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Silicon dioxide nanoparticles ameliorate the phytotoxic hazards of aluminum in maize grown on acidic soil

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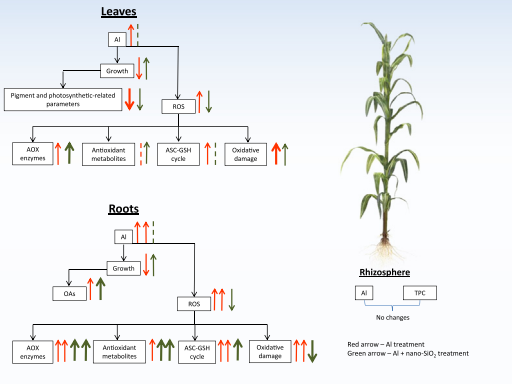
HIGHLIGHTS

- Aluminum (Al) reduced the growth and photosynthesis and induced oxidative stress in maize plants.
- Nano-SiO_2 (SNPs) did not affect Al accumulation but mitigated its phytotoxicity.
- SNPs induced organic acid exudation by roots and metal detoxification activity.
- SNPs reduced ROS production and improved ROS scavenging systems.
- Maize responses to Al and SNPs were in a dose- and organ-specific manner.

GRAPHICAL ABSTRACT



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ABSTRACT

Aluminum (Al) toxicity is a major constraint for crop production in acid soils. Therefore, looking for sustainable solutions to increase plant tolerance to Al toxicity is needed. Although several studies addressed the potential utilization of silica or silicon dioxide nanoparticles (SNPs) to ameliorate heavy metal phytotoxicity, the exact mechanisms underlying SNPs-induced stress tolerance are still unknown. The current study investigated how SNPs could mitigate Al toxicity in maize plants grown on acidic soil. The impact of Al alone or in combination with SNPs on Al accumulation and detoxification, plant growth, photosynthetic C assimilation and redox homeostasis has been investigated. Al accumulation in stressed-maize organs reduced their growth, decreased photosynthesis

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related parameters and increased production of reactive oxygen species, through induced NADPH oxidase and photorespiration activities, and cell damage. These effects were more pronounced in roots than in leaves. SNPs ameliorated Al toxicity at growth, physiological and oxidative damage levels. Co-application of SNPs significantly reduced the activities of the photorespiratory enzymes and NADPH oxidase. It stimulated the antioxidant defense systems at enzymatic (superoxide dismutase, catalase, ascorbate and glutathione peroxidases) and non-enzymatic (ascorbate, glutathione, polyphenols, flavonoids, tocopherols, and FRAP) levels. Moreover, SNPs increased organic acids accumulation and metal detoxification (i.e. glutathione-S-transferase activity) in roots, as a protective mechanism against Al toxicity. The SNPs induced-protective mechanisms was dependent on the applied Al concentration and acted in organ-specific manner. Overall, the current study suggests the promising application of SNPs as an innovative approach to mitigate Al phytotoxicity in acidic soils and provides a comprehensive view of the cellular and biochemical mechanisms underlying this mitigation capacity.

1. Introduction

Aluminum (Al) is one of the most dominant minerals in the earth's crust, representing about 8% of its mass. Fortunately, Al is mostly bounded in non-phytotoxic forms (Kochian et al., 2015), however, under acidic soil conditions (pH < 5.5), Al is solubilized into aluminum ion (Al^{3+}) and became biologically available (Sparling and Lowe, 1996). Leaching of alkaline cations, Ca^{2+} and K^{+}, from the soil, acidic rainfall, and acidic mine drainage are among the major causes for soil acidification and generation of the phytotoxic hazards of Al (Sparling and Lowe, 1996). Al^{3+} impairs the growth of many plant species at micromolar concentrations (50 μM) and, therefore, decreased crop productivity (Delhaize and Ryan, 1995). (Brunner and Sperisen, 2013). In Al-sensitive plants, such maize, Al toxicity strongly affects root growth and development features such as cell division, root elongation and cell membrane polarization (Arunakumara et al., 2013; Kochian et al., 2015). It also modifies nutrient acquisition processes, disturbs several metabolic pathways and causes oxidative damage (Arunakumara et al., 2013; Kochian et al., 2015). In leaves, Al toxicity induces a reduction in pigment content, photosynthetic rates, and PSII efficiency (Chen et al., 2010). Oxidative stress is also one of the well-known effects of Al toxicity (Panda and Matsumoto, 2007). Al increases reactive oxygen species (ROS) production, causing damage to membranes, proteins, and nucleic acids. For instance, Al stressed-maize, onion, rye, and cucumber seedlings, exhibited DNA and protein oxidation and lipid peroxidation (Boscolo et al., 2003; Achary et al., 2008; Pereira et al., 2010; De Sousa et al., 2016).

On the other hand, plants develop several mechanisms to cope with Al-induced stress. The best-documented mechanism of Al tolerance is the efflux of organic acids (OAs) from roots and/or accumulation of OAs within roots apoplast (Brunner and Sperisen, 2013; Kochian et al., 2015). Exudation of phenolics and polypeptides and secretion of mucilage are also involved in Al tolerance in several plants (Brunner and Sperisen, 2013; Kochian et al., 2015). Higher lignification levels were found in roots of sunflower plants in response to Al treatment (da Silva de Jesus et al., 2016). The activation of Al-tolerant metabolic pathways (e.g. antioxidant responses) were also recorded for several plant species (Giannakoula et al., 2010; Ma et al., 2012; Brunner and Sperisen, 2013; Kochian et al., 2015; Bojórquez-Quintal et al., 2017; Xu et al., 2017).

Silicon (Si) is not an essential element for plant growth and development, however, its beneficial role in development processes is well described (Marschner, 2011; DalCorso et al., 2014). Chemical amendment of soils with materials rich in Si can mitigate metal toxicity and improve plant growth (Chen et al., 2010). For instance, it enhanced the plant tolerance against several metals toxicity such as Al, As, Cd, Cr and Mn (Horiguchi, 1988; Li et al., 2009; Singh et al., 2011; Wang et al., 2016). Different protective mechanisms are suggested to be mediated by Si, including modifications of cell walls through deposition of SiO_2 to act as physical and mechanical protection (Luyckx et al., 2017; Wang et al., 2017). Si is believed to induce changes in plant cell metabolism, decrease heavy metal (HM) uptake by roots and induced exudation of certain compounds such as organic acids (OAs) and phenols (Kidd et al., 2001; Wang et al., 2004; Gu et al., 2011). However, deep research to understand the physiological role of Si in the regulation of abiotic and biotic stresses are still needed (Azeem et al., 2015; Coskun et al., 2016; Guerriero et al., 2016).

Nanotechnology is an emergent technology used in agricultural activities as a way to decrease the use of pesticides and to optimize nutrient management (Siddiqui et al., 2014). To present, the emphasis has been given to the protective role of bulk Si sources to plants; however, the use of nano-sized Si, such as SiO_2-nanoparticles (SNPs), in the mitigation of metal stress is much less studied. Despite having unique physicochemical properties, kinematics, and bioactivity, SNPs are also known to be taken up by plant roots (e.g. maize, wheat, lupin, *Arabidopsis*) via symplastic and apoplastic pathways, reaching the aerial parts through the xylem (Sun et al., 2016). Because of their higher dissolution, nano-sized metals could exert stronger effects compared to their bulk counterpart (Faisal et al., 2013). In this context, SNPs was found to be more effective in ameliorating ultra-violet (UV) stress than bulk Si in wheat seedlings (Tripathi et al., 2017). Moreover, the use of SNPs to mitigate the toxic effects of some stresses in plants, including salt and some HM, has been suggested (Tripathi et al., 2015a; Tripathi et al., 2015b; Tripathi et al., 2016; Gowayed et al., 2017; Soares et al., 2018). Nonetheless, mechanisms underlying the nanoparticle-mediated amelioration of metal stress are poorly understood. Thus, we attempted to evaluate SNPs as an innovative tool to mitigate Al-induced phytotoxicity in one of the most important crops, maize (*Zea mays* L.), grown on artificially acidified soil. Covering dosage and organ-specific responses were studied. We elucidated how acquired tolerance was reached by Al-stressed maize plants under SNPs co-treatment and how plants were able to partially restore their growth and reach a metabolic rebalancing.

2. Materials and methods

2.1. Characterization of silicon dioxide nanoparticles (SNPs)

Nano-SiO_2 (spherical and porous nanopowder with a particle size of 5–15 nm, surface area of 590-690 $m^2\ g^{-1}$ and 99.5% purity) was purchased from Sigma Aldrich (https://www.sigmaaldrich.com/catalog/product/aldrich/637246?lang=de&region=DE). To confirm the size and shape of nano-SiO_2, transmission electron microscopy (TEM) analyses using JEOL-JEM2100 (Hitachi Corporation, Japan) operated at an acceleration voltage of 200 kV, were performed (Faisal et al., 2013). The images were acquired by an Olympus KeenView digital Camera with the iTEM software. SNPs nanoparticles presented a spherical shape with an average size of 20 nm diameter, corroborating the manufacturer's information. In aqueous solution, these particles tended to

form coarse aggregates, which were avoided in the experiments by sonication (Supplementary data, Fig. S1).

2.2. Experimental design

Maize grains (*Zea mays* L. Giza 117, a commercial variety, Research Center of agriculture, Giza, Egypt) were surface sterilized with 35% (v/v) of sodium hypochlorite for 30 min. Healthy sterilized grains were germinated in petri plates and then grown in artificial soil (70% dry wt sand, 8% dry clay, 15% silt, 10% dry wt Sphagnum peat, 5.4% organic mater, 1.2 N (g/kg), 0.17 P (g/kg), 5.9 K (g/kg), 79 Ca (g/kg), 1.5 Mg (g/kg), 9 (cmol/kg) cation exchange capacity, 65% soil water capacity, adjusted to pH around 5 with $CaCO_3$) (OECD, 2006). The $AlCl_3$ solution was mixed with the soil at 0, 12 and 25 mg kg^{-1} Al^{+3} alone or in combination with SNPs suspension (4 mg/kg). To assure the bioavailability of Al^{+3}, the soil was maintained at pH 5 and soil water capacity of 65%. The maize plants were grown in a green house maintained at 25/18 °C (day/night), 55% relative humidity, 16/8 h photoperiod and photosynthetic active radiation (75 μmol m^{-2} s^{-1}). After 3 weeks of exposure to SNPs and/or Al^{+3}, the roots and leaves were harvested and their growth parametrs were measured. Some harvested materials were frozen in liquid N_2, and stored at −80 °C to be used for biochemical analysis.

2.3. Al level in maize tissues and soil

Al content was determined in dried plant organs and rhizosphere soil according to Giannakoula et al. (2008). Rhizosphere was obtained by shaking the excavated root very gently to separate from the bulk soil, then soil attached to the fine roots (0–2-mm thick layer) was obtained by brushing. Plant tissues (0.1 g) and 5 g of soil were digested in a nitric acid/perchloric acid solution (HNO_3/$HClO_4$) 4:1 (v/v) to get ready for analysis by inductively coupled plasma atomic emission spectrometry (ICP-AES). After the extraction, Al content was determined using ICP-AES (Perkin-Elmer Optima 3300XL Perkin-Elmer, USA). Rodhium was used as an internal standard, being added to samples and calibration solutions at known concentrations.

2.4. Organic acids (OAs)

Root tips (0.1 g) were ground in a MagNALyser (Roche, Vilvoorde, Belgium), and extracted with 0.1% phosphoric acid contained butylated hydroxyanisole. The internal standard (ribitol) was added during the extraction procedures. After centrifugation for 30 min at 14,000 rpm, the supernatant was transferred to new tubes for high-performance liquid chromatography (HPLC) quantification, SUPELCOGEL C-610H column coupled to UV detection system adjusted at 210 nm (LaChrom L-7455 diode array, LaChrom, Tokyo, Japan) (De Sousa et al., 2016). Samples were eluted using methanol as mobile phase A and mobile phase B was 5% potassium dihydrogen phosphate (pH 2.5) at 0.5 mL/min and the injection volume was 40 μL (De Sousa et al., 2016).

2.5. Photosynthetic parameters

Both light-saturated photosynthetic and respiration rates (μmol CO_2 m^{-2} s^{-1}) were measured (LI-COR LI-6400, LI-COR Inc., Lincoln, NE, USA) (Saleh et al., 2019). Stomatal conductance (gs, mol CO_2 m^{-2} s^{-1}) was measured using Leaf Porometer (Model SC1, Decagon Devices, Inc., Hopkins, Pullman, WA USA) on the abaxial side of the fully developed leaves. Chlorophyll fluorescence was measured on dark acclimated fully expanded leaves using FMS-2 pulse-modulated fluorometer (Han- satech Instruments, Norfolk, UK). The minimal fluorescence (F_0) and maximal fluorescence (F_m) were measured for 30 min dark acclimated leaves. The photochemical efficiency of PSII (F_v/F_m) for dark adapted leaves were calculated, where F_v (Maximal variable fluorescence) = F_m − F_0. Chlorophyll and carotenoid concentrations were assayed according to the method adopted by Zhao et al. (2008).

2.6. Oxidative markers

Photorespiration enzymes, glycolate oxidase (GO) and hydroxypyruvate reductase (HPR), were determined as previously described in De Sousa et al. (2017). Lipid peroxidation was determined using the thiobarbituric acid–malondialdehyde (TBA–MDA) assay. The absorbance was measured at 440, 532, 600 nm and TBARS content were calculated and expressed as nmol g^{-1} FW tissue (De Sousa et al., 2017). Hydrogen peroxide (H_2O_2) was extracted from fresh tissues with 0.1% (w/v) TCA. After centrifugation, the H_2O_2 concentration was measured as previously described in De Sousa et al. (2017). Nitric acid (NO) concentration was determined by measuring the production of methemoglobin (Murphy and Noack, 1994). Cell viability was evaluated by triphenyl tetrazolium chloride (TTC) reduction method (Casida Jr et al., 1964). Protein oxidation was assessed through carbonyl quantification using a 2,4-dinitrophenylhydrazine (DNPH)-based Protein Carbonyl Colorimetric Assay Kit (cat. 10,005,020; Cayman Chemical Company, Ann Arbor, MI, USA) (Levine et al., 1994). NADPH oxidase activity was assayed according to Sarath et al. (2007).

2.7. Antioxidant defense system

2.7.1. Total antioxidant capacity (TAC) and antioxidant metabolites

TAC was measured by a modified ferric ion reducing antioxidant power (FRAP) assay (De Sousa et al., 2017). Antioxidants were extracted from the frozen plant tissue (0.1 g) with ethanol 80% (v/v). After centrifugation (3000*g*, 4 °C, 15 min) the FRAP reagent (0.3 M acetate buffer, pH 3.6, 0.01 mM TPTZ in 0.04 mM HCl, 0.002 $FeCl_3.H_2O$) was mixed with the extracts and measured at 600 nm, using Trolox as a standard. Total phenolic content and flavonoids were extracted in 80% ethanol (v/v) and estimated as previously described in De Sousa et al. (2017). Tocopherols, ascorbate (ASC), glutathione (GSH), carotenoids and starch levels were measured by HPLC using the procedures previously described by De Sousa et al. (2017).

2.7.2. Enzymatic activities

Superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidases (POX), guaiacol peroxidases (GPX), catalase (CAT), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR) and glutathione-S-transferase (GST) were extracted in 50 mM potassium phosphate buffer (pH 7.0), containing 10% (w/v) polyvinylpyrrolidone (PVP), 0.25% (v/v) Triton X-100, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM ascorbate, by using a MagNALyser (Roche, Vilvoorde, Belgium). The SOD activity was determined by measuring the inhibition of nitroblue tetrazolium (NBT) reduction at 560 nm (De Sousa et al., 2017). APX, MDHAR, DHAR and GR activities were measured as previously described in De Sousa et al. (2017). POX activity was determined by the oxidation of pyrogallol (ε_{430} = 2.47 mM^{-1} cm^{-1}) (De Sousa et al., 2017). GPX activity was assayed by measuring the decrease in NADPH absorbance measured at 340 nm (ε_{340} = 6.22 mM^{-1} cm^{-1}) (De Sousa et al., 2017). The CAT activity was assayed by monitoring the decomposition of H_2O_2 at 240 nm (Aebi, 1984). GST activity was estimated by measuring the conjugation of GSH with the excess of 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm (ε_{340} = 0.0096 μM^{-1} cm^{-1}) (De Sousa et al., 2017). Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) was measured as previously described in De Sousa et al. (2017). All activity measurements were scaled down for semi-high throughput using a microplate reader (Synergy Mx, Biotek Instruments Inc., Winooski, VT, USA). Total soluble proteins concentration was estimated according to the method of Lowry et al. (1951).

2.8. Statistical analysis

The results were expressed as mean ± SD (standard deviation) and analyzed by three-way and two-way ANOVA using IBM SPSS Statistical 23 software package (SPSS® Inc., Chicago, IL, USA) for windows. Data were analyzed by the three-way ANOVA (SPSS Statistica 23, SPSS Inc., Chicago, IL, USA), with organ (O), treatment (T) and concentrations (C) as fixed variables (ANOVA results in Supplementary Table 1). Measurements performed only in soil, roots or leaves were analyzed by the two-way ANOVA with treatment (T) and concentrations (C) as fixed variables (ANOVA results in Supplementary Table 2). In cases of significant interactions between factors, one-way ANOVA was performed for each factor, and Tukey's multiple range tests were used to determine significant differences among means. A significance level of $P < 0.05$ was used for rejection of the null hypothesis. All experiments were carried out in four replicates ($n = 4$).

To explore the Organ specific responses to the different treatments, a cluster analysis was performed by using the Pearson distance metric using the MultiExperiment Viewer (MeV)™ 4 software package (version 4.5, Dana-Farber Cancer Institute, Boston, MA, USA).

3. Results

3.1. Al, Si and total polyphenol content

Al concentration increased in soil, roots, and leaves in a dose dependent manner (Fig. 1A, C, D). A 190- and 407-fold increase in Al levels was detected in roots of plants grown at mild (12 mg kg^{-1}) and severe (24 mg kg^{-1}) Al stress, respectively (Fig. 1A). In leaves, a 54- and 119-fold increase of Al levels was found in plants grown at 12- and 24 mg kg^{-1} of Al, respectively (Fig. 1D). SNPs treatment alone did not affected Al levels in both organs (Fig. 1A, D). Leaves and roots of Al + SNPs-treated plants exhibited a similar pattern of Al accumulation (Fig. 1A, D) ($P < 0.05$). Similar results were found for Al content in rhizosphere (Fig. 1C).

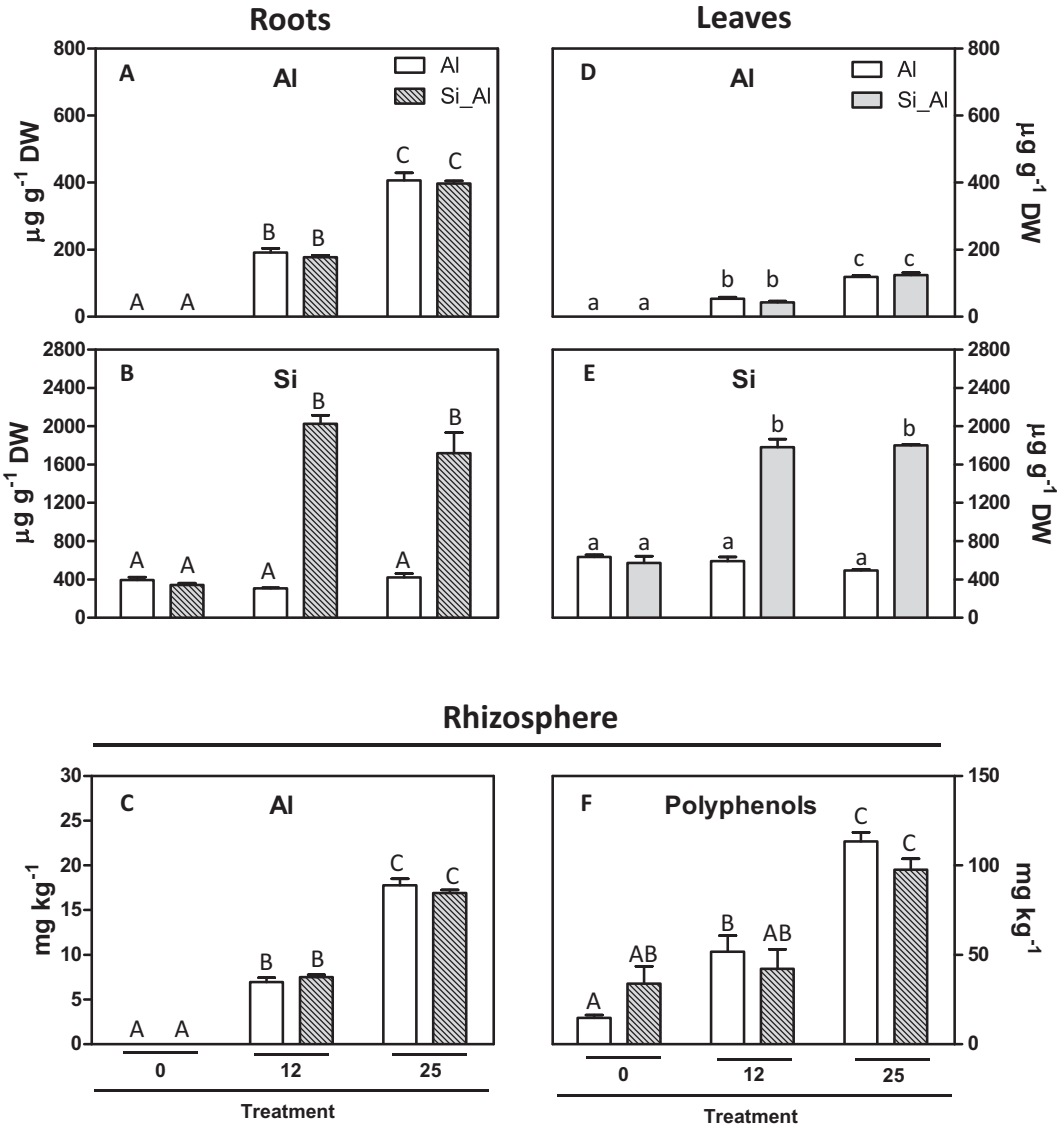


Fig. 1. Aluminum (Al)- and nano-SiO_2 (SNPs)-induced changes in Al and Si accumulation in maize tissues and in Al and polyphenols content in the rhizosphere. A, D: Al content in roots and leaves, B, E: Si content in roots and leaves, C: Al content in the rhizosphere, F: polyphenols levels in the rhizosphere. Data are the mean of 4 experiments (±SD). Letters indicate statistical difference ($p < 0.05$) (capital letters: roots; small letters: leaves).

Si levels were not affected by Al treatment in both organs (Fig. 1B, E). Also, SNPs alone treatment did not affected Si levels in both organs (Fig. 1B, E). However, SNPs co-treatment induced a 6- and 5- fold increase in Si levels in the roots of plants exposed to mild and severe Al stress, respectively. In the leaves, a 3-fold increase in Si was observed for Al-nanoSiO_2-treated plants (Fig. 1B, E) ($P < 0.05$).

A 4- and 8-fold increase was quantified for total polyphenol content (TPC) in soils treated with 12- and 24 mg kg^{-1} of Al, respectively (Fig. 1F), while SNPs treatment alone did not exert major effetcs. A positive effect of SNPs in TPC accumulation was only found in the soil of plants exposed to severe Al stress. Exudation of TPC was similar in both Al and Al-nanoSiO_2-treated plants (Fig. 1F) ($P < 0.05$).

3.2. Organic acids (OAs) levels

Accumulation of aconitic acid, lactate, and oxalate was not affected by Al treatment (Fig. 2D, E, F). However, plants exposed to severe Al conditions exhibited an increase of about 78%, 145% and 97% in citrate, malate and succinate levels, respectively (Fig. 2A, B, C). SNPs treatment did not affected OAs acumuation in rhizhospere. SNPs treatment significantly increased the accumulation of citrate, lactate, malate, and succinate in roots of plants exposed to severe Al stress (Fig. 2A, B, C, E). This pattern was also observed in Al-nanoSiO_2-treated maize plants (Fig. 2A, B, C, E). A significant interaction between treatment and concentrations was observed for accumulation of all OAs ($P < 0.05$, Supplementary Table 1).

3.3. Biomass production

Al exposure decreased biomass production in both organs (Fig. 3A–D), while SNPs alone did not has significant effects. However, SNPs co-application partially diminished the negative impact of Al on plant growth. The reduction in fresh weight and dry weight was less pronounced in roots (21% and 45%) ($P < 0.05$) and leaves (19% and 39%) ($P < 0.05$) of Al treated-maize plants (Fig. 3A–D). Foliar symptoms of Al toxicity appeared as marginal yellowing and death of leaf tips mainly in plants exposed to Severe Al stress (Supplementary data, Fig. S2). Overall, nanopriming of maize plants significantly mitigated the toxic effects of Al on biomass production.

3.4. Photosynthetic-related parameters

Maize plants leaves exposed to severe Al stress showed a decline in the photosynthetic rate (82%) (Fig. 4A) and a 200% increase in the respiration rate (Fig. 4B). Consistently, stomatal conductance decreased by about 67% and 85% in plants exposed to mild and Severe Al stresses, respectively (Fig. 4C). Chlorophyll fluorescence (Fv/Fm), as well as RuBisCo activity and total chlorophyll levels (Fig. 4D, E, F), were found

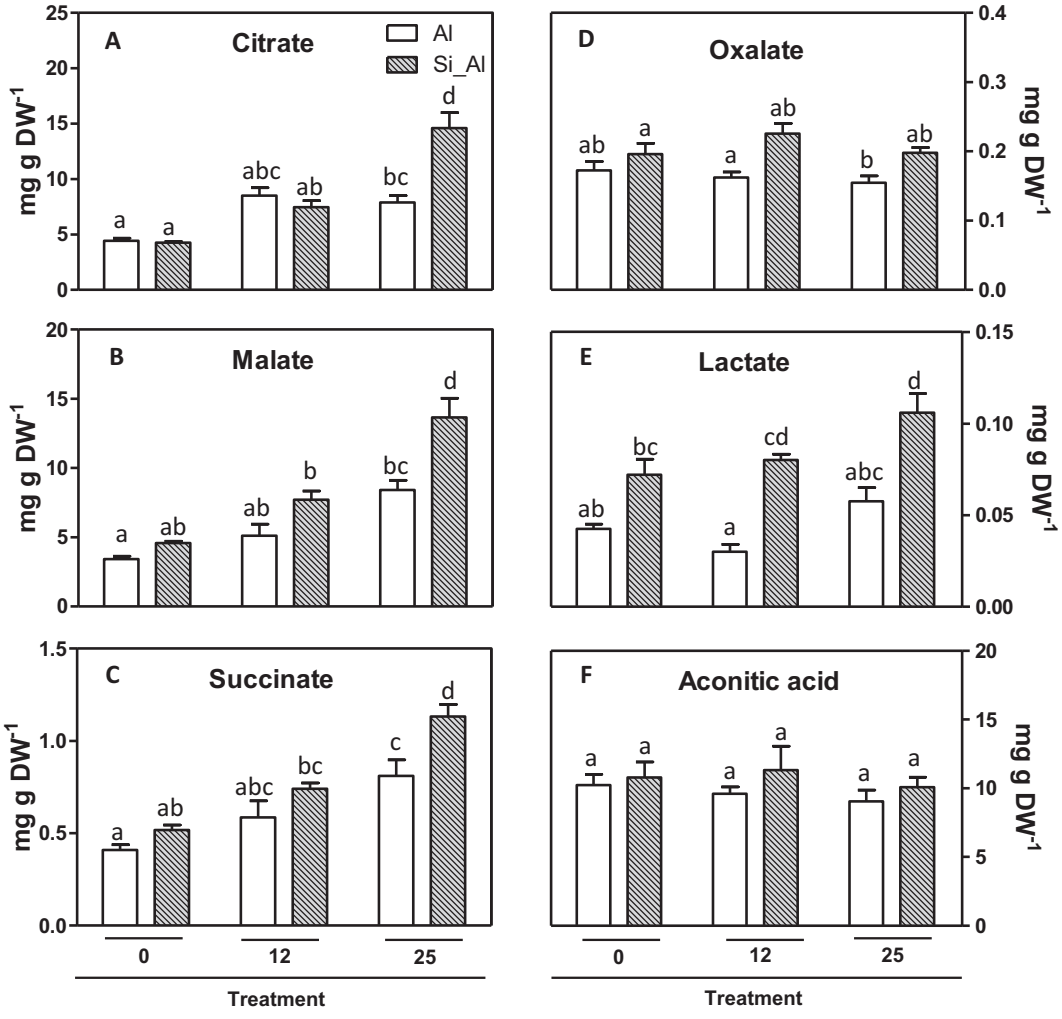


Fig. 2. Aluminum (Al)- and nano-SiO_2 (SNPs)-induced changes in organic acid accumulation in root tips of maize plants. A: citrate, B: malate, C: succinate, D: oxalate, E: lactate, F: aconitic acid. Data are the mean of 4 experiments (±SD). Letters indicate statistical difference ($p < 0.05$).

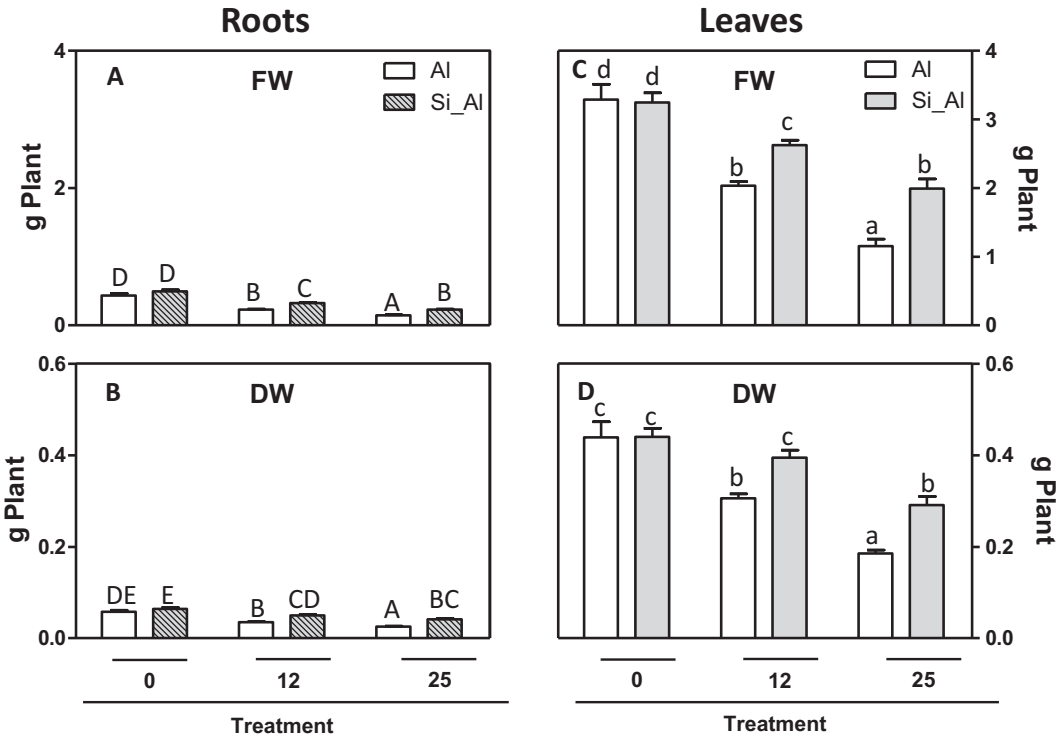


Fig. 3. Aluminum (Al)- and nano-SiO_2 (SNPs)-induced changes in biomass production. Fresh and dry weight was determined in roots (A, B) and leaves (C, D) of maize plants. Data are the mean of 4 experiments (±SD). Letters indicate statistical difference ($p < 0.05$) (capital letters: roots; small letters: leaves).

to be decreased in Al-treated plants. Carotenoids levels increased in leaves (58%) of severely stressed plants, while starch levels decreased by 38% (Fig. 4G, H). All above described parameters, enzymes and metabolites were not affected by SNPs alone treatment.

Photosynthetic rates decreased to a less extent in plants co-treated with nano-SiO_2 ($P < 0.05$), while respiration rates increased ($P < 0.05$) (Fig. 4A, B). Stomatal conductance and Rubisco activity decreased in Al-nanoSiO_2-treated plants ($P < 0.05$) (Fig. 4C, E), but again to a less extent compared with plants treated solely with Al. Also, Fv/Fm ratios increased by 6% in Al-nanoSiO_2-treated plants compared to Al treatment alone ($P < 0.05$) (Fig. 4D). Moreover, pigment levels and Rubisco catalytic activity tended to increase in maize plants after nano-SiO_2 treatment ($P < 0.05$) (Fig. 4E, F). At starch level, nanoSiO_2 co-treatment did not affect starch accumulation in maize stressed plants ($P < 0.05$) (Fig. 4H). Interestingly, a significant interaction between treatment and concentrations was observed for all photosynthesis related parameters ($P < 0.05$, Supplementary Table 2).

3.5. Oxidative stress markers

Lipid peroxidation decreased by 62% and 100% in leaves of mild- and severely stressed plants, respectively (Fig. 5F) and similar results were observed for protein oxidation in severely stressed plants (Fig. 5H). H_2O_2 and NO levels increased in both organs of maize plants exposed to mild and Severe Al stresses (Figs. 5B, G and 8C, D). Consequentially, cell viability reduced by 48% in Al-treated roots (Fig. 5D).

Nanopriming of maize plants had no significant effect on oxidative stress-related parameters under control conditions. On the other hand, SNPs co-application counteracted the toxic effects of Al in almost all assessed parameters, particularly under severe stress conditions ($P < 0.05$) (Figs. 5A–J and 8C, D).

NADPH oxidase activity significantly increased in both organs of the Al-treated plants (Fig. 5E, J), while the activities of GO and HDR were increased by 148% and 167% in severely stressed leaves, respectively (Fig. 6A, B). Also, increased Gly/Ser ratios (51%) were observed in leaves for the same group of plants (Fig. 6C). SNPs treatment did not affected NADPH oxidase, GO and HDR activities as well as Gly/Ser ratio. Interestingly, co-application of SNPs attenuated the effects of severe Al toxicity on NADPH oxidase, GO and HDR activities as well as on Gly/Ser ratios (Figs. 5E, J, and 6A–C) ($P < 0.05$).

3.6. Antioxidant defense systems

3.6.1. Non-enzymatic antioxidants

Regarding all the non-enzymatic antioxidants assayed (Fig. 7A–H), only flavonoids levels increased (73%) in roots of Al-severely stressed plants (Fig. 7C). Maize plants exposure to SNPs alone did not cause any significant change in accumulation of non-enzymatic metabolites in both organs. SNPs co-treatment partially reversed the Al-induced toxicity, since increases between 45% and 88% were found among non-enzymatic antioxidants in roots and leaves of plants exposed to severe Al stress, respectively (Fig. 7A–H) ($P < 0.05$).

3.6.2. Antioxidant enzymes

Roots and leaves of maize plants exhibited a significant increase, of about 64% and 69%, respectively, in SOD activity when exposed to severe Al stress (Fig. 8A, B). Similar results were found for the H_2O_2-scavenging enzymes, CAT, APX, GPX and POX (Fig. 8E–L). Antioxidant enzyme activities (SOD, CAT, APX, GPX and POX) remainded unaltered upon SNPs treatment alone in both organs. Upon SNPs co-treatment, about bifold in SOD activity was observed for both roots and leaves of the plants exposed to severe Al stress (Fig. 8A, B). Similar results were also found for APX, POX, CAT and GPX activities (Fig. 8E–L). SNPs co-treatment generally enhanced the activity of ROS scavenging enzymes (Fig. 8A, B, E–L) ($P < 0.05$).

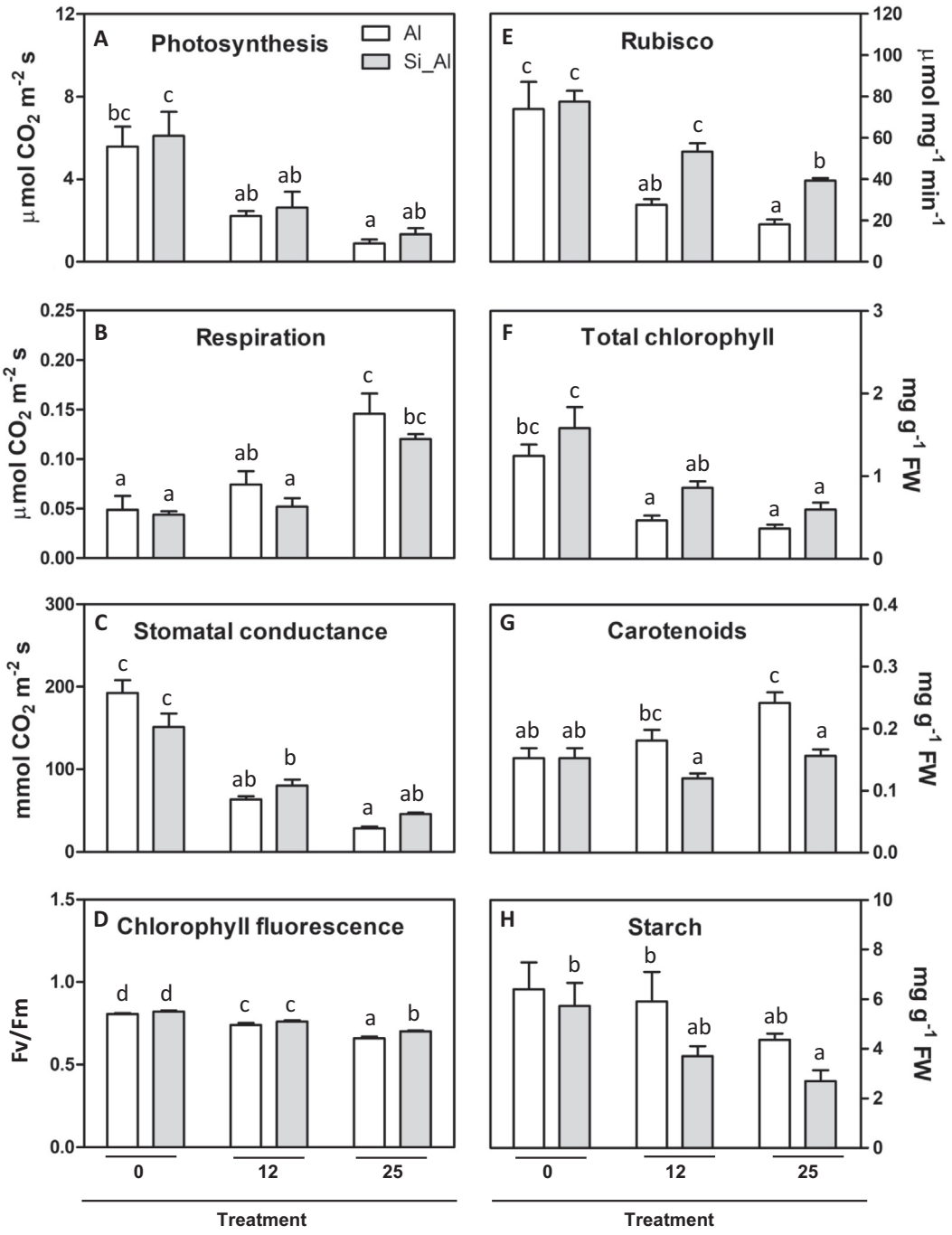


Fig. 4. Aluminum (Al) - and nano-SiO_2(SNPs)-induced changes in photosynthetic-related parameters and pigments. A: photosynthetic rate, B: respiration rate; C: stomatal conductance, D: chlorophyll fluorescence, E: RuBisCo activity, F: total chlorophyll, G: carotenoids and H: starch levels. Data are the mean of 4 experiments (±SD). Letters indicate statistical difference ($p < 0.05$).

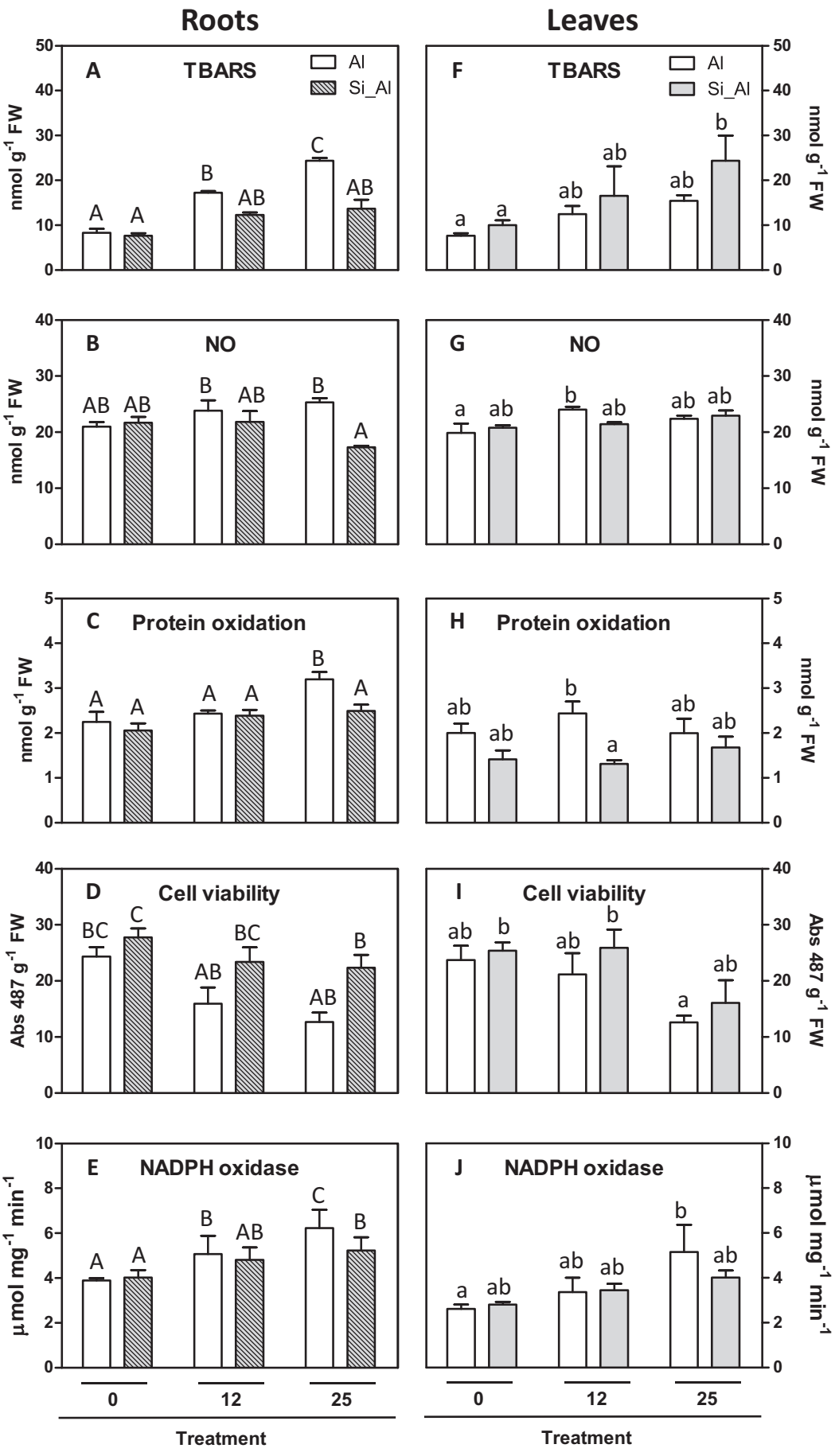
3.6.3. ASC and GSH recycling

Al stress did not exert any significant effect on ASC content (Fig. 8M) and its redox status (data not shown) in roots, while leaves of plants exposed to severe Al stress exhibited an increase of about 94% in ASC levels (Fig. 8N). In relation to MDHAR and DHAR activities, no significant changes were observed in leaves and roots (Fig. 8O–R). GSH levels increased by 200% in roots of Severely stressed plants (Fig. 8W); however, no changes were observed in GSH redox status (data not shown). GR activity increased significantly in roots and leaves (152% and 110%, respectively) of plants exposed to severe Al stress (Fig. 8U, V). As for antioxidant enzymes, no significant changes were found for MDHAR, DHAR and GR activities upon SNPs treatment alone. The same was observed for ASC and GSH levels.

In general, almost no significant effects were reported for SNPs treatment in control conditions. Nonetheless, co-treatment of Al stressed plants with SNPs increased ASC and GSH levels (Fig. 8M, N, W, X). A significant increase in the activity of DHAR in roots was also observed (Fig. 8Q) ($P < 0.05$).

3.6.4. Metal detoxification (GST activity)

GST activity increased 3- and 4-fold in roots of maize plants exposed to mild and severe stress conditions (Fig. 8S), whereas in leaves this


Roots
Leaves
A
TBARS
Al
Si_Al
nmol g-1 FW
F
TBARS
B
NO
G
NO
C
Protein oxidation
H
Protein oxidation
D
Cell viability
Abs 487 g-1 FW
I
Cell viability
E
NADPH oxidase
µmol mg-1 min-1
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0
12
25
Treatment

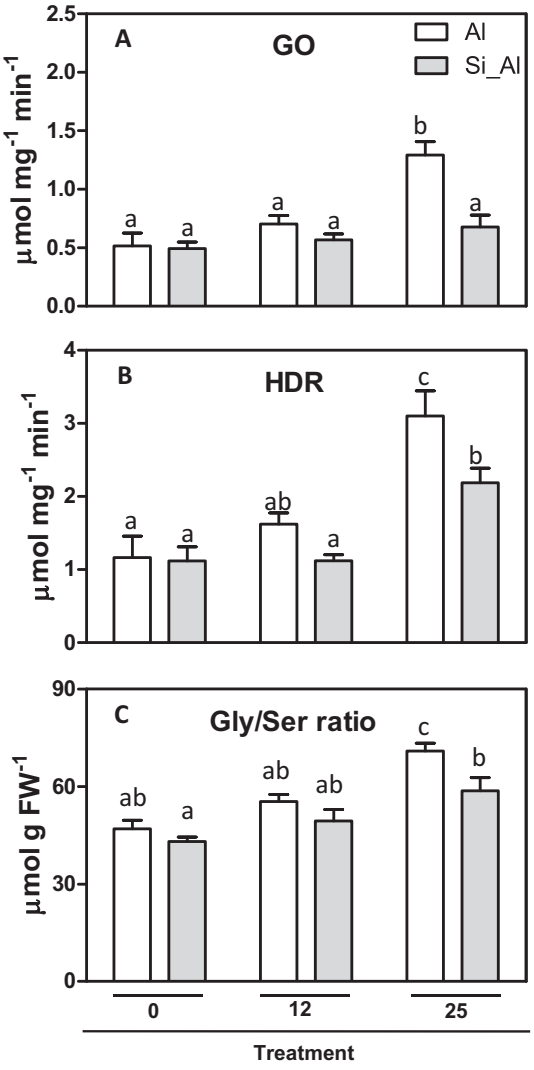

Fig. 6. Aluminum (Al)- and nano-SiO_2 (SNPs)-induced changes in photorespiration-related parameters. A: glycolate oxidase, B: hydroxypyruvate reductase, C: Gly/Ser ratios. Data are the mean of 4 experiments (±SD). Letters indicate statistical difference ($p < 0.05$).

increase was only observed in plants exposed to severe Al stress (4-fold) (Fig. 8T). GST activity remainded unchanged under SNPs treatment alone.In contrast, SNPs alone did alter GST activity in control and in stressed plants, except for roots under severe stress conditions (Fig. 8S, T) ($P < 0.05$).

3.6.5. Hierarchical cluster analysis

Hierarchical clustering of growth, physiological and biochemical parameters revealed variations in their relative levels in maize roots and leaves and in their responsiveness to Al and/or SNPs treatments (Fig. 9). There were four major clusters: those were higher in leaves than roots under control conditions and altered by Al and/or SNPs (antioxidants ASC, GSH, and phenols, cluster 1); those were lower in Severely stressed maize leaves and this effect was reduced by SNPs application (photosynthetic related parameters, cluster 2); those increased by Al stress and SNPs further increased this effect, particularly in maize roots (antioxidant enzymes, cluster 3,); and those were higher in stressed roots and leaves and SNPs mitigated Al effects (oxidative markers and photorespiration, cluster 4). Interestingly, we also found significant interactions between treatment, Al concentrations, and organs (Supplementary Table 1).

4. Discussion

4.1. SNPs did not affect Al uptake but induced its detoxification in roots

It's well known that Si is able to overcome toxic metal such as Al (Song et al., 2011; Ali et al., 2013; Farooq et al., 2013;). Although compartmentalization of HM in roots is a common tolerance mechanism mediated by Si that prevents metal translocation to leaves (Adrees et al., 2015), we found that Al level in maize tissues was not affected by SNPs treatment. Therefore, we hypothesized that there are other mechanisms underlying the protective effects of SNPs reported herein, compared with their bulk counterparts. For instance, Al tolerance can be achieved by the exudation of OAs from root tips, the primary site of Al toxicity in maize (Ryan et al., 1993), or by its accumulation within root apoplasts (Delhaize and Ryan, 1995; Kochian, 1995; Ma et al., 2001; Kinraide et al., 2005). Citrate and malate showed the highest absolute changes in plants exposed to Severe Al stress. Citrate, compared to another organic acid, is more effective in Al detoxification (Ma, 2000; Pineros et al., 2002; Yang et al., 2013). These increases in citrate level have been also reported for maize, rye and soybean cultivars (Pellet et al., 1995; Li et al., 2000; Silva et al., 2001). Interestingly, SNPs co-treatment further stimulated OAs accumulation, suggesting that OAs play a significant role in Si-mediated amelioration of Al toxicity. Such results were not previously recorded in maize and wheat after Si pre-treatments (Cocker et al., 1998; Kidd et al., 2001; Wang et al., 2004).

Additional mechanisms other than OAs may be involved in Al tolerance and one of them comprises the exudation of phenolic compounds by roots. Kidd et al. (Kidd et al., 2001) reported that Si-induced amelioration of Al toxicity in the Al-tolerant genotype was due to the active exudation of phenolic compounds. In our study, we observed that both Al and SNPs treatments induced the release of phenols from roots, which was dependent on the applied Al concentrations.

Hence, we suggest that SNPs similar to Si can stimulate the exudation of OAs and polyphenols ameliorating the toxic effects of Al in maize plants. This presents a new outcome since SNPs-induced exudation of OAs and polyphenols in response to stressors has not reported.

4.2. SNPs improved maize growth and photosynthetic reactions under Al stress

It is well known that Al significantly decreased plant biomass and metabolism and These decrease were correlated with reduced photosynthesis (Ryan et al., 1993; Yamamoto et al., 2001). The decrease in photosynthesis can be explained by the changes at stomatal and non-stomatal (e.g., chlorophyll content, F_m/F_v ratios and Rubisco activity) factors. Similarly, Al-induced responses in stomatal conductance were recorded before (Moustakas et al., 1996; Pereira et al., 2000; Peixoto et al., 2002). Regarding non-stomatal responses, Al could damage photosystem, for instance, Moustakas et al. (Moustakas et al., 1995) found that Al exposure can alter chlorophyll content by displacing Mg ion from chlorophyll molecules (Mihailovic et al., 2008; Yang et al., 2015). Moreover, we and others found that Al reduced PSII efficiency and RuBisCo activity (Chen et al., 2010). High respiration was also observed in stressed plants, which may be a consequence of increased energy demand for the production of specific metal-binding proteins and their

Fig. 5. Aluminum (Al)- and nano-SiO_2(SNPs)-induced changes in oxidative stress markers in roots and leaves. A, F: TBARS (lipid peroxidation); B, G: NO (nitric oxide); C, H: protein oxidation; D, I: cell viability, E, J: NADPH oxidase activity. Data are the mean of 4 experiments (±SD). Letters indicate statistical difference ($p < 0.05$) (capital letters: roots; small letters: leaves).

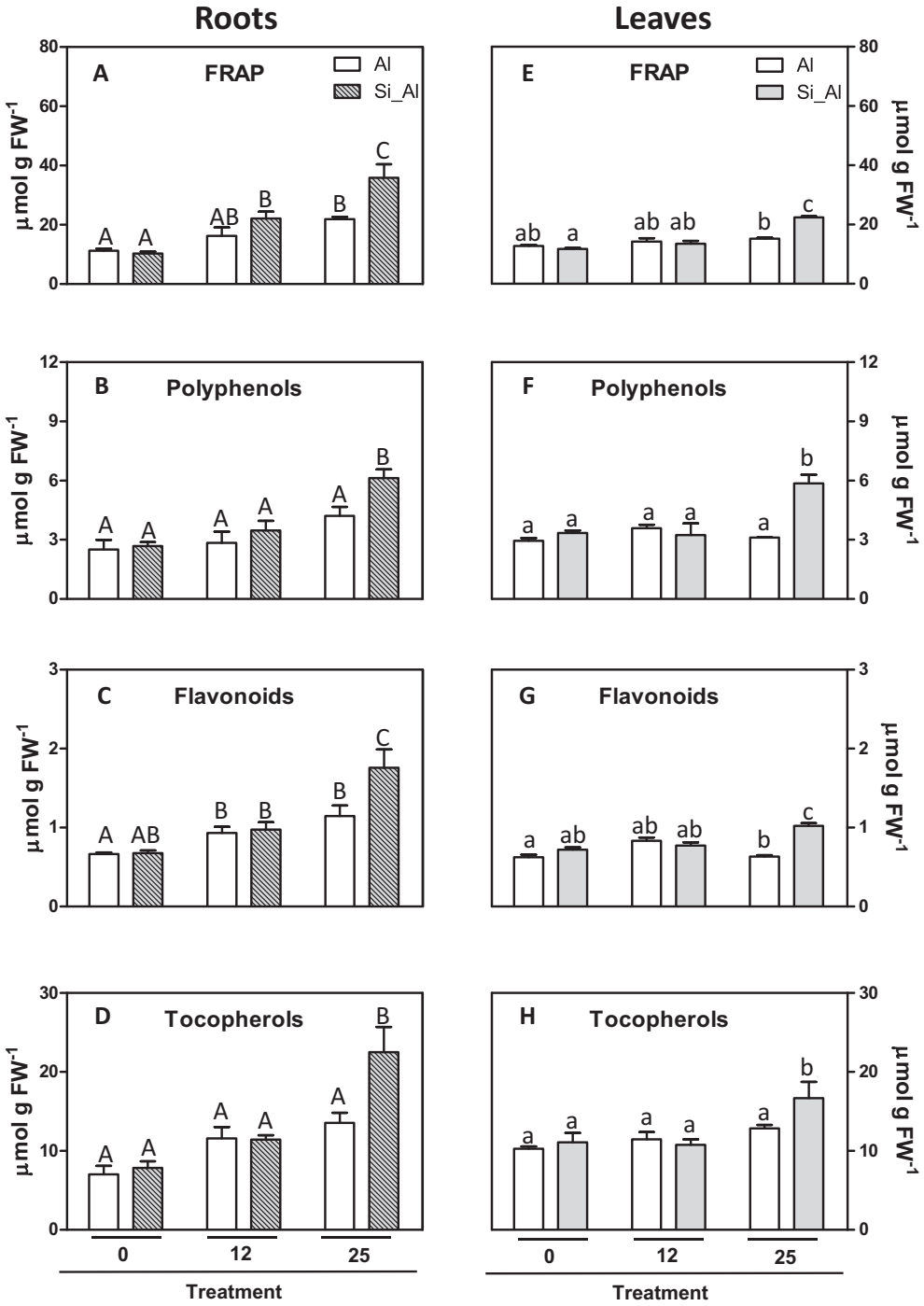

Fig. 7. Aluminum (Al)- and nano-SiO_2 (SNPs)-induced changes in non-enzymatic metabolites in roots and leaves. A, E: FRAP; B, F: polyphenols; C, G: flavonoids; D, H: tocopherols. Data are the mean of 4 experiments (±SD). Letters indicate statistical difference ($p < 0.05$) (capital letters: roots; small letters: leaves).

sequestration into vacuoles. This finding was also observed for plants treated with Cd and Zn (Prasad, 2013).

In this work, we show that SNPs co-application reduced Al toxicity at a growth level. This protective effect was attributed to enhanced photosynthetic reactions. SNPs co-application improved both stomatal and non-stomatal factors, e.g., stomatal conductance as well as pigment levels, Fm/Fv ratios and Rubisco activity. Under control condition, SNPs improved photosynthetic rate by increasing the activity of carbonic anhydrase and the synthesis of pigments (Xie et al., 2012). Corroborating with our results, application of SNPs also mitigated abiotic stress-induced photosynthesis inhibition by increasing the level of chlorophyll and stomatal conductance (Siddiqui et al., 2014).

4.3. SNPs regulate production and scavenging of reactive oxygen species (ROS) under Al stress

At oxidative stress level, SNPs decreased lipid peroxidation and protein oxidation in leaves of Al stressed plants. In this regard, our results interestingly showed that SNPs down-regulated Al induced-ROS production by reducing the activities of the photorespiration related enzymes (GO and HPR) and glycine/serine ratio as well as NADPH oxidase. GO and HPR are key enzymes catalyzing the photorespiratory reactions, and glycine/serine ratio is frequently used as an index to estimate photorespiration (Kebeish et al., 2007). Consequently, we observed low reactive oxygen species and reduced oxidative damage in

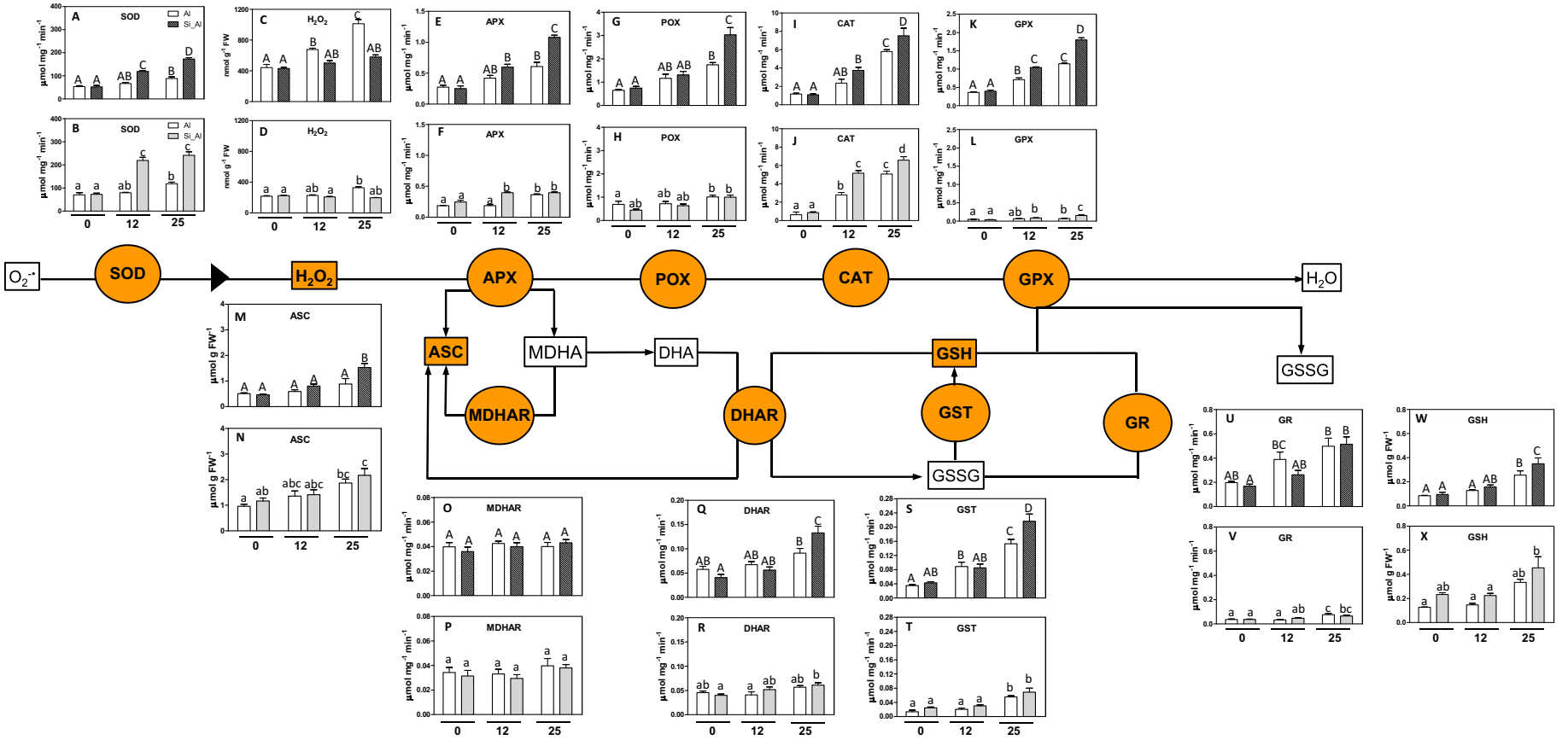

Fig. 8. Aluminum (Al)- and nano-SiO_2 (SNPs)-induced changes in antioxidant defenses. A, B: superoxide dismutase (SOD); C, D: hydrogen peroxide (H_2O_2); E, F: ascorbate peroxidase (APX); G, H: peroxidase (POX); I, J: catalase (CAT); K, L: guaiacol peroxidase (GPX); M, N: ascorbate (ASC); O, P: monodehydroascorbate reductase (MDHAR); Q, R: dehydroascorbate reductase (DHAR); S, T: glutathione-S-transferase (GST); U, V: glutathione reductase (GR); W, X: glutathione (GSH). Data are the mean of 4 experiments (±SD). Letters indicate statistical difference ($p < 0.05$) (capital letters: roots; small letters: leaves).

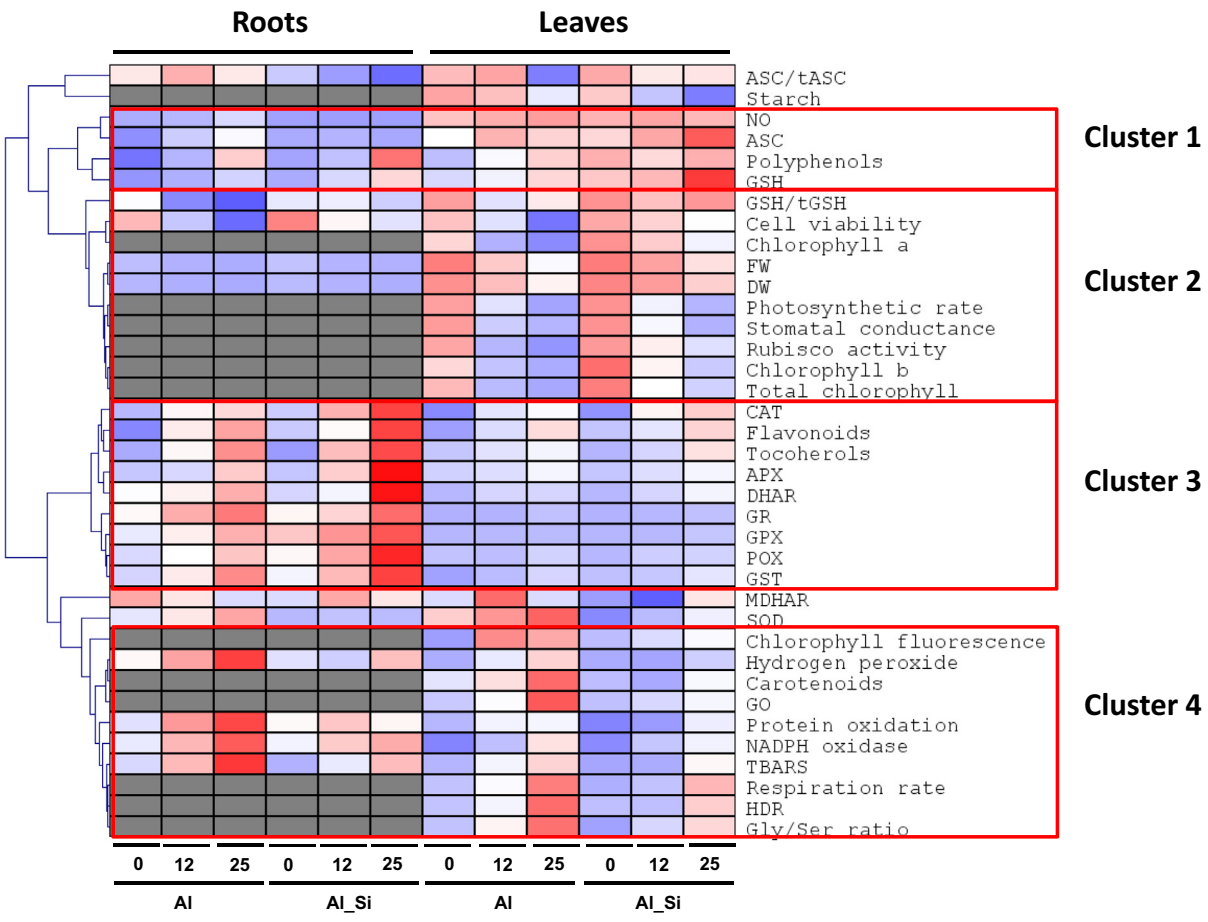

Fig. 9. Hierarchical clustering analyses of growth and physiological related parameters as well as metabolites levels in maize root and leaves treated with Al and/or SNPs. The relative increases/decreases patterns are shown in the heat map based on the average value ($n = 5$). Blue and red colors indicate lower and higher levels, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

SNPs treated plants. Similarly, squash abiotic stressed Seedlings treated with SNPs exhibited reduced lipid peroxidation and H_2O_2 levels (Kebeish et al., 2007).

Stimulation of the antioxidant defense system in roots and leaves by SNPs co-application seems to be held responsible for the decrease in the oxidative markers. As a matter of fact, we observed that almost all antioxidant enzymes (SOD, CAT, APX, and GPX) and antioxidant metabolites (polyphenols, flavonoids, tocopherols, and FRAP) were enhanced in leaves and roots by SNPs exposure. In previous studies, SNPs were also shown to protect wheat, pea and barley Seedlings against UV-B, Cr and Ni stress by the enhancement of the antioxidant defense system (Tripathi et al., 2015a; Tripathi et al., 2017; Soares et al., 2018). In this context, SNPs-mitigated oxidative stress, by a more proactive performance of several antioxidant metabolites and enzymes activities. For instance, foliar application of SNPs reduced Cd toxicity in rice Seedlings by improving antioxidant SOD, CAT and APX enzymes activities (Wang et al., 2015). Thus, These increases in defense system maintain the membrane integrity of stressed plants as demonstrated by reduced lipid peroxidation.

It is also worth noticing that SNPs enhanced ASC and GSH contents providing a strong defense against Al-induced oxidative stress in these organs. In this regard, both Si and SNPs could reduce arsenate toxicity in maize plants though enhancing the components of the ascorbate-glutathione cycle (ASC-GSH cycle) (Tripathi et al., 2016). SNPs induced GSH level as a mechanism to protect the plant against oxidative stress was also previously suggested (Wang et al., 2015). Also, In the study of Tripathi et al. (2015a), enhancement of APX, GR, and DHAR activity after SNPs application on heavy metal-stressed pea counteract metal-mediated oxidative damage. GSH is not only necessary for maintaining GSH/GSSG redox balance and regeneration of ASC, but it is also important for detoxification of toxic compounds in a reaction catalyzed by GST (Yadav et al., 2010). Similarly, improved HM detoxification by GST activity was also involved in the stress mitigating impact of SNPs activity. Taken together, severe Al stress-mitigating SNPs effects operated through controlling ROS production and scavenging, as well as increasing Al detoxification by GST activity.

4.4. SNPs mitigated Al toxicity showed organ- and dose-specific responses

Al was accumulated in both organs of maize plants, however, its levels were higher in roots than in leaves. In agreement with these findings, preferential accumulation of Al in the radicular system of several plant species was reported (see the review by Kochian et al. (2015)). This increase of Al level induced more oxidative stress in root as indicated by higher H_2O_2, TBARS levels, protein oxidation and NAPDH activity, on the other hand, SNPs protective effect was more pronounced in roots. Compared to leaves, roots of maize plants showed higher antioxidant defense system under severe stress conditions (cluster 4, Fig. 9). The greater impact of SNPs on antioxidants e.g., GPX, CAT, APX, and POX activities, and polyphenols, flavonoids, and tocopherols contents were also observed for root.

Although, roots rather than leaves increased the activities of enzymes involved in the ASC-GSH cycle (MDHAR, DHAR, and GR) in an attempt to control ROS levels and maintain cellular redox, stressed leaves, increases in ASC and GSH levels (cluster 1, Fig. 9) could tame the Al-induced oxidative damage. Overall, a dose-specific response was

found since significant changes occurred at severe Al stress for almost all measured parameters.

5. Conclusions

The role of SNPs in plant response to abiotic stress is not well known and the attempts to associate these nanoparticles with physiological or metabolic activities have been inconclusive. Here we hypothesized that SNPs treated plants have a greater capacity for Al-detoxification in roots through accumulation and exudation of organic acids and polyphenols and enhanced GST activity. Moreover, SNPs treatment enhances the plant tolerance to Al-induced oxidative stress through regulating ROS production and detoxification. Therefore, SNPs could be used in agricultural practices to improve the production of economically important plants under numerous environmental constraints including Al stress.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.133636.

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