo FisherScientific Inc., Waltham, MA, USA). To 20 µL of the sample or standard solution, 180 µL of the adjusted ABTS•+ solution was added. The mixture was then incubated for 5 min at 30°C, the absorbance was measured at 734 nm and the results were obtained by calculating the reduction of percentage in sample absorbance (ASPL) with respect to the control (ACTL) using Equation 1. Trolox was used as a standard to prepare a calibration curve and the results were expressed as mmol Trolox equivalents/g DW. All reactions were performed in triplicate, and the radical stock solution was freshly prepared and filtered with a 0.45 µm syringe filter.

$$\text{ABTS scavenging (\%)} = (A_{CTL} - A_{SPL}) * \frac{100}{A_{CTL}} \quad (1)$$

2.3.3 | Total phenolics and flavonoids

Determination of total phenolic contents was performed using the Folin-Ciocalteu reagent following Ramos et al. (2019), with slight modifications. Briefly, 20 µL of extract or standard was mixed with 80 µL of Folin-Ciocalteu reagent previously diluted 1:10 (v/v), and 100 µL of 7.5 % (w/v) sodium carbonate was added to the mixture in a 96-well plate. After 60 min at room temperature and dark conditions, absorbance was measured at 750 nm. Results were expressed as mg gallic acid equivalents/g of fresh weight (mg GAE/g FW). Each reaction was performed in triplicate.

Total flavonoids were quantified through the aluminum chloride method adapted from Zafar et al. (2016). Briefly, an aliquot of each extract (40 µL) was mixed with 20 µL of 10% (w/v) aluminium chloride and 20 µL of 1 M potassium acetate solutions. Thereafter, distilled water was added to obtain a final volume of 200 µL in a 96-well plate. After 30 min of incubation in dark conditions and room temperature, the absorbance at 415 nm was measured by using a microplate reader. The calibration

curve was drawn by using quercetin as standard and the flavonoid concentration was established in μg quercetin equivalents/g FW (μg QE/g FW).

2.4 | Enzymatic assay

2.4.1 | Enzyme extraction and activity quantification: SOD, CAT, and APX

The extraction of the AOX enzymes was performed under cold conditions according to Fidalgo et al. (2011), using frozen samples of around 250 mg. After centrifugation, the supernatants were collected and used for both protein and enzyme quantification following the protocols described by Soares et al. (2019a). Briefly, protein quantification was performed as described by Bradford (1976), using bovine serum albumin as standard. Superoxide dismutase (SOD, EC.1.15.1.1) activity was quantified with a spectrophotometric assay based on the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) (Donahue et al., 1997). Absorbance was read at 560 nm and results were expressed as units of SOD/mg of protein. Catalase (CAT, EC.1.11.1.6) activity was determined spectrophotometrically by measuring CAT-mediated H_2O_2 degradation ($\varepsilon_{240\,nm} = 39.4\ mM^{-1}\ cm^{-1}$) (Aebi, 1984). Absorbance measurements were recorded every 5 s for 60 s, and results were expressed as mmol H_2O_2 min^{-1} mg of protein. Ascorbate peroxidase (APX, EC.1.11.1.11) activity was determined by monitoring the oxidation of ascorbic acid (AsA) for 1 min, with readings every 5 s ($\varepsilon_{290\,nm} = 0.49\ M^{-1}\ cm^{-1}$) (Nakano and Asada, 1981); results were expressed as mmol dehydroascorbate (DHA) min/mg of protein. The protocols for quantifying CAT and APX activity were adapted to UV-microplates following the adaptation from Soares et al. (2019a).

2.5 | Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). The effects of deficit level and silicon supply on plant growth parameters and AOX system were analysed with a two-way ANOVA, using deficit level, silicon supply and their interaction as fixed factors. Mean separation was based on Fisher's protected least significant difference (LSD) test at $p = 0.05$ using IBM SPSS Statistics 26 and GraphPad Prim 8.

3 | Results

3.1 | Plant growth

The deficit level significantly ($p < 0.001$) affected all plant growth parameters (Table SM1, Figures 1 and 2), but Si supplementation significantly ($p < 0.001$) increased root length at all deficit levels (21% CTR, 18% N, 30%W, 37% N+W). (Table SM1, Figure 2b). Deficit level and Si supplementation showed a significant interaction for RDW ($p = 0.002$) and TDW ($p = 0.021$). When Si was added to plants grown under W deficit a significant reduction of 15% occurred in both RDW and TDW. On the other hand, plants grown under N+W deficit and supplemented with Si significantly increased their RDW by 29% and their TDW by 23%.

3.2 | Total antioxidant activity (ABTS assay), non-enzymatic components: total phenolics and flavonoids concentration

Neither the deficit level, the silicon supplementation, nor their interaction significantly affected the roots or leaves total antioxidant activity (ABTS assay) ($p > 0.05$, Table SM2 and Figure 3a and b). However, phenolics concentration in roots was significantly affected either by the Si supplementation ($p = 0.024$) or the interaction between the

deficit level and the Si supply (p = 0.003) (Table SM2). When Si was added to CTR plants a significant increase of 83% was found (Figure 3c). A significant (p = 0.030) interaction between the deficit level and the Si supplementation was also found for the flavonoid's concentration in the leaves, which was decreased in plants grown in CTR conditions and supplemented with Si by 31% (Table SM2 and Figure 3f).

3.4 | Enzymatic components: SOD, CAT, APX

All enzyme's activity, both in roots and leaves, was significantly ($p < 0.001$) affected by the deficit level. Si supplementation alone only significantly affected CAT roots activity ($p < 0.001$) and APX and SOD activity in leaves (p = 0.003 and $p < 0.001$, respectively) (Table SM2).

Deficit level and Si supplementation showed a significant interaction for APX, both in roots and leaves ($p < 0.001$ and p = 0.003, respectively; Table SM2). While in CTR plants Si addition led to a 52% significantly lower activity, in plants grown under N or N+W deficit and supplemented with Si APX activity was actually increased by 98% or by 48%, respectively (Figure 4a). An interaction between both factors also occurred for the APX activity in the leaves (p = 0.003; Table SM2), being significantly higher (by 103%) in CTR plants grown with Si supplementation (Figure 4b). The activity of CAT in roots was also significantly increased by the interaction between deficit level and Si supplementation ($p < 0{,}0001$; Table SM2). The N+W plants showed the highest increase (by 263%), followed by N (by 176%) and W (by 24%) deficits (Figure 4c). Concerning SOD activity, only in leaves an interaction between both factors was found ($p < 0{,}0001$; Table SM2). An increase in CTR (by 18%) and in W deficit (by 14%) plants occurred in SOD activity when Si was supplied in the nutrient solution (Figure 4f)

4 | Discussion

To the best of our knowledge, this is the first study that compares the benefits of Si application on the AOX response of young tomato plants subjected to individual N or W deficit or combined N+W deficit. By studying a set of plant growth traits and AOX biochemical parameters, we showed that individual and combined N+W deficit hampered plant growth, particularly when no Si was added to the nutrient solution (Table SM1; Figures 1 and 2) and that tomato plants under N+W deficit were able to modulate their AOX network in the presence of Si (Table SM1; Figure 4).

It has been reported that Si plays an important role in plant functioning under stress conditions, yet under optimal conditions, its role is often reported as being minimal or non-existent (reviewed by Coskun et al., 2019). Accordingly, concerning plant growth, in our study, the addition of Si did not cause significant effects in CTR plants, except for root length which increased (Table SM1 and Figures 1 and 2). The positive effects of Si on root morphological traits, including root length was already observed for several crops and was recently reviewed by Tripathi et al. (2021). Oppositely, Si addition positively affected growth and biomass production in several plant species under environmental stress conditions (Coskun et al., 2019; Bokor et al., 2021). However, the literature is very scarce on the effects of Si supplementation in Si-excluder plants, such as tomato, subjected to N deficiency. In our study, Si addition to N-stressed plants did not significantly benefit any of the analyzed growth traits (Table SM1 and Figures 1 and 2). The same was previously reported by Haddad et al. (2018), who also found that for the Si-excluder *Brassica napus* under N starvation, the Si treatment did not affect plant biomass. Furthermore, Leal et al. (2021) came to the same conclusion for cotton plants subjected to N deficiency and supplemented with Si. On the other hand, the positive

effects of Si in mitigating the W deficit are widely reported (recently reviewed by Thorne et al., 2020; Bokor et al., 2021; Malik et al., 2021; Wang et al., 2021; Verma et al., 2022). However, in our work, Si addition to plants under W deficit alone did not show noticeable effects, except for RDW and TDW where a significant reduction was found, and for root length which was increased (Table SM1 and Figures 1 and 2). This difference in the results could be due to different experimental design conditions such as the form and concentration of silicon given to the plants, the type of substrate used, or even using different tomato genotypes. For example, Zhang et al. (2018) and Shi et al. (2016) used potassium silicate instead of sodium silicate to conduct their experiment while Shi et al. (2014) utilized sodium silicate but in a lower concentration of 0.5 mM. Furthermore, a study by Ali et al. (2018) demonstrated that tomato genotypes subjected to drought stress responded differently to the application of sodium silicate in a concentration of 1.5 mM, with the tolerant genotype having no beneficial effects on growth upon Si application. Finally, in most of the studies (Shi et al., 2014, 2016; Ali et al., 2018; Zhang et al., 2018), drought stress was imposed to tomato plants using polyethylene glycol and not through the decrease of watering *per se* which may also influence the results. Interestingly, although under individual deficit (N or W deficit) Si addition to the nutrient solution did not result in significant benefits, under combined N+W deficit it led to an improvement in growth traits such as increased RDW, TDW, and root length (Table SM1 and Figure 1 and 2). This, in turn, may be linked to the stimulation of the AOX system (Table SM2 and Figure 4) as already observed for other crops under abiotic stress (Shi et al., 2014; Bu et al., 2016; Kim et al., 2017; Geng et al., 2018).

It is known that under abiotic stress, plants have a complex AOX system for scavenging toxic ROS, which can be classified as non-enzymatic and enzymatic AOX

systems, that work *in tandem* to counteract the overproduction of these by-products (Soares, et al 2019b, Laxa et al., 2019). Si application has been reported as a sustainable way of increasing the tolerance of plants under several abiotic stresses by boosting plant defence responses, such as those of the AOX system (reviewed by Kim et al., 2017). In our work, the total AOX capacity (ABTS) and the non-enzymatic system (phenolics and flavonoids) did not significantly change after Si addition, except for phenolics content in the roots, which was increased, and for the flavonoid content in the leaves, which was decreased, in CTR plants (Figure 3). Increasing evidence shows that Si influences the concentration of phenolic compounds (Hajiboland et al., 2017) which is supported by our findings in CTR plants. Such increase has also been reported for unstressed strawberry plants (Hajiboland et al., 2018).

However, the enzymatic system responded both to the imposed deficit as well as to the Si supplementation, demonstrating a higher importance of the enzymatic part of the AOX system on the deficits studied (Table SM2 and Figure 4). In CTR plants supplemented with Si, APX was decreased in roots but increased in leaves, while CAT did not significantly differ in any plant organ, and SOD only increased in leaves (Figure 4). This suggests that Si impacted the tomato AOX response even in CTR conditions, as previously reported by Vega et al. (2019) after observing that Si application stimulated the AOX system of two healthy barley cultivars. Interestingly, although after Si addition, APX and CAT were increased in the roots of plants subjected to all the imposed deficits, it was in the N+W deficit where the Si application showed a higher impact (Figure 4a and 4c). Shi et al. (2016) observed that Si improved the growth and development of tomato plants under drought stress by improvement of root hydraulic conductance due to an increase of the AOX system response. In this study, the general improvement of the AOX system on the roots of combined stress tomato plants may therefore be the

reason why this group of plants responded better to the imposed stress. On the other hand, Si addition increased the activity of SOD in leaves of plants subjected to W deficit, but not in the other stress treatments, where no significant changes were observed (Figure 4c and f)Although Si was already shown to increase the activities of SOD, CAT, and APX in crops such as wheat, tomato, chickpea, rapeseed, sugarcane and sunflower under drought conditions (Gunes et al., 2007, 2008; Shi et al., 2016; Xu et al., 2017; Verma et al., 2019), we could not find literature reports about the effect of Si on the AOX system of plants subjected to individual N or combined N+W deficit. Our results suggest that Si differentially affects the response of the enzymatic system in plants under individual stress or in a combination of stresses, the latter occurring most often. While for individual W stress, Si seems to have stimulated the enzymatic machinery partly in roots and partly in the leaves, for combined N+W stress, and to a certain extent for N stress, it seems to have acted mostly at the root level, where a high increase was seen in the activity of APX and CAT enzymes. This greater impact of Si on the roots of plants under combined N+W stress may be due to the fact that both the N deficit and the W deficit are primarily perceived by roots (Bengough et al., 2011; Sun et al., 2020). On the other hand, since Si was also applied via the root, it may have led to a greater activation here, as a first line of defence, which could only later be extended to the leaf. Indeed, the significant role of Si on root development during stress has been previously noted (Hattori et al., 2005) and was also observed in our study (Figure 2b). The root growth stimulation by Si application may be the result of root elongation by increasing the extensibility of cell walls (Hattori et al., 2003).

5 | Conclusions

Root Si application on tomato seedlings can partly alleviate the stress caused by combined nitrogen and water deficit as demonstrated by the increase in growth traits such as RDW, and root length which ultimately contributed to their higher TDW. The better performance of plants in combined N+W deficit supplemented with Si seems to be related, at least in part, to the AOX system of the roots (where the stresses were first experienced), shown as increased activity of CAT and APX enzyme. Plants' response to combined stresses cannot be extrapolated from the responses observed for the individual deficits, because for plants subjected to either N or W deficit the impact of Si was not so evident. These results are highly relevant if we consider that: 1) plants often do not face only one individual abiotic stress, but rather a combination of them and 2) combined stress was the treatment where the highest decreases in growth traits were found, and therefore the treatment where the application of Si would bring more benefit. Comprehension of the full mechanisms involved in the complex interactions between these two abiotic stresses and Si application is still in its infancy. It is also not clear yet to what extent the present findings can be extrapolated to other crops and stress levels.

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Author contribution statement

SMPC, MWV, BB, MV and EH were responsible for the conception and design of the experimental work. JM performed seed germination, plant growth and sampling as well as morphological attributes evaluations. JM, and APGF were responsible for non-enzymatic and enzymatic components quantification. All authors contributed to data analysis and interpretation. JM wrote the manuscript, and all authors provided critical revision of the manuscript and approved the final version. SMPC, as JM grant supervisor, assumes the responsibility for the integrity of the present work, from inception to the finished article.

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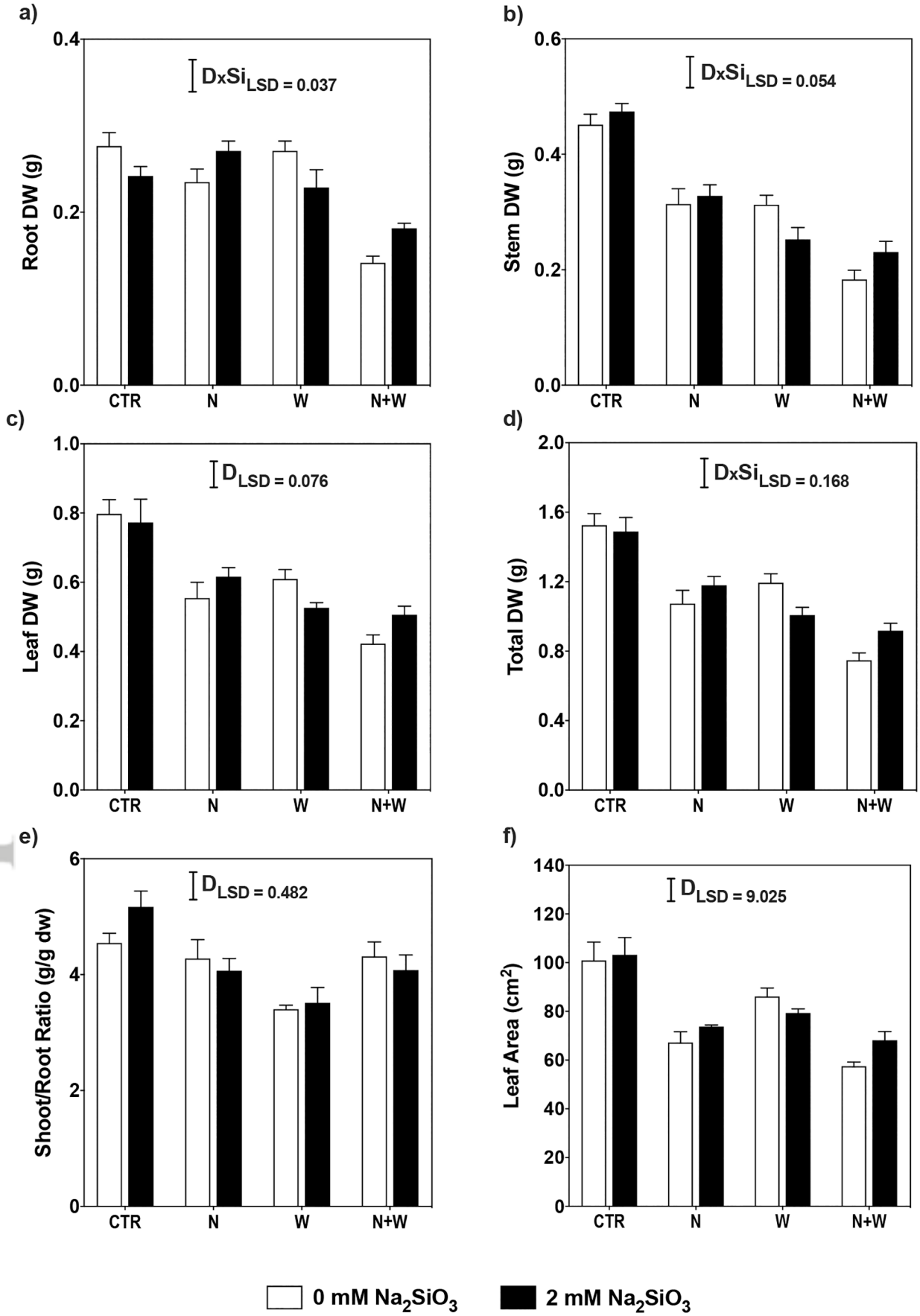

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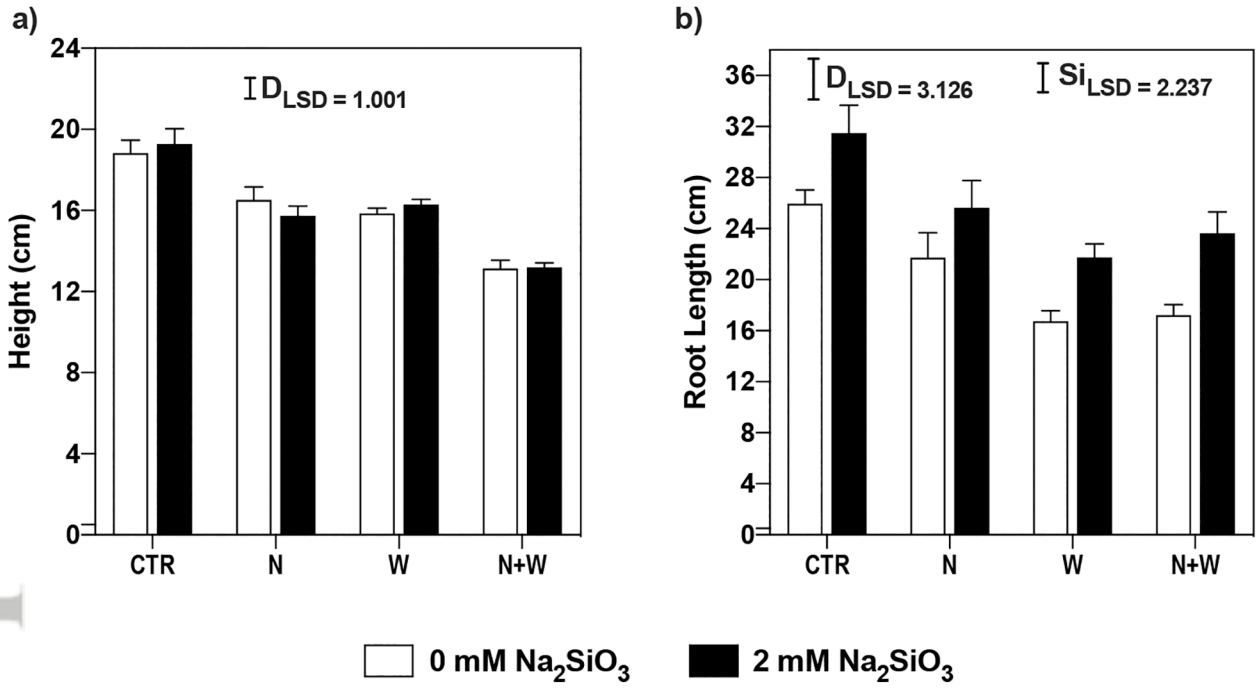

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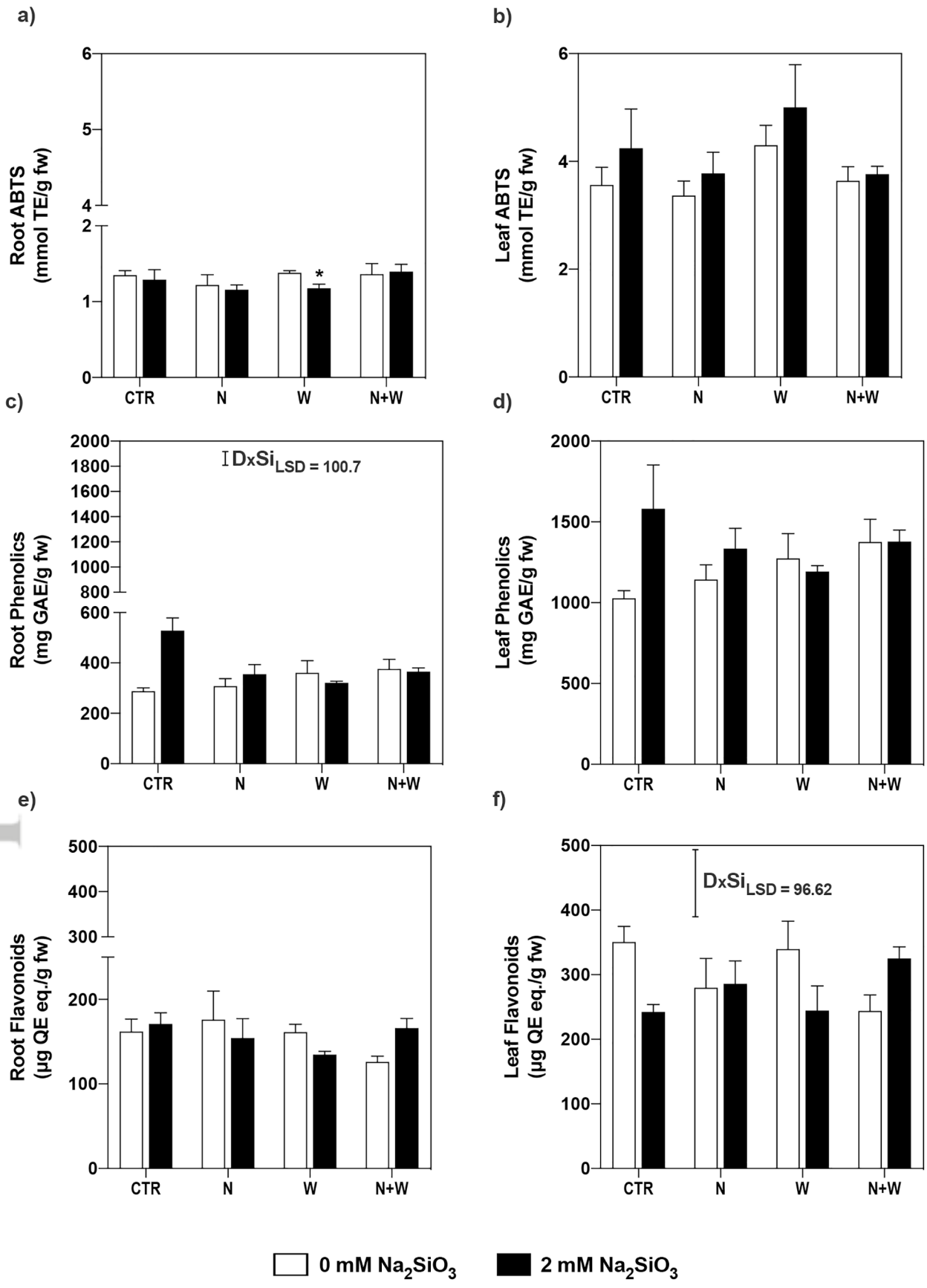

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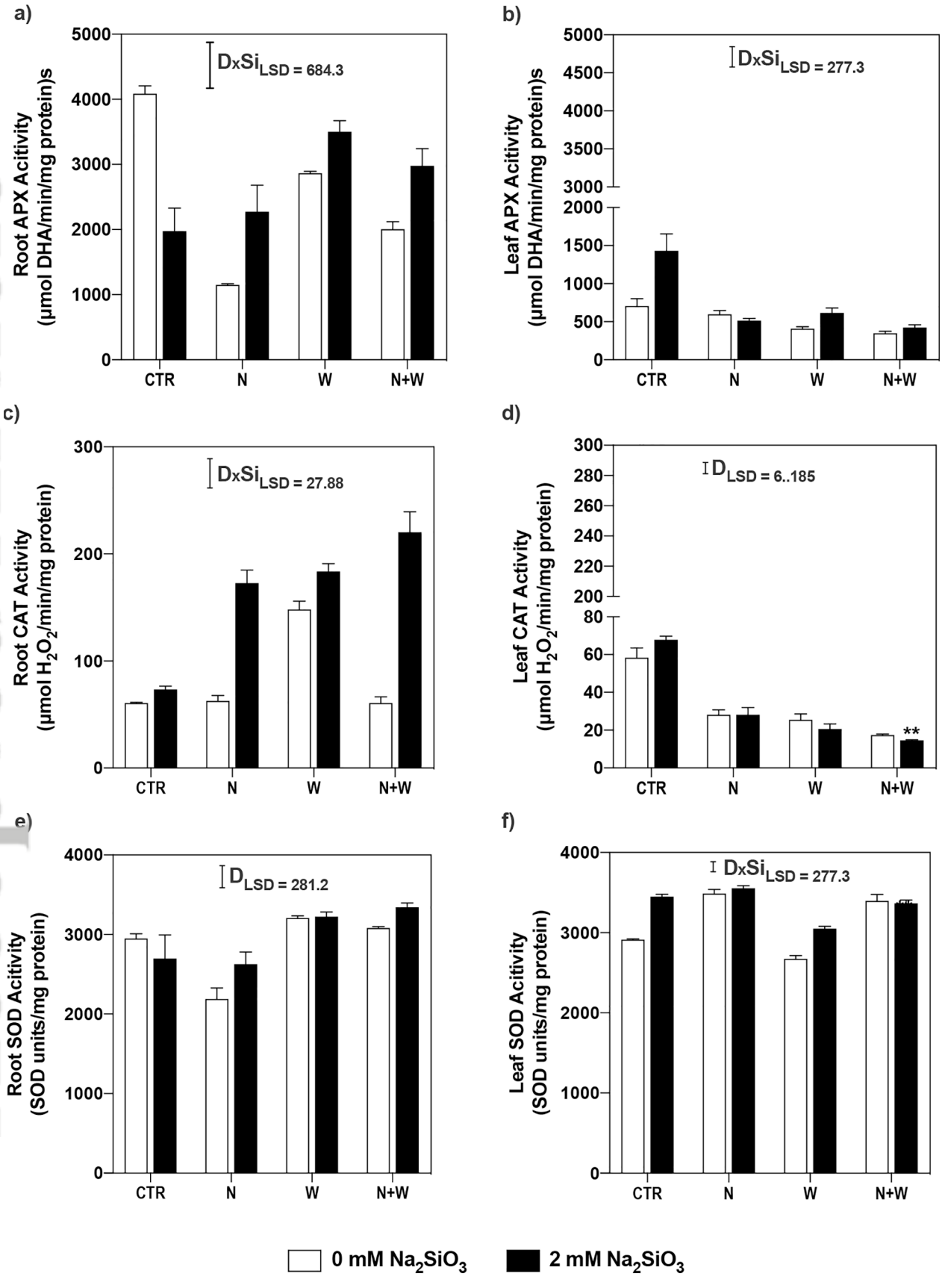

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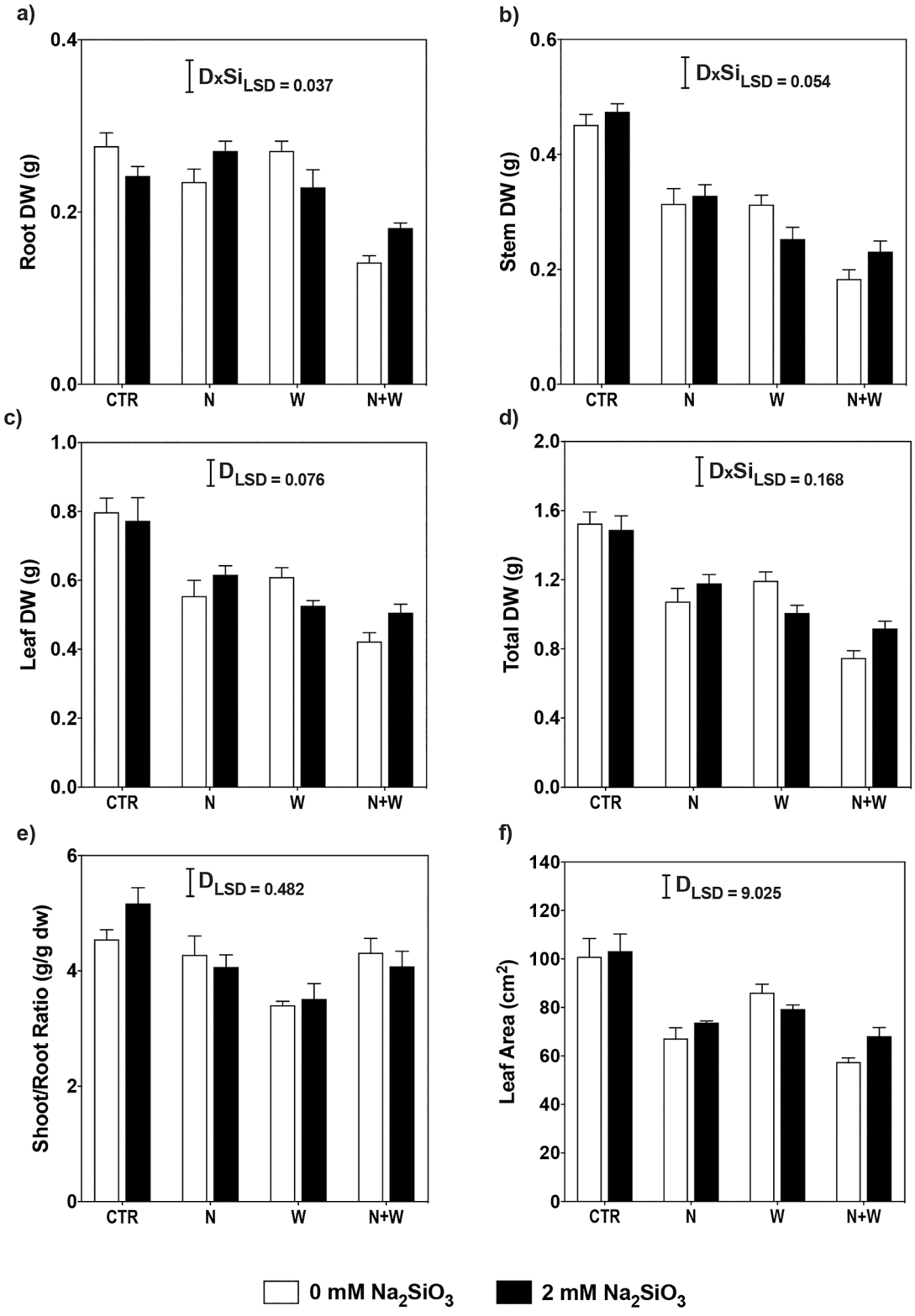

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Figure 1

Root dry weight (a), stem dry weight (b), leaf dry weight (c), total dry weight (d), shoot-to-root ratio (e) and leaf area (f) of tomato plants cv. 'Micro-Tom' grown for 27 days under four levels of deficit [control (CTR; 100% N + 100% W), nitrogen deficit (N; 50% N + 100%W), water deficit (W; 100% N + 50% W) and combined nitrogen and water deficit (N+W; 50% N + 50% W)] without (0 mM Na_2SiO_3) or with (2mM Na_2SiO_3) of silicon (Si) supplementation. Data presented are mean ± SEM (n = 6). Bars represent Fisher's protected LSD test (p = 0.05) for the significant interaction deficit level × silicon supplementation and for the independent effects (D, deficit level; Si, silicon supplementation) when the interaction was not statistically significant.

Figure 2

Plant height (a) and root length (b) of tomato plants cv. 'Micro-Tom' grown for 27 days under four levels of deficit [control (CTR; 100% N + 100% W), nitrogen deficit (N; 50% N + 100%W), water deficit (W; 100% N + 50% W) and combined nitrogen and water deficit (N+W; 50% N + 50% W)] without (0 mM Na_2SiO_3) or with (2mM Na_2SiO_3) of silicon (Si) supplementation. Data presented are mean ± SEM (n = 6). Bars represent Fisher's protected LSD test (p = 0.05) for the significant interaction deficit levels × silicon supplementation and for the independent effects (D, deficit level; Si, silicon supplementation) when the interaction was not statistically significant.

Figure 3

Antioxidant capacity measured by ABTS in roots (a) and leaves (b), total phenolics in roots (c) and leaves (d), and total flavonoids in roots (e) and leaves (f) of tomato plants cv. 'Micro-Tom' grown for 27 days under four levels of deficit [control (CTR; 100% N + 100% W), nitrogen deficit (N; 50% N + 100%W), water deficit (W; 100% N + 50% W) and combined nitrogen and water deficit (N+W; 50% N + 50% W)] without (0 mM Na_2SiO_3) or with (2mM Na_2SiO_3) of silicon (Si) supplementation. Data presented are mean ± SEM (n = 3). Bars represent Fisher's protected LSD test (p = 0.05) for the significant interaction deficit levels × silicon supplementation and for the independent effects (D, deficit level; Si, silicon supplementation) when the interaction was not statistically significant

Figure 4

APX activity in roots (a) and leaves (b), CAT activity in roots (c) and leaves (d), and SOD activity in roots (e) and leaves (f) of tomato plants cv. 'Micro-Tom' grown for 27 days under four levels of deficit [control (CTR; 100% N + 100% W), nitrogen deficit (N; 50% N + 100%W), water deficit (W; 100% N + 50% W) and combined nitrogen and water deficit (N+W; 50% N + 50% W)] without (0 mM Na_2SiO_3) or with (2mM Na_2SiO_3) of silicon (Si) supplementation. Data presented are mean ± SEM (n = 3). Bars represent Fisher's protected LSD test (p = 0.05) for the significant interaction deficit levels × silicon supplementation and for the independent effects (D, deficit level; Si, silicon supplementation) when the interaction was not statistically significant

Graphical Abstract

This paper shows that silicon mitigated some of the adverse effects of combined water and nitrogen deficit, increasing several traits related to plant growth. The combined deficit, silicon-treated plants could efficiently modulate their antioxidant system. Better performance seems to be related to the root´s antioxidant system.