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Zinc oxide nanoparticle-mediated changes in photosynthetic efficiency and antioxidant system of tomato plants

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Abstract

The present study was carried out to assess the role of zinc oxide nanoparticles (ZnO-NPs) in tomato plants on growth, photosynthetic efficiency, and antioxidant system. At 20-d stage of growth, roots of tomato plants were dipped into 0, 2, 4, 8, or 16 mg(ZnO-NPs) L^{-1} for 15, 30, and 45 min and then seedlings were transplanted in their respective cups and allowed to grow under natural environmental conditions. At 45-d stage of growth, the ZnO-NPs treatments significantly increased growth, photosynthetic efficiency together with activities of carbonic anhydrase and antioxidant systems in a concentration- and duration-dependent manner. Moreover, the treatment by 8 mg(ZnO-NPs) L^{-1} for 30 min proved to be the most effective and resulted in maximum activities of antioxidant enzymes, proline accumulation and the photosynthetic rate. We concluded that presence of ZnO-NPs improved the antioxidant systems and speeded up proline accumulation that could provide stability to plants and improved photosynthetic efficiency.

Additional key words: antioxidant enzyme; gas exchange; growth; micronutrient.

Introduction

Naturally occurring or engineered materials with at least one dimension and less than 100 nm in size are called nanomaterials. These nanomaterials are also characterized by a very high surface area to volume ratio contributing to their unique physio-chemical properties. It is estimated that more than 12,480 commercial products use nano-materials including biological systems (Poma and Di Giorgio 2008, Berube *et al*. 2010). Since such materials are being extensively used, their global production has also increased dramatically, making it immensely important to monitor a response of living systems to such material exposure. Limited reports are available dealing with the effect of nanomaterials on plants and related ecosystems (Bernhardt *et al*. 2010). Although plants and microbes are continuously exposed to naturally occurring nano-materials, exposure to engineered nanomaterials is relatively new and requires appropriate attention (Chinnamuthu and Boopathi 2009).

In the last decade, various researchers showed that nanomaterials affect plant growth and development and assessed its use in sustainable agriculture practices. Castiglione and Cremonini (2009) reported that effect of NPs can be beneficial or harmful to plants depending on the type of nanomaterials used and their mode of application. Studies have demonstrated the uptake of NPs by different plants led to their accumulation in subcellular locations (Wang *et al*. 2012, Schwab *et al*. 2016), to alterations of various physiological processes, and induced plant growth and development (Garcia-Sanchez 2015, Ge *et al*. 2012). Moreover, Nair *et al*. (2011) revealed that silica-NPs induced seed germination, whereas the treatment with cadmium-selenide quantum dots restricted the germination. Lin and Xing (2007) showed that the higher concentrations of nano-sized Zn (35 nm) and ZnO (20 nm) inhibited the germination in ryegrass and corn, respectively. In addition to this, Ma *et al*. (2010) reported

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Abbreviations: ASH-GSH cycles – glutathione-ascorbate cycle; CA – carbonic anhydrase; CAT – catalase; CeO_2-NPs – cerium oxide nanoparticles; C_i – intercellular CO_2 concentration; DAS – days after sowing; DDW – double distilled water; E – transpiration rate; g_s – stomatal conductance; LSD – least significant difference; NPs – nanoparticles; NR – nitrate reductase; P_N – net photosynthetic rate; POX – peroxidase; ROS – reactive oxygen species; SiO_2-NPs – silicon oxide nanoparticles; SOD – superoxide dismutase; SPAD – soil and plant analysis development; ZnO-NPs – zinc oxide nanoparticles.
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that at particular concentration of CeO_2-NPs did not induce any changes in root elongation, whereas La_2O_3, Gd_2O_3, and Yb_2O_3 affected the root growth. The inhibitory effects of these NPs were observed at different stages of growth. The global production of ZnO-NPs is ever increasing to its extensive use in various industrial products (Piccinno *et al*. 2010) making it one of the most produced NPs. These NPs end up in various habitats including soil (Keller *et al*. 2013). Hence, the effect of these NPs on plants and soil ecosystem should be studied. Therefore, the phytotoxic behaviour of the NPs needs to be addressed scientifically before utilizing them for agriculture practices. On the other hand, Oancea *et al*. (2009) believed that controlled release of active plant growth stimulators and other chemicals encapsulated in nanocomposites made of layered double hydroxides (anionic clays) could be feasible option for organic agriculture. Uptake of nanoparticles by plants, their translocation, and effect on plants were reported by various researchers (Dietz and Herth 2011). Moreover, positive effects of nanoparticles in various plants are shown in various species, such as peanut (Parasad *et al*. 2012), wheat (Ramesh *et al*. 2014), and cotton (Venkatachalam *et al*. 2017).

With the above cited reports regarding the NPs in general and ZnO-NPs in particular, the present study was designed to characterise the plant profile in terms of growth biomarkers, photosynthetic efficiency, and anti-oxidants capacity of tomato plants with different concentrations of ZnO-NPs applied through roots, which has not been reported earlier. Moreover, transplantation is a common practice for vegetables, including tomato. Therefore, dipping of roots in ZnO-NPs at the time of transplantation could be established as an effective mode of nanoparticles application for vegetables.

Materials and methods

Plant materials: The seeds of tomato (*Lycopersicon esculentum L.* cv. PKM-1) were procured from *National Seed Corporation Ltd.*, New Delhi, India. The seeds of healthy looking and uniform size were surface-sterilized with 1% sodium hypochlorite solution for 10 min, followed by repeated washing with double distilled water (DDW).

Source of nanoparticles: Zinc oxide nanoparticles (ZnO-NPs) were synthesized and characterized as described by Khan *et al.* (2016). Characterization refers to the study of material features, such as its composition, structure, and various properties (physical, electrical, magnetic, *etc*.) There is a number of techniques which are used in the process of characterization, such as separation, microscopy, spectroscopy, *etc*. (Fabrega *et al.* 2011).

Required quantity (2, 4, 8, or 16 mg L^{-1}) of ZnO-NPs was dissolved in 10 ml of DDW in a 100 ml volumetric flask and final volume was made up to the mark by using deionised water. Surfactant (*Tween-20*) was used prior to treatment. The roots were washed with 0.01% *Tween 20* for 1 min before dipping in NPs.

Treatment pattern and experimental design: The experiment was conducted under randomized block design with 75 plastic cups (350 mL in size). The sterilized seeds were sown in a plastic tray (28 × 40 × 16 cm) filled with an equal quantity of sandy loam soil mixed with farmyard manure in a ratio of 6:1. At 20 d after sowing (DAS), seedlings were transplanted to plastic cups (350 mL in size) filled with acid-washed sand allowed to germinate under natural environmental conditions in the net house of Department of Botany, Aligarh Muslim University, Aligarh, India. Cups (n = 75) were divided into 3 groups for 15, 30, and 45 min-treatment duration, whereas 5 sets in each group represented 100 ml of 0 (control), 2, 4, 8, or 16 mg(ZnO-NPs) L^{-1}, respectively, and each treatment was replicated five times. All the seedlings were transplanted in their cups and allowed to grow under natural environmental conditions with the supply of full nutrient solutions (Hewitt 1966) on alternate days. On 45 DAS, the plants in all the sets of each group were assessed for various growth and leaf gas-exchange traits as well as biochemical parameters.

Determination of growth biomarkers and leaf area: The growth biomarkers [shoot and root length, shoot and root fresh (FM) and dry mass (DM)] were determined by the method followed by Khan *et al*. (2015).

The leaf area was measured by using a portable leaf area meter (*ADC Bioscientific*, UK).

Determination of chlorophyll (SPAD value): The SPAD values of chlorophyll (Chl) in the leaves were measured under natural conditions by using the *SPAD* chlorophyll meter (*SPAD-502*; *Konica*, *Minolta Sensing, Inc*., Japan).

Determination of leaf gas-exchange traits: Photosynthetic traits were determined on the third fully expanded attached leaves before collection of leaf sample for other parameters between 11:00 and 12:00 h by using an infra-red gas analyzer (IRGA) portable photosynthetic system (*LI-COR 6400*, *LI-COR*, Lincoln, NE, USA). In order to measure the net photosynthetic rate (P_N) and its related attributes [stomatal conductance (g_s), intercellular CO_2 concentration (C_i), transpiration rate (E)], the air temperature, relative humidity, CO_2 concentration, and PPFD were maintained at 25°C, 85%, 600 μmol mol^{-1}, and 800 μmol mol^{-2} s^{-1}, respectively.

Biochemical analysis: Fresh leaves (1 g) were weighed and homogenized in a cold extraction buffer (70 mM phosphate buffer; pH 7.0, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.5% *Triton X-100*, and

2% polyvinylpyrrolidone (PVP) with the help of a precooled mortar and pestle. The homogenate was centrifuged at 12,000 × *g* for 20 min at 4°C and the supernatant was stored at –20°C. This supernatant was utilized for analysis of a protein content and activities of antioxidant enzymes catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD).

The total protein content of leaves was determined by the method followed by Bradford (1976). The Bradford reagent (2 ml) was added to 100 µl of supernatant and mixed gently and thoroughly. The samples were incubated at 25°C for 5–10 min and the absorbance at 595 nm measured by a spectrophotometer (*Spectronic 20D*, Milton Roy, Rochester, NY). A graph of absorbance *vs*. different known concentrations for standard solutions of bovine serum albumin (BSA) was plotted and a standard linear equation was derived. The amount of protein in the samples was calculated from the standard linear equation. The amount of protein was expressed as mg g^{-1}(FM).

The activity of carbonic anhydrase (CA, 4.2.1.1) in the leaves was measured following the method described by Dwivedi and Randhawa (1974). The leaf samples were cut into small pieces in cysteine hydrochloride solution. The leaf samples were blotted and transferred in a test tube, followed by the addition of phosphate buffer (pH 6.8), 0.2 M $NaHCO_3$, bromothymol blue, and the methyl red indicator, at the last. This reaction was titrated against 0.5 N HCl. The activity of enzyme was expressed on the basis of fresh mass in the form of mol(CO_2) g^{-1}(FM) s^{-1}. The activity of nitrate reductase (NR, 1.6.6.1) was measured following the method of Jaworski (1971). The fresh leaf samples were cut into small pieces and transferred to plastic vials, containing phosphate buffer (pH 7.5), KNO_3, and isopropanol, and incubated at 30°C for 2 h. After incubation, sulfanilamide and N-1-naphthyl-ethylenediamine hydrochloride solutions were added. The absorbance was read at 540 nm on a spectrophotometer (*Spectronic 20D*; Milton Roy, USA). The activity of enzyme was expressed on the basis of fresh mass in the form of nM(NO_2) g^{-1}(FM) s^{-1}.

The activities of various enzymes such as catalase (CAT, 1.11.1.6), peroxidase (POX, 1.11.1.7), superoxide dismutase (SOD, 1.15.1.1) and contents of proline were analysed as described in our previous study (Khan *et al*. 2015). The activity of enzyme was expressed on the basis of fresh mass in the form nM(H_2O_2) decomposed g^{-1}(FM) for CAT, U g^{-1}(FM) for POX, U g^{-1}(FM) for SOD and mg g^{-1}(FM) for protein.

Statistical analysis: Data were statistically analyzed using *SPSS*, *17.0* for *Windows* (*SPSS*, Chicago, IL, USA). Standard error was calculated and analysis of variance (*ANOVA*) was performed on the data with 5 replicates to determine the least significance difference (LSD) between treatment means with the level of significance at $p \leq 0.05$.

Results

Growth biomarkers: Plants grown with ZnO-NPs of the average size of 35 nm, irrespective of durations and treatments, showed a positive increase in growth biomarkers (length of shoot and root, FM and DM of shoots and roots, and leaf area) in comparison with control plants. Moreover, the maximum increase of growth parameters was reported in the plants with the roots exposed to 8 mg(ZnO-NPs) L^{-1} of for 30 min before transplantation; values for shoot length (35.8%), root length (28.6%), shoot FM and DM (21.9 and 27.6%, respectively), FM and DM of roots (19.9 and 27.7%, respectively), and leaf area (27.9%) were higher than their respective controls (Figs. 1, 2*A*). The pattern of growth parameters after 30-min root dipping followed for various concentrations an order of 8 > 16 > 4 > 2 > 0 mg(ZnO-NPs) L^{-1}.

Chl content (SPAD units): All the treatments under different durations of exposure showed an increase in the Chl content (SPAD) in the plants and their response was both concentration- and duration-dependent (Fig. 2*B*). Out of various treatments by ZnO-NPs and after 30-min exposure of roots before transplantation, the concentration of 8 mg(ZnO-NPs) L^{-1} showed a maximum value for the Chl content over all the other treatments and durations.

Leaf gas-exchange traits: The plants with the roots dipped for 30 min in ZnO-NPs (8 mg L^{-1}) before transplantation showed the highest values of P_N (50.7%), g_s (34.4%), C_i (27.9%), and E (32.0%) in comparison with their control plants. The different duration of root dipping showed varied responses, while the 30-min exposure proved to be the most effective with the concentration pattern of 8 > 16 > 4 > 2 > 0 mg L^{-1}.

Activities of CA and NR: Activities of CA and NR increased under different durations of ZnO-NPs treatment (Fig. 3*A,B*). The 30-min treatment duration proved to be the most effective together with 8 mg(Zn-NPs) L^{-1} and increased the CA activity by 38.5% and NR activity by 31.2% in comparison with their respective controls. The minimal effect was noted for the 2 mg(ZnO-NPs) L^{-1} treatment for 30 min over all the other treatments and duration of root dipping.

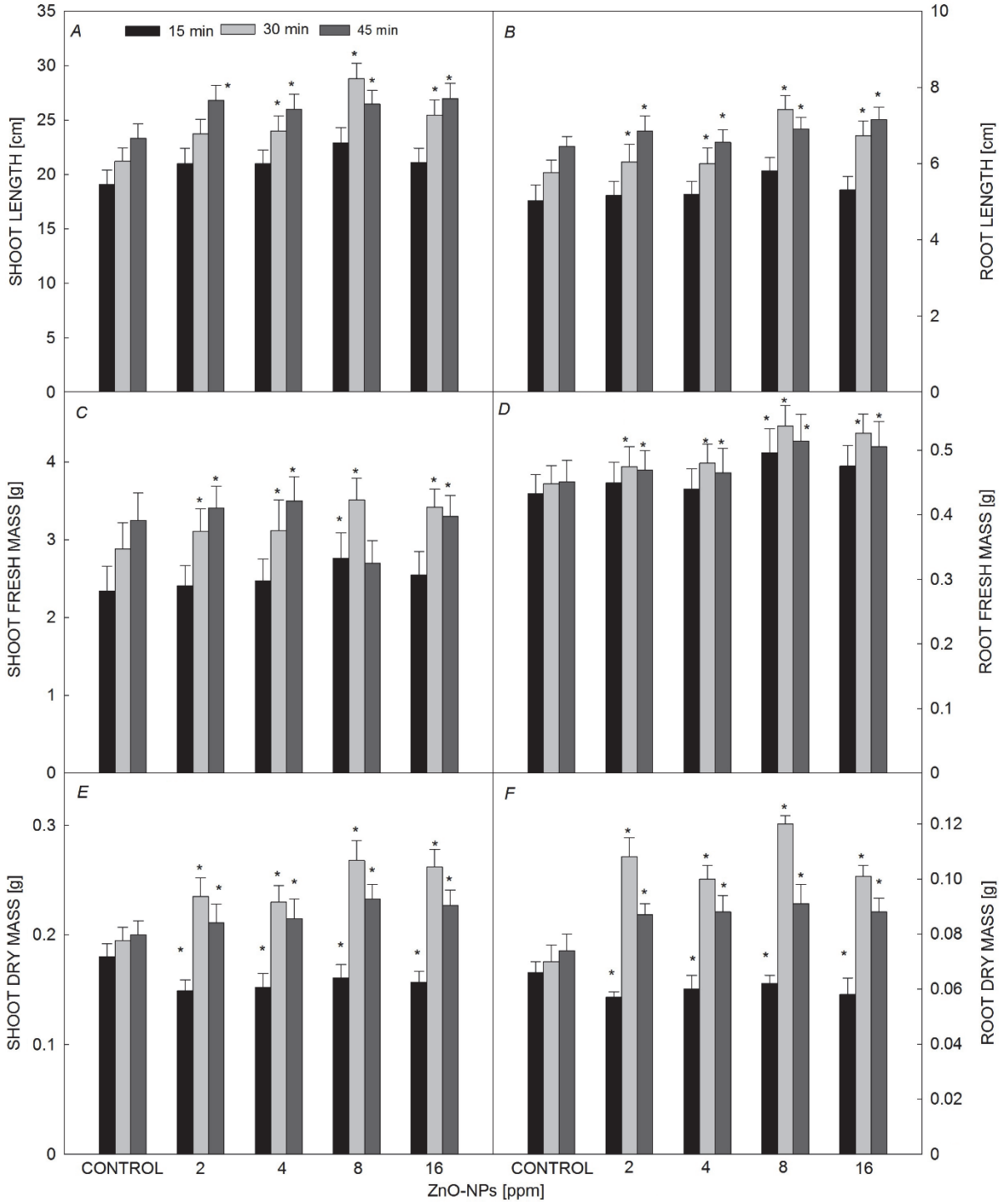

Fig. 1. Effect of ZnO-nanoparticles on the shoot (*A*) and root length (*B*), shoot (*C*) and root fresh mass (*D*), shoot and (*E*) root dry mass (*F*) of tomato plants at 45 DAS. All the data are the mean of five replicates (n = 5) and *vertical bars* show standard errors (± SE). * – significant difference between the control of different durations and their respective treatments ($p \leq 0.05$).

Protein content: Under various ZnO-NPs treatments, the treatment of the plants with ZnO-NPs (8 mg L^{-1}) for 30 min increased the content of proteins in the leaves by 45.0 %, compared with their control and other treatments (Fig. 4). Moreover, the increase of protein content depended on the durations and on ZnO-NPs concentrations. The pattern for the protein content in the plants treated for 30 min by root dipping in ZnO-NPs was in order of $8 > 16 > 4 > 2 > 0$ mg L^{-1}.

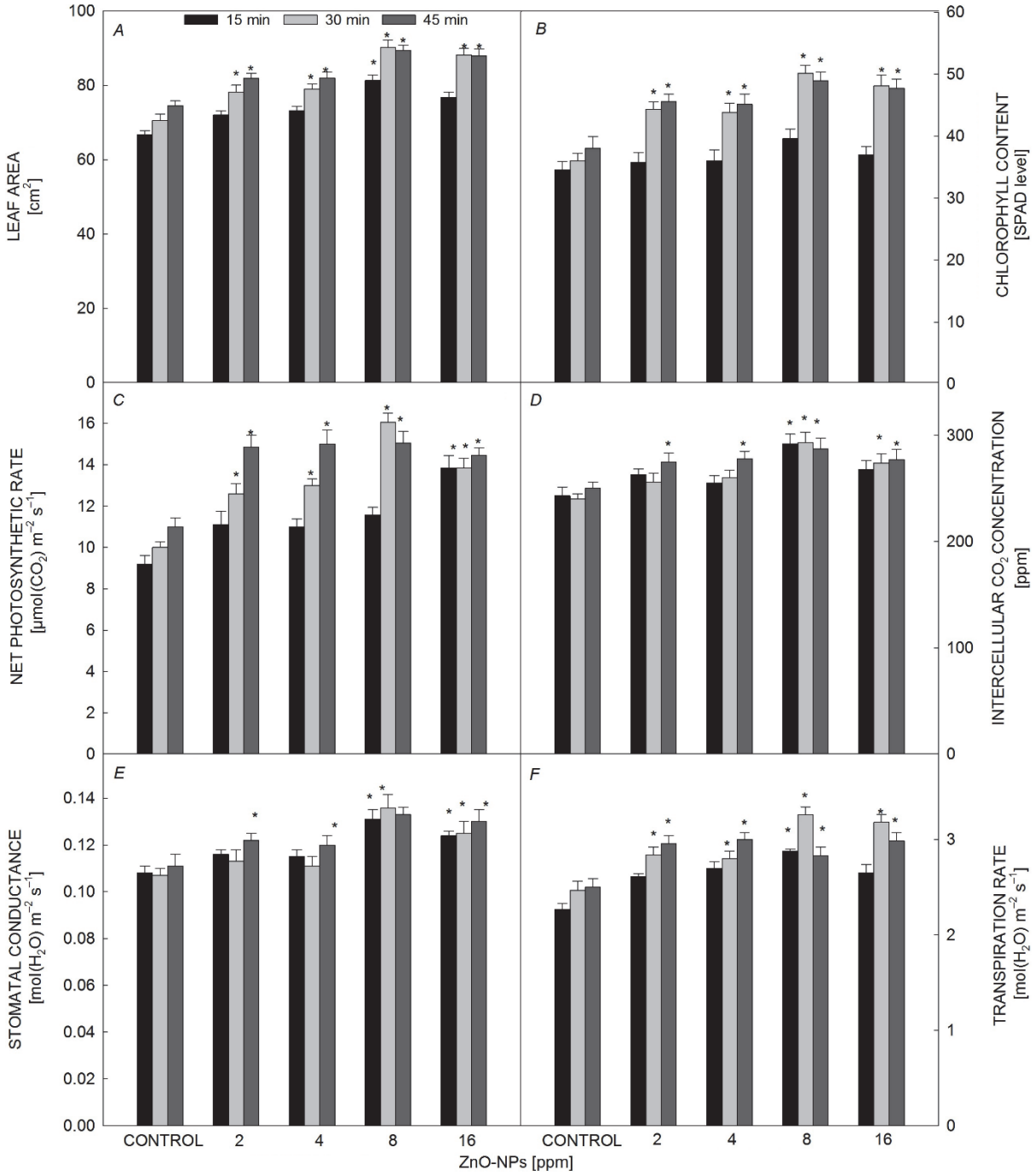

Fig. 2. Effect of nanoparticles (NPs) on the leaf area (*A*) chlorophyll content (*B*), net photosynthetic rate (*C*), internal CO_2 concentration (*D*), stomatal conductance (*E*), and transpiration rate (*F*) of tomato plants at 45 DAS. All the data are the mean of five replicates (n = 5) and *vertical bars* show standard errors (± SE). * – significant difference between the control of different durations and their respective treatments ($p \leq 0.05$).

Activity of antioxidant enzymes: Our results (Fig. 3*C*–*E*) clearly revealed a significant increase in the activity of antioxidative enzymes (CAT, POX, and SOD) after the treatment of ZnO-NPs under various durations of root dipping. Control plants possessed a minimum activity of these enzymes. The maximum activity of these enzymes was noted in the plants treated with 8 mg(ZnO-NPs) L^{-1} for 30 min. The activity of CAT increased by 69.7%, POX by 65.0%, and SOD by 80% at 45 DAS compared with the control plants.

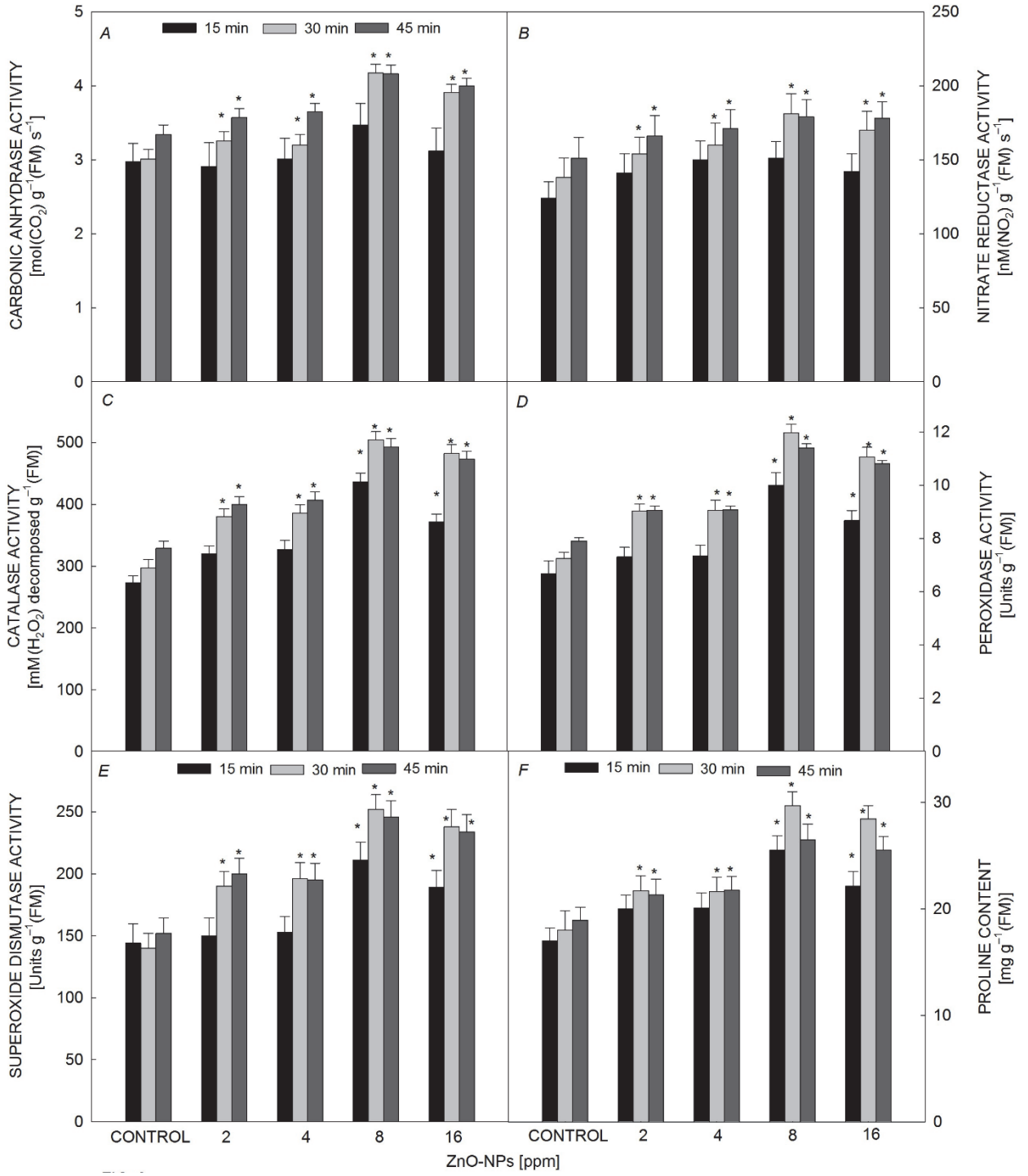

Fig. 3. Effect of nanoparticles (NPs) on the carbonic anhydrase activity (*A*), nitrate reductase activity (*B*), catalase activity (*C*), peroxidase activity (*D*), superoxide dismutase (*E*), and proline content (*F*) of tomato plants at 45 DAS. All the data are the mean of five replicates ($n = 5$) and *vertical bars* show standard errors (± SE). * – significant difference between the control of different durations and their respective treatments ($p \leq 0.05$).

Proline content: The proline content in the leaves of tomato plants increased by the root treatment with ZnO-NPs irrespective of its concentrations and durations. The roots of stock plants dipped in ZnO-NPs (8 mg L^{-1}) for 30 min possessed the highest proline content. This treatment increased the proline content by 65.0% over their control and other treatments. The pattern of proline accumulation in plants with different concentrations after 30-min root treatment was as follows: 8 > 16 > 4 > 2 > 0 mg(ZnO-NPs) L^{-1}.

Discussion

In the present study, treatments of various ZnO-NPs concentrations *via* roots significantly increased the growth biomarkers dependent on the concentration and also duration of the treatment. We believed that nanoparticles induced various morpho-physiological changes in root length, shoot length, root and shoot FM as well as DM, photosynthetic attributes, and biochemical parameters depending on its chemical composition, size, surface-contact, reactivity, and most significantly on the dose of nanoparticles (Khadakovskaya *et al*. 2012). Moreover, Zn plays a pivotal role in protecting and maintaining structural stability of cell membranes (Welch *et al*. 1982, Cakmak 2000). It is also used for protein synthesis, membrane function, cell elongation, and tolerance to environmental stresses (Cakmak 2000, Ajouri *et al*. 2004). In addition, Prasad *et al*. (2012) revealed that treatment of groundnut seeds with ZnO-NPs resulted in a significant increase in the germination and other growth biomarkers. Treatments by ZnO-NPs showed also significant increase in plant biomass, shoot and root growth, and root area in *Solanum lycopersicum* (Raliya *et al.* 2015). Seedling roots of *Vigna radiata* and *Cicer arietinum* absorbed ZnO-NPs and promoted their length of roots and shoots and its biomass (Mahajan *et al*. 2011). Release of zinc ions from ZnO-NPs has also been demonstrated (Fukui *et al*. 2012).

Researchers are trying hard to enhance the efficiency of crops by modulating their biochemical and physiological traits. In the present study, 30-min treatment by 8 mg(ZnO-NPs) L^{-1} through roots significantly increased P_N and its related attributes along with Chl and enhanced activity of CA in tomato plants. Govorov and Carmeli (2007) reported that metal NPs can induce the efficiency of chemical energy production in photosynthetic systems. Moreover, Noji *et al.* (2011) reported that nanosized silica compound bound to PSII induced stable activity of a photosynthetic oxygen-evolving reaction, indicating the light-driven electron transport from water to the quinone molecules, and they suggested that PSII conjugate might have properties to develop photosensors and artificial photosynthetic system. SiO_2-NPs improves photosynthetic rate by improving activity of CA and synthesis of photosynthetic pigments (Siddiqui *et al.* 2014, Xie *et al*. 2012). Govorov and Carmeli (2007) showed that metal NPs induced the efficiency of chemical energy production in photosynthetic systems. The cumulative effect of all these modified processes might improve the photosynthetic machinery in the plants exposed to ZnO-NPs (8 mg L^{-1}). These observations are in line with the earlier findings of An *et al.* (2008) who demonstrated that NPs increased the ascorbate and Chl contents in the leaves of *Asparagus*.

In plants exposed to any external stimuli, such as some environmental factors, phytohormones, *etc*., metal-based NPs induced uncontrolled production of reactive oxygen species (ROS) at different sites of plants. In order to

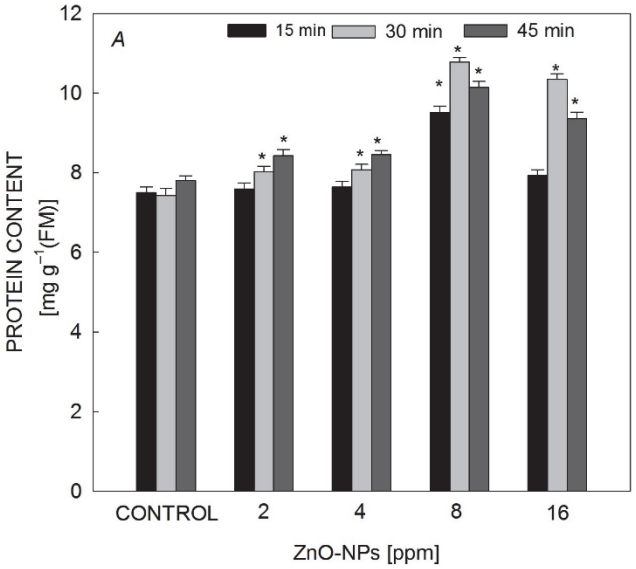

Fig. 4. Effect of nanoparticles (NPs) on the protein content of tomato plants at 45 DAS. All the data are the mean of five replicates (n = 5) and *vertical bars* show standard errors (± SE). *Asterisks above the bar* indicate a significant difference between the control of different durations and their respective treatments ($p \leq 0.05$).

counter this uncontrolled production of ROS, plants cells and organelles evolved defence system, *i.e*., antioxidant systems (Gill and Tuteja 2010). SOD is an effective enzymatic antioxidant in all aerobic organisms prone to ROS-mediated oxidative stress. CAT is the enzyme with potential to dismutate directly H_2O_2 into H_2O and O_2 and it is crucial for ROS detoxification during unfavourable conditions (Garg and Manchanda 2009). Peroxidase play a pivotal role in protecting cells of higher plants by scavenging H_2O_2 in water-water and glutathione-ascorbate cycles (Gill and Tuteja 2010). Moreover, in the present study, the treatment of roots with ZnO-NPs significantly enhanced the antioxidant enzymes (CAT, POX, and SOD; Fig. 3*C,D,E*). It is well documented that Zn plays a critical role in stabilizing the stability of biomembranes and proteins by balancing the scavenging ROS production (Khan *et al.* 1998). Treatment of Au-NPs improved the antioxidant system in *Arabidopsis thaliana* and modified the levels of micro RNAs (miRNA) expression that regulates various metabolic processes in plants (Christou *et al.* 1988). Moreover, Lei *et al.* (2007) reported that nanosized TiO_2 improved antioxidant systems under abiotic stress by declining the accumulation of H_2O_2, malondialdehyde content, and increasing activities of SOD, CAT, ascorbate peroxidase, and guaiacol peroxidase in spinach plants. These findings strengthened our finding that exposure of tomato roots to the nanoparticle ZnO improved the enzymatic antioxidant system (CAT, POX, and SOD; Figs. 3*C–E*) to scavenge the excessive ROS. It

has been also reported SiO_2-NPs improved the seed germination in tomato and also enhanced the antioxidant system under stress conditions (Haghighi *et al.* 2012, Siddiqui *et al.* 2014). Salama (2012) revealed that treatment of NPs increased growth biomarkers in *Brassica juncea* plants with the increase of biochemical traits (Chl, carbohydrate, protein content, and antioxidant enzymes). Our study also showed similar findings in terms of the increased protein content and growth biomarkers (Fig. 1) when tomato plants were exposed to ZnO-NPs.

Proline acts as an nonenzymatic antioxidant that has ability to stabilize the subcellular structures, such as that of proteins and cell membranes, scavenging free radicals and buffering redox potential under stress conditions; it also has the ability of molecular chaperones that protect the integrity of proteins and enhances the activity of different enzymes, such as protection of nitrate reductase under abiotic stress conditions (Szabados and Savoure 2009). Moreover, among various compatible solutes, proline is the only molecule that has been shown to protect plants against singlet oxygen and free radical-induced damages resulting from excess ROS (Alia and Mohanty 1997). These reports support the present observations, where the treatment of ZnO-NPs enhanced the accumulation of proline (Fig. 3*F*). It has been reported that ZnO-NPs supplemented with MS media in banana induced proline synthesis, activity of SOD, CAT, and POX and improved a tolerance to biotic stress (Helaly *et al.* 2014). SiO_2-NPs also increased proline accumulation along with Chl content in basil (*Ocimum basilicum*) (Kalteh *et al.* 2014, Siddiqui *et al.* 2014). Therefore, it can be suggested that elevated proline content induced by ZnO-NPs may play a significant role in growth and development of plants. However, the mechanism and the reasons for proline accumulation and enhanced antioxidant systems in plants exposed to ZnO-NPs have not been fully investigated. Possible mechanisms behind the nanoparticle-mediated changes should be further explored in plants for sustainable agriculture practices.

Conclusions: From our present investigation, we concluded that ZnO-NPs-mediated response was concentration- and mode-dependent. Moreover, roots of tomato plants treated with 8 mg L^{-1} for 30 min showed the most promising response and increased the growth, enhanced photosynthetic efficiency of plants, while other concentrations (2, 4, or 16 mg L^{-1}) and duration (15 or 45 min) of ZnO-NPs treatment did not show such a promising response. Antioxidant systems and proline accumulation enhanced by 8 mg L^{-1} for 30 min provided better ROS protection to plants. Therefore, we believe that root dipping to ZnO-NPs (8 mg L^{-1}) could be exploited to improve the productivity and as a potent nano-micronutrient for tomato plants.

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