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Titanium Dioxide Nanoparticles Increase Tissue Ti Concentration and Activate Antioxidants in *Solanum lycopersicum* L.

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Abstract

This work was aimed at characterizing the effects of foliarly applied rutile (TiO_2) nanoparticles (NPs) on Ti translocation as well as biomass production and antioxidant system in tomato (*Solanum lycopersicum* L.). The seeds were germinated and grown on a substrate in individual pots in a growth chamber. The TiO_2 NPs were characterized using transmission electron microscopy (TEM), Raman spectroscopy, dynamic light scattering (DLS), and laser doppler velocimetry (LDV). Titanium dioxide NPs had a rod-shaped form and were moderately prone to agglomeration. The TiO_2 NPs treatments were applied at 0, 5, 10, 20, 40, 80, and 160 mg L^{-1} by foliar spraying on 20-day-old *S. lycopersicum* plants. After 7 days of treatment exposure, tissue Ti concentration was determined by inductively coupled plasma-mass spectrometry (ICP-MS). Nanoparticle treatments increased tissue Ti concentration; Ti was translocated from leaves to stem, triggering a significant decrease in biomass production. With respect to the control, an increase in total reducing capacity (1.7-fold), antioxidant activity (1.5-fold), and superoxide dismutase activity (2-fold) were observed in the treatments with intermediate to high doses. The TiO_2 NPs triggered an increase in tissue Ti concentration, increasing the antioxidant system activity and lipid peroxidation at low to intermediate doses, and decreasing biomass production at intermediate to high doses.

Keywords Translocation · TiO_2 nanoparticles · Tomato · Oxidative stress · Total reducing capacity

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1 Introduction

The nanoparticles (NPs) may have natural or anthropogenic origin, ranging in size between 1 and 100 nm, and can be dispersed as individual particles in liquid, solid, or gaseous media (Schmid 1994; Ghosh and Pal 2007). In plants, metallic NPs are associated with either negative (inducing structural and/or functional damages), neutral, or positive responses (Hong et al. 2005; Da Costa and Sharma 2016; Sarmast and Salehi 2016; Tripathi et al. 2017; Tighe-Neira et al. 2018). Some metallic NPs causing negative responses in plants are Ag NPs in *Triticum aestivum* L. (Da Costa and Sharma 2016) and Cu NPs in *Oryza sativa* L. (Karimi and Mohsenzadeh 2017). In both species, a decrease in growth and biomass and an increase in lipid peroxidation was observed. In particular, Cu NPs had a strong negative impact on photosynthetic performance (Da Costa and Sharma 2016). In contrast, positive responses to TiO_2 were reported in *Ocimum basilicum* L. regarding photosynthetic parameters and biomass production depending on TiO_2 NPs rate and treatment duration (Tan et al. 2018). Similarly, TiO_2 NPs had a positive effect in *Brassica napus* L. (photosynthetic parameters, antioxidant system, and biomass production) (Li et al. 2015). Hence, there are contradictory reports on the TiO_2 NPs impacts on plants (Gogos et al. 2012; Zahra et al. 2017; Manesh et al. 2018). Indeed, the effects of NPs in plants may be influenced by multiple and complex interactions dependent on four factors: (i) the NPs properties (e.g., shape, size, surface, coating, crystal chemistry); (ii) plant species characteristics (genotype, phenological state, etc.); (iii) environmental conditions of plant growth (mainly soil/substrate properties and light intensity); and (iv) NPs application and absorption by roots (soil-grown, seed imbibition, and/or hydroponic systems) or foliage (Moaveni et al. 2011; Conway et al. 2015; Cox et al. 2017; Tan et al. 2017; Tripathi et al. 2017).

The exposure of plants to NPs via roots or leaves influences internal translocation and concentration of the relevant element in various plant tissues (e.g., Ti for TiO_2) (Silva et al. 2017). Upon TiO_2 application to the root medium, the Ti concentration in roots and shoots of *T. aestivum* was found to vary in a dose-dependent manner (Jiang et al. 2017). In contrast, there was no change in Ti concentration in *Hordeum vulgare* L. tissues using the same exposure pathway (Marchiol et al. 2016). In addition, Larue et al. (2012) reported difference in Ti concentration in plant tissues of *T. aestivum* and rapeseed at the same dose of TiO_2 applied to the root medium. In *Solanum lycopersicum* L., grown in sludge-amended soil containing TiO_2 NPs, the Ti concentration in stem, leaves, and fruit was similar and lower than the control in case of leaves (Bakshi et al. 2019). Regarding the impact of TiO_2 on growth, a decrease in root biomass of *T. aestivum* was found in the treatments with high doses of TiO_2 NPs, whereas shoot biomass was unchanged (Jiang et al. 2017). In contrast,

Tan et al. (2017) observed a reduction (more than 30%) in shoot biomass of *O. basilicum* treated with TiO_2 NPs compared to the control. Others studies reported no change in biomass production in species such as *Phaseolus vulgaris* L. and *T. aestivum* (Jacob et al. 2013; Larue et al. 2012).

The antioxidant system is used by plants to counteract possible toxic effects associated with application of TiO_2 NPs. Ultraviolet light exacerbates generation of ROS under TiO_2 NPs exposure (Kőrösi et al. 2019), differentially activating the enzymatic and non-enzymatic antioxidative mechanisms in plants (Silva et al. 2019). In general, the antioxidant system has been activated by TiO_2 in several crops, such as *O. sativa* (Wu et al. 2017), *T. aestivum* (Silva et al. 2017), *S. lycopersicum* (Tiwari et al. 2017), and *Vicia narbonensis* L. (Castiglione et al. 2014).

Solanum lycopersicum L. is an important edible crop grown worldwide (Tiwari et al. 2017). This species has been recognized as a terrestrial plant model for toxicity studies testing chemicals in the stages of seedling emergence and seedling growth (OECD 2003). In this context, *S. lycopersicum* has been used in the evaluation of toxicity, absorption, transport, and accumulation of TiO_2 NPs applied in doses ranging from 0 to 5000 mg L^{-1}. In addition, the TiO_2 effects have been evaluated on seed germination and on seedlings, including growth, biomass production, photosynthesis, water conductance, transpiration, and antioxidant system (Qi et al. 2013; Song et al. 2013; Raliya et al. 2015; Singh et al. 2016; Tiwari et al. 2017). Nevertheless, there is a paucity of information on Ti translocation and concentration in *S. lycopersicum* plants exposed to TiO_2 NPs (Raliya et al. 2015). In addition, no study could be found about foliar TiO_2 exposure and the interaction between Ti concentration in tissues with biomass production and antioxidant system activity in *S. lycopersicum* as the model species.

The foliar application of NPs is an interesting mode of exposure because of multiple potential entry pathways, e.g., through stomata, trichomes, cuticle, and hydathodes (Khan et al. 2019; Rodríguez-González et al. 2019). The working hypothesis was that the foliar-applied NPs would induce an increment in Ti accumulation and its translocation within the seedlings, as well as activate the antioxidant system and decrease biomass production. Therefore, this work was aimed at characterizing the effects of foliar-applied TiO_2 NPs on Ti absorption and translocation, biomass production, and the antioxidant system in *S. lycopersicum* L.

2 Materials and Methods

2.1 Physical Characterization of Nanoparticles

The TiO_2 NPs used in this work (catalog #637262, Sigma Aldrich Co., St. Louis, USA) had <100 nm, 99.5% purity,

specific surface area 50 m^2 g^{-1}, and 4.17 g mL^{-1} density at 25 °C. The characterization of these NPs was performed according to Nanogenotox (2011). In brief, TiO$_2$ NPs were dispersed in 0.5% v/v ethanol and subsequently suspended in 0.05% w/v filtered bovine serum albumin and dissolved in autoclaved Milli-Q® water. Then, they were sonicated at 10% of amplitude for 16 min. To corroborate TiO$_2$ nanoparticles were rutile, Raman spectroscopy was carried out on a WITec alpha 300 R Confocal Raman Microscope (WITec GmbH, Germany) using a laser at 785 nm. Transmission electron microscopy (TEM) (JEOL JEM-1400, Jeol LTD, Tokyo, Japan) was used to determine size and morphology of dried nanoparticles. The hydrodynamic size and Z-potential were determined by dynamic light scattering (DLS) and laser Doppler velocimetry (LDV) in a Malvern Zetasizer Nano-ZS ZEN3600 device (Malvern, UK) at 0, 24, and 48 h after sonication.

2.2 Plant Material and Growth Conditions

The assay was performed in the plant tissue culture laboratory at Universidad Católica de Temuco (38° 42′ 08.4″ S 72° 32′ 53.5″ W, and 149 m altitude).

Six *S. lycopersicum* seeds were germinated and then grown on a substrate (peat + perlite, volumetric ratio 2:1) in individual pots (500 mL) placed in a large box (3 L) for irrigation with distilled water by capillarity. *S. lycopersicum* was grown in a control-environment chamber under 200-μmol photons m^{-2} s^{-1}, 23 ± 1 °C, 50% relative humidity, and 16/8 h photoperiod for 20 days followed by treatment application and additional 7 days of growth.

2.3 Treatments

Titanium dioxide NPs used were rutile in the crystalline form; these NPs are considered thermodynamically more stable than anatase and with higher hardness (Muscat et al. 2002; Pacheco et al. 2014). These characteristics give some advantages in terms of less agglomeration, and a lower photocatalytic activity to protect the plant system from photodegradation (Gogos et al. 2012). In addition, there is a precedent of using rutile in *S. lycopersicum* research (Tiwari et al. 2017).

The NPs were suspended in ultrapure water and stirred for 30 min, followed by breaking up possible aggregates for further 30 min in a sonicator (Elmasonic VC300) at room temperature, just before application. The treatments were applied 20 days after sowing, and lasted for 7 days. The doses of TiO$_2$ NPs applied were 0, 5, 10, 20, 40, 80, and 160 mg L^{-1} (with ultrapure water serving as the control) by spraying 2.5 mL per plant foliarly to ensure the complete wetting. All the treatments were applied in the morning from 09:00 to 11:00 h, and the substrate was covered with aluminum foil to avoid contact with NPs.

2.4 Biomass Production

For the biomass measurement, after 7 days of exposure, plants were separated into leaves, stems, and roots, rinsed with abundant deionized water, blotted gently with absorbent paper, and weighed. Subsequently, they were dried in a forced-air oven to constant weight. Dry matter (DM) was calculated according to Balestri et al. (2014).

2.5 Samples for Analysis

The plants were harvested in the morning (from 09:00 to 11:00 h), pooled for each experimental unit, and rinsed three times with deionized water; subsequently, subsamples created for different assays were stored at −20 or −80 °C according to the established protocols for lipid peroxidation and other biochemical assays, respectively.

2.6 Titanium Concentration in Plant Tissues

Titanium concentration was determined according to Gao et al. (2013); to remove TiO$_2$ NPs from the leaf surface, the leaves were washed with tap water and distilled water. Subsequently, samples were dried at 100 °C for 72 h and digested in concentrated HNO$_3$ at 115 °C for 1 h. The measurement of Ti concentration was performed using ICP-MS (Thermo iCAP RQ model) at the Geosciences Institute of the Universidad Austral de Chile.

2.7 Lipid Peroxidation

Oxidative stress was determined according to the protocol described by Heath and Packer (1968), based on the reaction with thiobarbituric acid. For extraction, the samples stored at −20 °C for 1 week were used, and the absorbance was measured at 532, 600, and 440 nm in a UV-Vis spectrophotometer to correct for the interference generated by TBARS-sugar complexes. The peroxidation was expressed in nanomoles of malondialdehyde per gram of fresh weight (nmol MDA g^{-1} FW).

2.8 Antioxidant Activity

The radical scavenging was determined using methanolic extracts and the free 2.2-diphenyl-1-picrylhydrazyl (DPPH) method according to Chinnici et al. (2004). The samples frozen at −80 °C for 2 weeks were ground and homogenized in 80% v/v methanol solution, centrifuged at 13,000g at 4 °C for 5 min, and 200 μL of the supernatant was used for reaction with the DPPH reagent. The absorbance was measured at 515 nm using a spectrophotometer (Thermo Scientific Spectronic Genesys 10 UV-Vis Scanning, Madison, WI, USA) using Trolox as standard.

2.9 Total Reducing Capacity

The total reducing capacity was measured by the Folin-Ciocalteu method as described by Slinkard and Singleton (1977), with chlorogenic acid used as a standard. The extraction was performed as described above, and 10 μL of the supernatant was used for mixing with the reagent, followed by measurements at 765 nm using the UV-Vis spectrophotometer. The total reducing capacity was expressed in milligrams of chlorogenic acid equivalents per g of fresh weight (mg CAE g^{-1} FW).

2.10 Superoxide Dismutase Activity

The superoxide dismutase (SOD, EC 1.15.1.1) activity was measured in fresh leaves stored at −80 °C for 2 weeks. The SOD activity was determined according to Giannopolitis and Ries (1977) with minor modifications (Mora et al. 2009), based on the photochemical inhibition of nitroblue tetrazolium (NBT) reduction. The SOD values were standardized by the total protein content, which was determined according to Bradford (1976). One unit of SOD activity (U g^{-1}) was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT measured at 560 nm by the UV-Vis spectrophotometer.

2.11 Experimental Design and Statistical Analysis

The experiment was set up in a randomized complete block design with three replicates composed of three plants each. The data were tested using one-way ANOVA and Tukey test for the multiple comparisons. Correlation analysis was used to establish relationships between the variables. All statistical analyses were done using JMP Software 5.01®.

3 Results

3.1 Nanoparticle Characterization

In the Raman spectrum of TiO_2 NPs, the two characteristic peaks of rutile were observed at 446 and 608 Raman shift (cm^{-1}) (Fig. 1a). The TiO_2 NPs had a rod form, ranging in size from 30 to 60 (width) to 60–90 nm (length). The average width and length of TiO_2 rod-shaped nanoparticles were 52 nm and 77 nm, respectively (Fig. 1b and c).

The main hydrodynamic parameters of TiO_2 rod nanoparticles are summarized in Table 1. In general, TiO_2 nanoparticles showed a moderate stability (with some trend to agglomeration), given a hydrodynamic diameter of 236 nm and a Z-potential of −13 mV in an aqueous medium. In addition, PDI index was relatively low, indicating that particles showed homogeneous distribution in aqueous media.

3.2 TiO_2 NPs and Ti Translocation

TiO_2 NPs were applied to the leaves at different concentration. After 7 days of exposure, Ti concentration was increased in leaves and stem in the 160 mg L^{-1} treatment (Fig. 2). Significantly, higher Ti concentration was observed in leaves compared to stem and roots. With respect to the control, significant differences in Ti concentration ($p < 0.05$) for leaves and stem were observed at 160 mg L^{-1} dose. The Ti accumulation was not observed in roots regardless of the dose applied (Fig. 2).

3.3 Biomass Production

Dry matter was measured after 7 days of the treatment (Fig. 3). Compared to the control, in the root dry matter (DMr), a significant decline occurred in the treatments with 5 and especially 10 mg L^{-1}. Stem dry matter (DMs) was higher at 10 mg L^{-1} compared to the control, and was about 40% lower at 20 mg L^{-1} compared to the 10 mg L^{-1} treatment. Leaf dry matter (DMl) and total dry matter (DMt) showed a decreasing trend with the doses from 10 to 160 mg L^{-1}. The high values for DMl and DMt were observed in the 10 mg L^{-1} treatment, being 33% and 40% higher (respectively) than the low values measured at 20 mg L^{-1}.

3.4 Lipid Peroxidation and Antioxidant System

Lipid peroxidation was measured in leaves after 7 days of exposure (Fig. 4A). There was an increasing trend from control to higher doses. However, significantly higher lipid peroxidation was recorded only at 20 mg L^{-1} (Fig. 4A).

Regarding the enzymatic antioxidant system, the SOD activity showed an increase in the treatment with 20 mg L^{-1} (about 2-fold compared with the control) and a significant decrease between 20 and 160 mg L^{-1} (Fig. 4B). Similar trends were observed for antioxidant activity and total reducing capacity across the treatments (Fig. 4C and D). Compared with the control, the dose of 40 mg L^{-1} produced significantly higher antioxidant activity (1.5-fold) and total reducing capacity (1.7-fold). The dose of 80 mg L^{-1} showed a slight increase compared to the control.

4 Discussion

TiO_2 NPs used in this work had a rutile crystalline structure based on the two main peaks in the Raman spectrum near 446 and 610 (cm^{-1}), characteristic for rutile TiO_2 NPs (Choi et al. 2005; Moreno et al. 2019). These NPs had a moderate-to-high tendency to agglomerate, duplicating its initial size in suspension. These NPs were also characterized by García-Rodríguez et al. (2018), who observed similar properties using TEM,
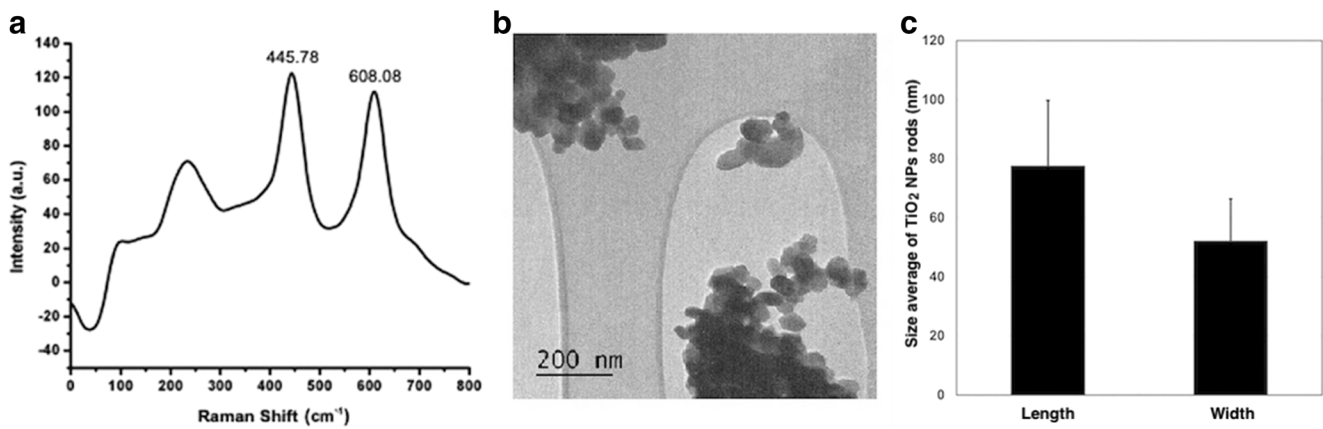
Fig. 1 Titanium dioxide nanoparticles. **a** Raman spectrometry. **b** TEM image indicating shape and size. **c** Size ranges

with rod sizes ranging from 70 to 40 nm, similar to our values 77.4 nm (length) and 52 nm (width) in suspension. The TiO_2 NPs agglomeration is an intrinsic property in aqueous media, where the hydrodynamic diameter of particles can be increased 50-fold (Jiang et al. 2009). This natural trend for NPs to agglomerate varies with the NPs type, its crystalline form, and the suspension medium (Reches et al. 2018).

In our work, the hydrodynamic size was a relevant parameter because the NPs TiO_2 were used in an aqueous suspension. The aggregation would influence absorption and Ti translocation inside the plant. This could be a reason why Ti was found in leaves at higher concentration than in stem and roots, especially at the high doses. Our data showed Ti concentration in *S. lycopersicum* exposed to 160 mg L^{-1} being around 3.7-fold higher in leaves and 2-fold higher in stem but only 1.08-fold higher in roots compared with the control. The presence of Ti in roots might have been an effect of Ti present in the substrate and not due to the foliar application of TiO_2 because root Ti concentration did not differ significantly among the treatments, except being slightly lower in the 40 mg L^{-1} treatment. In the other study, the tissue distribution of Ti applied at 1000 mg kg^{-1} as foliar spray to *S. lycopersicum* resulted in around 33-fold higher concentration in leaves, around 8-fold in roots, and around 5-fold higher in stem compared with the control (Raliya et al. 2015). Thus, we observed lower translocation in our work compared to that reported by Raliya et al. (2015), which can be explained by the lower maximum dose (160 mg L^{-1}) we used. In contrast, the distribution of Ti in *O. basilicum* plants was around 5-fold greater in roots and 1.1-fold greater in the shoot with respect to the control, using the dose of 750 mg kg^{-1} applied to soil (Tan et al. 2017). Likewise, *H. vulgare* seedlings showed Ti concentration in roots fluctuating from 35- to 412-fold with respect to the control and from 7.8- to 26-fold in the shoot at 1000 and 2000 mg L^{-1} of TiO_2 applied to seeds (Mattiello et al. 2015). These variations in Ti transport and concentration in plant tissues are related mainly to the dose and the mode of application, physicochemical properties of NPs, and the plant physiology (Tan et al. 2018; Verano-Braga et al. 2014). Moreover, the species-specific relationships between plant and NPs have been reported Gruyer et al. (2014).
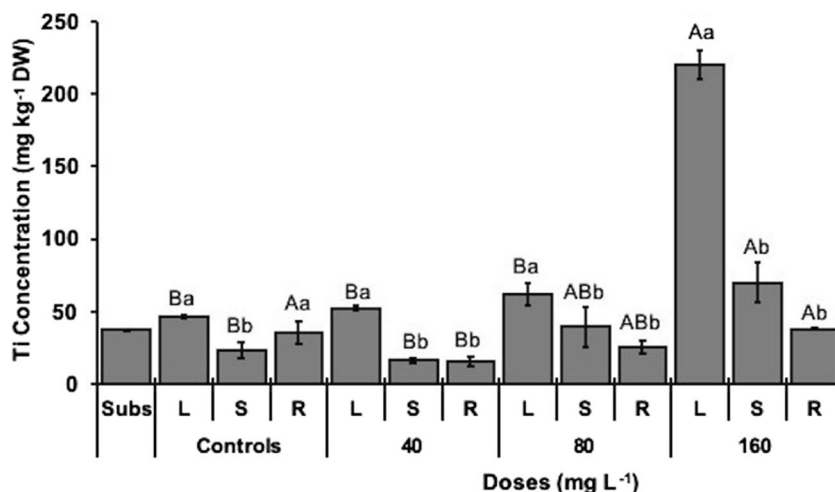
The transport and the availability of NPs within organisms are associated with biotransformation processes (Montes et al. 2012). In *S. lycopersicum* in the present study, although we did not evaluate biotransformation, this phenomenon could explain in part the TiO_2 immobilization in leaves and its poor Ti transport toward stem and roots. The only work that evaluated TiO_2 biotransformation in crop plants did not found any evidence for it in *Cucumis sativus* L., with TiO_2 staying in that form during its transport from roots to trichomes (Servin et al. 2012).

In our study, the high Ti content in the substrate (37.4 mg kg^{-1}) may have resulted in plant absorption of Ti, which would explain the small difference in Ti concentration in the specific organ among the treatments, and among plant organs for the same treatment, with such differences being accentuated only at the high foliar doses (e.g., between 80 and 160 mg L^{-1}). It is important to bear in mind that Ti content in the substrate we used, mainly in peat (mosses of the genus *Sphagnum*), probably was due to the natural absorption, given that Ti is the ninth most abundant element in the earth's crust, and the second most abundant transition metal (after Fe) (Buettner and Valentine 2012).

Table 1 Zetasizer measurements of TiO_2 rod-shaped nanoparticles in aqueous medium. The values are averages ($n = 3$) ± standard deviation (± SD)

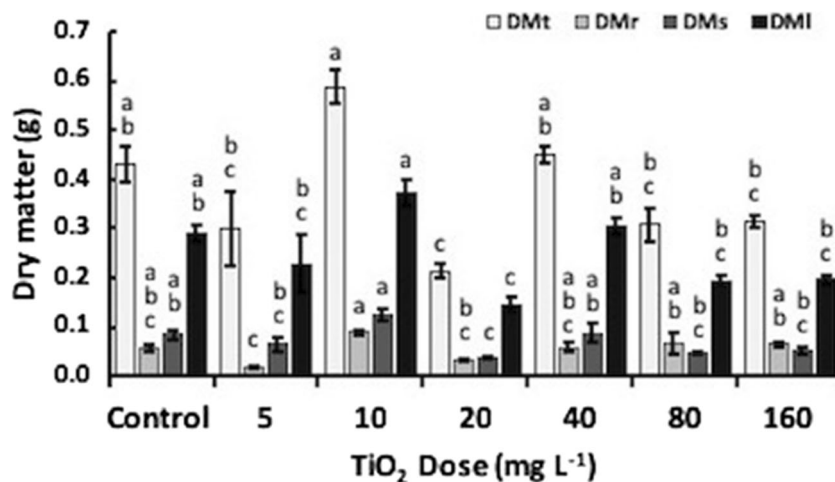| Z-average (d nm)	Polydispersity index (PDI)	Zeta potential (mV)	Mobility (μm cm V s^{-1})	Conductivity (mS cm^{-1})
236±1.01	0.219 ± 0.011	−13.15 ± 2.48	−1.03 ± 0.273	12.1 ± 0.057

In our work, the exposure to TiO$_2$ NPs for 7 days was sufficient to generate a significant decreasing trend in biomass of leaves and the whole plant, concomitant with an increase in the doses applied, mainly from 10 to 160 mg L^{-1}. This decrease was negatively correlated (Pearson's correlation) with Ti concentration in leaf biomass ($r = -0.71$; $p = 0.045$) and total biomass ($r = -0.62$; $p = 0.048$). Similar studies by Raliya et al. (2015) in the same species over the whole phenological cycle found the opposite results, with a biomass increment in the treatment with 250 mg kg^{-1} TiO$_2$ NPs. This opposite plant response may be associated with TiO$_2$ application to the root medium and/or the higher dose (56% greater than the highest dose used in our work) used by Raliya et al. (2015).
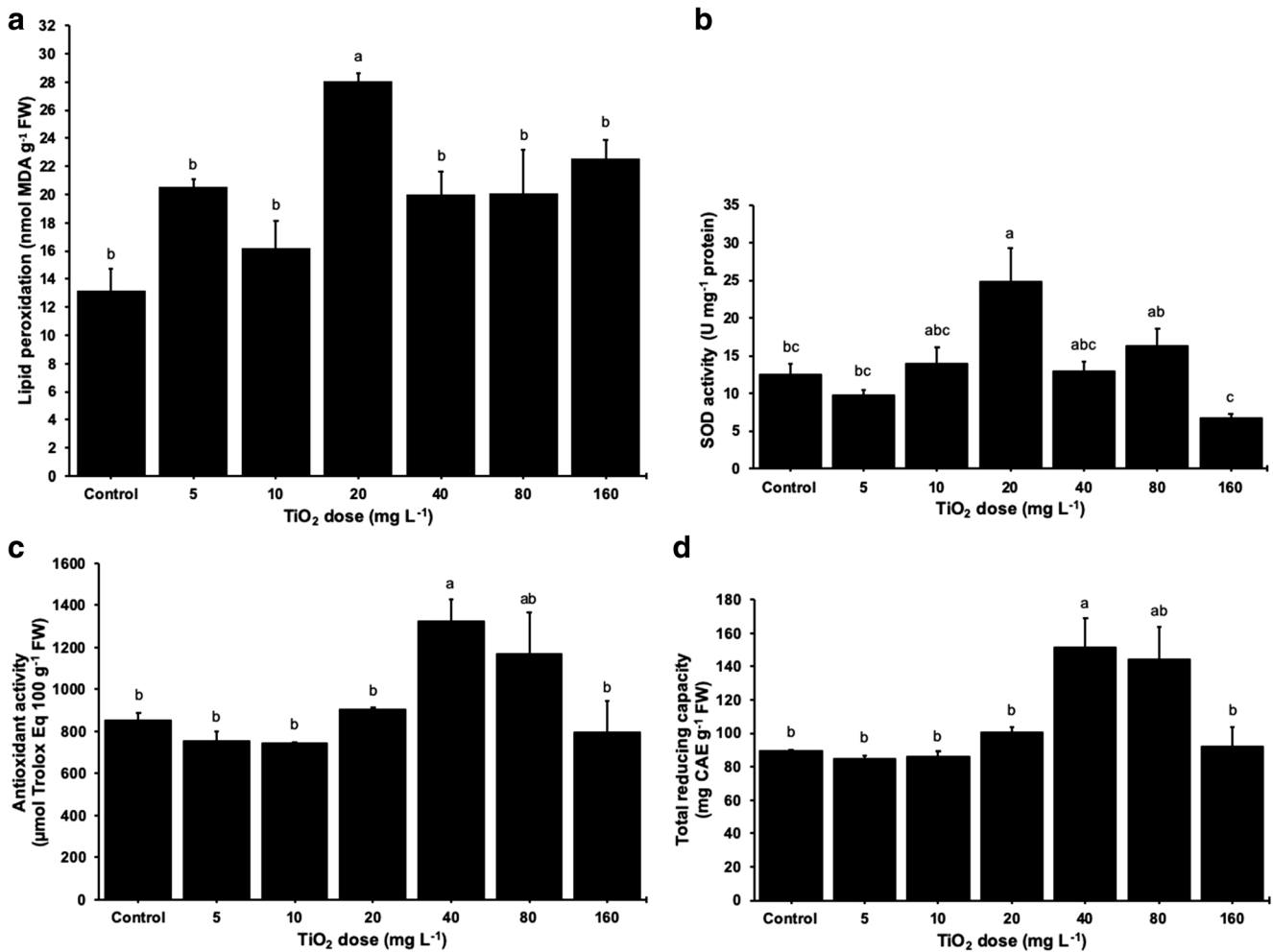
Activation of SOD was recorded at low dose (20 mg L^{-1}) (Fig. 4B), and high total reducing capacity (non-enzymatic mechanism) was noted at high doses (40 and 80 mg L^{-1}) (Fig. 4D). Only SOD was negatively correlated (Pearson's correlation) with leaf Ti concentration ($r = -0.70$; $p = 0.01$). An increase in antioxidant parameters is a common response in plants treated with TiO$_2$ NPs. For example, in

S. lycopersicum, an increase in SOD activity was observed in plants treated with 5000 mg kg^{-1} TiO$_2$ NPs (Song et al. 2013). Also, in *Vicia narbonensis*, TiO$_2$ NPs gradually and differentially activated non-enzymatic and enzymatic antioxidant responses (Castiglione et al. 2014), which is in accordance with our observations. Nevertheless, despite a rising trend of oxidative stress in leaves (Fig. 4A) with an increase in the TiO$_2$ NPs dose in our study, no significant correlation was observed. Little information exists in the literature regarding oxidative stress induced by TiO$_2$ NPs; however, some reports with application of bulk TiO$_2$ showed an increase in oxidative stress in *Vicia faba* L. (Castiglione et al. 2016). However, several reports in animal models have noted oxidative stress caused by TiO$_2$ NPs in specific tissues of rats (Liang et al. 2009; Chen et al. 2020), fish (Federici et al. 2007), mussels (Huang et al. 2018), etc.

In the present study, the integrated metabolic response of *S. lycopersicum* to the TiO$_2$ NPs treatments apparently were triggered at the exposure doses from 10 to 80 mg L^{-1}, with a rise at 20 mg L^{-1} and then a decrease at the highest dose. This response was potentially due to an unavoidable trend of NPs

Fig. 4 Antioxidant system in leaves of *S. lycopersicum* 7 days after TiO$_2$ foliar application at various doses. (**A**) lipid peroxidation, (**B**) superoxide dismutase activity, (**C**) antioxidant activity, and (**D**) total reducing capacity. Different lowercase letters indicate a significant difference ($p < 0.05$) among treatments by Tukey test ($p \leq 0.05$). Means ± SE, $n = 3$

agglomeration, which was greater at higher concentrations. However, it should be borne in mind that Degabriel et al. (2018) pointed out that the rod-shaped TiO$_2$ NPs (similar to those used in the present work) had a lower critical coagulation (aggregation) concentration than other crystalline shapes (such as spheroids).

5 Conclusions

The titanium dioxide nanoparticles used in this work were rod-shaped; applied foliarly, they generated an increase in leaf concentration of Ti, but also translocation from leaves to stem. After 7 days of plant exposure to TiO$_2$, there was a decrease in biomass production at intermediate and high doses; however, the activation of the antioxidant metabolism was present only at intermediate doses (20 mg L^{-1} regarding superoxide dismutase activity and at 40 mg L^{-1} for antioxidant activity) suggesting an increase of toxicity in this range. The moderate

trend of agglomeration of these nanoparticles could explain these observations partly. However, further studies are necessary to verify titanium dioxide translocation (by transmission electron microscope analysis) and its particle sizes inside plant tissue, as well as to evaluate the effects of long-term titanium dioxide exposure at higher doses.

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