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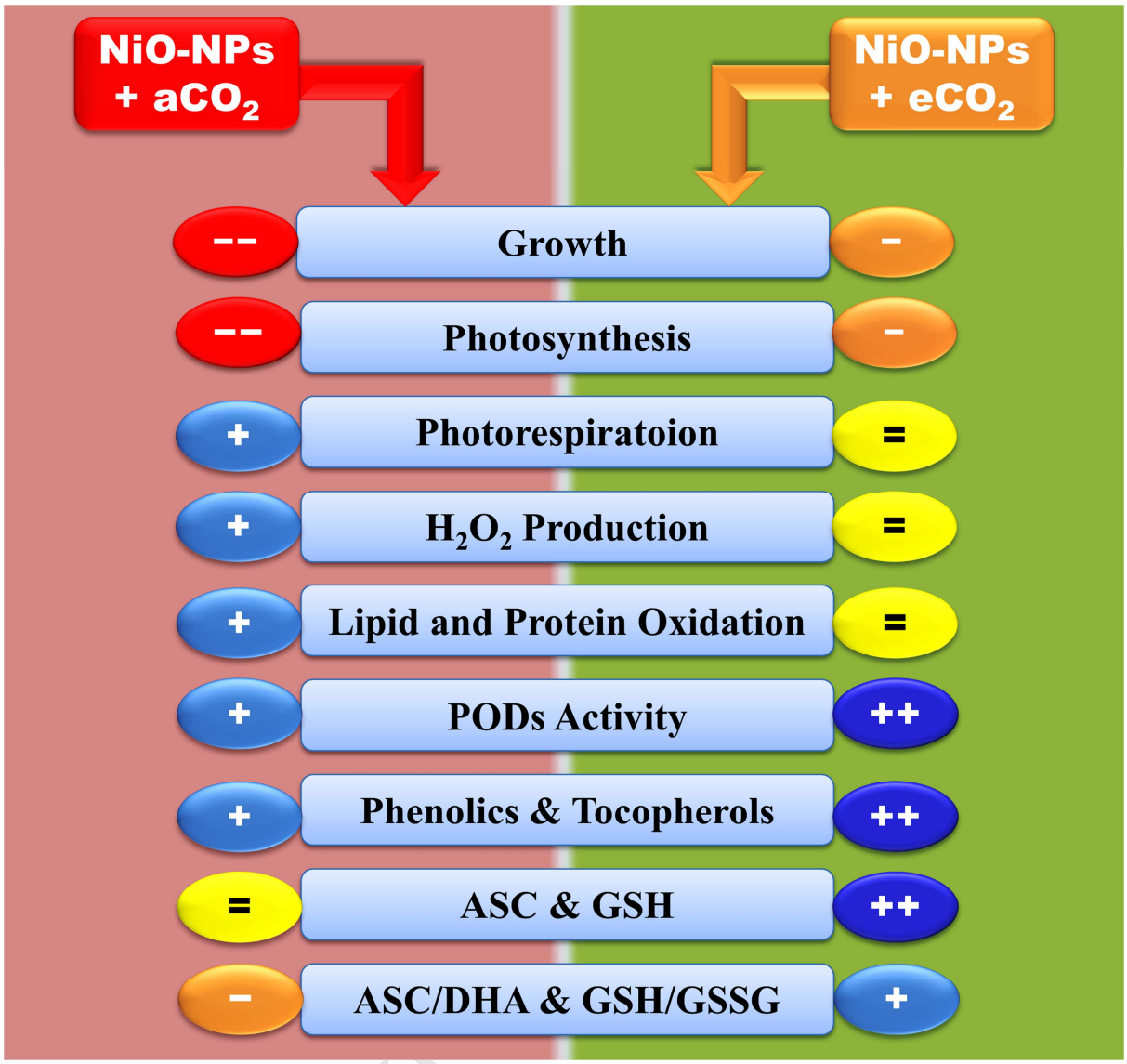

NiO-nanoparticles induce reduced phytotoxic hazards in wheat (*Triticum aestivum* L.) grown under future climate CO_2

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eCO_2 mitigates NiO-NPs-induced oxidative stress in wheat

Keywords

CO_2; NiO nanoparticles; Photosynthesis; Photorespiration; Antioxidants; Glutathione; Ascorbate

Abstract

Due to industrialization and expansion of nanotechnology, ecosystem contamination by nanoparticles is likely. Overall, nanoparticles accumulate in environmental matrices and induce phytotoxicity, however future climate (elevated CO_2 (eCO_2)) may affect the distribution of nanoparticles in ecosystems and alter their impact on plants. In the current study, nickel oxide nanoparticles (NiO-NPs) with an average diameter of 54 nm were synthesized using Triton X-100 and characterized by scanning electron microscopy (SEM), UV-VIS spectroscopy and Fourier transform infrared spectroscopy (FTIR). We have investigated the impact of NiO-NPs at a concentration of 120 mg kg^{-1} soil, selected based on the results of a preliminary experiment, on accumulation of Ni ions in wheat (*Triticum aestivum* L.) and how that could influence plant growth, photosynthesis and redox hemostats under two CO_2 scenarios, ambient (aCO_2, 400 ppm) and eCO_2 (620 ppm). NiO-NPs alone reduced whole plant growth, inhibited photosynthesis and increased the levels of antioxidants. However, improved defense system was not enough to lessen photorespiration induced H_2O_2 accumulation and oxidative damage (lipid and protein oxidation). Interestingly, eCO_2 significantly mitigated the phytotoxicity of NiO-NPs. Although, eCO_2 did not affect Ni accumulation and translocation in wheat, it promoted photosynthesis and inhibited photorespiration, resulting in reduced ROS production. Moreover, it further improved the antioxidant defense system and maintained ASC/DHA and GSH/GSSG redox balances. Organ specific responses to NiO-NPs and/or eCO_2 were indicated and confirmed by cluster analysis. Overall, we suggest that wheat plants will be more tolerant to NiO-NPs stress under future climate CO_2.

1. Introduction

Over the last decades, there has been a growing advance of consumer products based on nano-sized heavy metal. For instance, due to its unique properties, nano-sized nickel oxide (NiO-NPs) is utilized for several products, such as batteries, gas sensors and electrochromic devices (Kim and Lee, 2014; Salimi et al., 2007; Zhu et al., 2012). Inevitably, these nano-sized heavy metals are likely released both from point and nonpoint sources leading to deterioration of environmental matrices and toxification of biota (Nowack and Bucheli, 2007). In fact, because of their higher dissolution, nano-sized heavy metals represent a stronger environmental hazard, as compared with their bulk form (Faisal et al., 2013; Katsumiti et al., 2016). Therefore, risk assessment and amelioration of biological toxicity of nano-sized heavy metals have received much attention (Savolainen et al., 2010; Torabifard et al., 2018). In this regard, many investigations have addressed the toxicological behavior of nano-sized heavy metals on animals, however much less efforts have been devoted for their phytotoxicity (Meena et al., 2015; Sager et al., 2016). Beside their importance in sustenance of food for human, plants are essential component of the ecosystem and are frequently subjected to environmental pollutants, such as nano-sized heavy metal (Antisari et al., 2015). Therefore, substantial efforts should be given to develop efficient approaches for alleviating the impact of such pollutants on plant productivity and limiting their accumulation in the edible parts of the plant.

Despite of the considerable difference in their physical properties, once inside plants nano-sized heavy metals impose the same toxicological mechanisms as their bulk form (Faisal et al., 2013; Soares et al., 2018; Yusuf et al., 2011). It is well recognized that Ni is an essential micronutrient for plants, it is utilized as a cofactor for several metalloenzymes such as urease and glyoxalase I (Brown et al., 1987). However, at higher concentrations of Ni, 10 mg/Kg dry mass

in sensitive species, phytotoxicity symptoms such as chlorosis, necrosis and wilting are evident (Yusuf et al., 2011). The deleterious effect of Ni on plant growth, could be ascribed to its negative impact on photosynthesis, dark respiration and mineral nutrition (Seregin and Kozhevnikova, 2006; Srivastava et al., 2012). By considering its impact on photosynthesis, Ni has been reported to inhibit the synthesis of photosynthetic pigments, replace the Mg ion of chlorophyll molecule, induce damage in the thylakoid membrane, restrain electron transport chain, and inhibit the activity of ribulose-1,5-biphosphate carboxylase/oxygenase (rubisco) and other Calvin cycle enzymes (Soares et al., 2016; Srivastava et al., 2012; Yusuf et al., 2011). Owing to its critical role in metabolism, such suppression of the photosynthetic C assimilation is inevitably reflected on other metabolic processes as well as plant growth and development. Although Ni is not a redox active metal, but it is known to impose oxidative stress, mainly through impairing redox homeostasis leading to accumulation of reactive oxygen species (ROS) that is normally produced during metabolic processes (Baccouch et al., 2006; Pandey and Gopal, 2010). Under these circumstances, the increased levels of ROS can result in damage of cell compartments, by oxidation of lipids and proteins, and disturbance of cellular functions (Apel and Hirt, 2004). Therefore, to tolerate the toxic levels of Ni, plants have to develop an efficient antioxidant defense system and to maintain proper performance of the critical physiological processes, such as photosynthesis.

Unlike its bulk counterpart, limited number of studies have addressed the stress imposed by nano-sized Ni, e.g. NiO-NPs, on plants. In this regard, Faisal et al. (2013) assessed the phytotoxicity of NiO-NPs using roots of tomato seedlings as an *in vivo* model. They reported that NiO-NPs treatment reduced root elongation, increased the levels of ROS, lipid peroxidation, glutathione (GSH) and superoxide dismutase (SOD) activities, and induced cell death as

indicated by the increased number of apoptotic and necrotic cells. They ascribed the oxidative damage caused by NiO-NPs to dissolution, uptake and accumulation of Ni ions in the plant tissues, which was significantly higher as compared to the bulk form of NiO. Recently, cultivation of barley in artificial soil treated with NiO-NPs (120 mg kg^{-1}) resulted in significant reduction in growth and photosynthesis related parameters (Soares et al., 2018). They found that NiO-NPs triggered oxidative damage in barley, as revealed by overproduction of ROS and higher lipid peroxidation.

Another factor that represents a future challenge for plants is the progressive increase in the concentration of atmospheric CO_2 (IPCC, 2013). Interestingly, within a physiological range, elevated CO_2 (eCO_2) has been reported to improve plant growth by enhancement of the photosynthetic C assimilation; thus providing the building blocks and energy required for plant growth and development (Misra and Chen, 2015; Tuba et al., 2003; Watanabe et al., 2014). Moreover, it has been documented that eCO_2 could mitigate the adverse effect exerted by various stress factors on plants (Abdelgawad et al., 2016; Pérez-López et al., 2009; Zinta et al., 2014). eCO_2 could not only affect plant growth and physiology, but it may also alter the distribution of contaminants, such as heavy metals, in the ecosystem. In this regard, some investigations have addressed the impact of elevated CO_2 on the uptake, accumulation and partitioning of some heavy metals in hyperaccumulator and nonaccumulator plant species (Guo et al., 2011; Li et al., 2010). In addition, some efforts has been devoted to study the combined effect of eCO_2 and limited number of heavy metal (Cd, Cu and to less extent Pb) on growth, photosynthesis and some parameters related to the antioxidant defense systems in hyperaccumulator plants (Guo et al., 2015; Jia et al., 2010; Pietrini et al., 2016). On contrary, much less attention has been given for the effect of eCO_2 on the phytotoxicity induced by nano-sized heavy metals. In this context,

92 eCO_2 was reported to intensify the adverse effect of TiO_2-NPs on growth of rice and wheat by
93 improving the uptake and translocation of Ti (Du et al., 2017; Jiang et al., 2017). Moreover,
94 Yadav et al. (2014) found that eCO_2 treatment improved the accumulation of Fe and Zn in
95 different parts of rice plant grown in hydroponic cultures supplemented with different
96 concentrations of Fe_2O_3-NPs and ZnO-NPs, respectively. However, none of these studies had
97 investigated the impact of eCO_2 on the antioxidant defense system of plants under nano-sized
98 heavy metal contamination. Moreover, so far there are no studies investigating the concurrent
99 effect of nano-sized Ni and eCO_2 on plants

100 In the current study, we have addressed the impact of NiO-NPs, alone or in combination
101 with future climate CO_2, on growth and redox homeostasis in one of the most important crops for
102 human food, bread wheat (*Triticum aestivum* L.). The uptake and accumulation of Ni ions and
103 the triggered changes in photosynthesis, photorespiration, molecular ROS scavengers and
104 antioxidant enzymes as well as the detailed changes in ASC-GSH cycle were studied in wheat
105 subjected to NiO-NPs (120 mg/Kg soil) under two levels of CO_2, ambient (aCO_2, 400 ppm) and
106 eCO_2 (620 ppm).

107

2. Materials and methods

2.1. Preparation *of Nickel oxide nanoparticles (NiO-NPs)*

NiO-NPs were synthesized following the method adopted by Kooti and Jorfi (2009). To 200 ml of $NiCl_2.6H_2O$ (0.1 M), 5ml of Triton X-100 was added and the solution was stirred for 15 min. Then, 200ml of sodium hypochlorite solution containing 0.1M of NaOH was added drop wise under continuous stirring for 20 min. The black precipitate of NiO_2 was performed at 6000 rpm for 10 min, then washed with distilled water. The collected precipitate was placed into a beaker containing 50 mL of methanol and stirred for 2 h till the black solid turned to dark olive green indicating the formation of NiO. The green precipitate was collected by centrifugation and washed with distilled water and methanol and then dried in an oven at 90°C for 5 h, then calcined at 300°C for 6h to afford nanosized NiO.

2.2. Characterization of NiO-NPs

2.2.1. Size and morphology

The size and morphology of synthesized NiO-NPs powder were characterized using JEOL, JSM-6510 scanning electron microscope (SEM). The instrument was operated at 30 keV and scanning electron imaging was taken with a resolution of 50,000 and a width of 250 mm on a 0.5 µm scale.

2.2.2. Ultraviolet-Visible Spectroscopy (UV-VIS)

The optical features of NiO-NPs were determined on PE-UV Lambda 950 UV/VIS/NIR in the wavelength range of 250-800 nm. NiO-NPs powders were suspended in Dimethylformamide (DMF) with the help of a magnetic stirrer for 30 min prior analysis by UV-VIS spectrophotometer.

2.2.3. Fourier transform infrared spectroscopy (FTIR)

FTIR analysis of NiO-NPs powder was performed using a Bruker IFS66V FTIR spectrometer for the frequency range between 4000 and 400 cm^{-1} at room temperature. NiO sample was mixed with potassium bromide, which were ground and pressed into a transparent pellet with a diameter of 13 mm.

2.3. Experimental setup

Uniform wheat seeds (*Triticum aestivum* L., Sids 13, a commercial variety) were surface sterilized with 35 % (v/v) of commercial bleach for 30 min. Sterilized seeds were grown in pots contained artificial soil with 5% organic matter and pH 6 (OECD, 2006). Pots were transferred to controlled-growth rooms under varied climate conditions, viz: 1) ambient CO_2 (aCO_2) (400 ppm), 2) aCO_2 + NiO-NPs (120 mg/Kg soil), 3) elevated CO_2 (eCO_2) (620 ppm), 4) eCO_2 + NiO-NPs (120 mg/Kg soil). NiO-NPs were applied after 1 week from sowing in the form of suspension. Other climate conditions included: light intensity of 150 µmol m^{-2} s^{-1}, 16/8 h day/night photoperiod, 21/18 ^{o}C air temperature and 60/70% humidity. After 3 weeks of NiO-NPs exposure, roots and leaves were harvested. Harvested materials were immediately frozen in liquid N_2, and stored at -80 °C for biochemical analysis. The concentration of NiO-NPs was chosen based on the results of preliminary assays on the impact of a range of NiO-NPs doses (60-480 mg/Kg soil) on growth and accumulation of stress markers in wheat plants, under both aCO_2 and eCO_2 (supplementary materials).

2.4. Determination of photosynthesis rate and stomatal conductance

For measuring the rates of photosynthesis and stomatal conductance the protocols described in our previous work (Al Jaouni et al., 2018) were followed up. Photosynthesis at saturating light (Asat, µmol CO2 m^{-2} s^{-1}) were determined (LI-COR LI-6400, LI-COR Inc., Lincoln, NE, USA) on the youngest fully expanded. A minimum of 5 min of leaf equilibration

was set at each step before data were logged. LI-COR leaf chamber conditions were set at 400 or 620 ppm CO_2, 22 ºC (block temperature) and saturated photon flux density (1500 µmol m^{-2} s^{-1}). Stomatal conductance (gs, mol CO_2 m- m^{-2} s^{-1}) was measured on the abaxial side of fully developed leaves with a Leaf Porometer (Model SC-1, Decagon Devices, Inc., Hopkins, Pullman, WA USA). The average vapor pressure deficit and leaf temperature were 0.37±0.02 and 20±2.02, respectively. Chlorophyll fluorescence was measured on dark acclimated fully expanded leaves using FMS-2 pulse-modulated fluorometer (Hansatech Instruments, Norfolk, UK). The minimal fluorescence (F_0) and maximal fluorescence (F_m) were measured for 30 min dark-adapted leaves. The photochemical efficiency of PSII (F_v/F_m) for dark adapted leaves were calculated, where F_v (Maximal variable fluorescence) = $F_m - F_0$.

2.5. Photorespiration

Glycolate oxidase (GO) and hydroxypyruvate reductase (HPR) activities were measured according to (Feierabend and Beevers, 1972; Schwitzguebel and Siegenthaler, 1984), respectively. Moreover, to calculate glycine/serine ratio, frequently used as an index to estimate photorespiration (Kebeish et al., 2007), glycine and serine were quantified using a Waters Acquity UPLC-tqd system (Al Jaouni et al., 2018).

2.6. Stress markers

Lipid peroxidation was determined according to the thiobarbituric acid assay (Hodges et al., 1999). Protein oxidation was assessed via carbonyl quantification (Levine et al., 1994). Xylenol orange method was employed to quantify Hydrogen peroxide (H_2O_2) in TCA (0.1%) extract of plant samples (Murphy and Noack, 1994). The concentration of nitric oxide was determined by measuring the production of methemoglobin (Feelisch and Noack, 1987).

176 *2.7. Total antioxidant capacity (TAC)*

177 TAC was assessed according to the FRAP (ferric reducing antioxidant power) method
178 described by Benzie and Strain (1999) using trolox (Sigma-Aldrich, St. Louis, MO, USA) as a
179 standard.

180 *2.8. Triphenyltetrazolium chloride–dehydrogenase activity (TTC-DHA) assay*

181 The activity of dehydrogenase was measured by TTC reduction method (Casida Jr et al.,
182 1964). Samples were treated with 0.1 M TTC and incubated for 24 h at 37°C. Formed triphenyl
183 formazan was extracted and assayed at 485 nm. *TTC-DHA* was expressed as µg formazan/g
184 tissue

185 *2.9. Antioxidant metabolites*

186 Reduced ascorbate (ASC) and reduced glutathione (GSH) were quantified by HPLC.
187 Total ascorbate (ASC+DHA) and glutathione (GSH+GSSG) concentrations were determined
188 after reduction with DTT as described in Zinta et al. (2014). Polyphenols and flavonoids were
189 quantified using Folin-Ciocalteu and aluminum chloride assays, respectively (Mohamed et al.,
190 2017). Separation and quantification of tocopherols were conducted by HPLC (Shimadzu,
191 Hertogenbosch, Netherlands) using normal phase conditions (Particil Pac 5 µm column mate-
192 rial, length 250 mm, i.d. 4.6 mm).

193 *2.10. Antioxidant enzyme activities*

194 Superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione peroxidase
195 (GPX), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate
196 reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione-S-transferase (GST)
197 activities were assayed according to the protocols described in our previous work (Abdelgawad
198 et al., 2015).

199 *2.11. Statistical analyses*

200 Data analyses were performed using the procedures provided in Statistical Analysis
201 System (SPSS Inc., Chicago, IL, USA). Data normality and the homogeneity of variances were
202 checked using the KolmogoroveSmirnov test and Levene's test, respectively. All the data were
203 subjected to one-way Analysis of Variance (ANOVA). Tukey's Test ($p < 0.05$) was carried out
204 as the post-hoc test for mean separations. Number of replicates for each experiment were three (n
205 $= 3$). Cluster analysis was performed by using Pearson distance metric of the MultiExperiment
206 Viewer (MeV)™ 4 software package (version 4.5, Dana-Farber Cancer Institute, Boston, MA,
207 USA).

3. Results

3.1. Characterization of NiO-NPs

The size and morphology of the synthesized NiO-NPs is shown in Figure 1a. SEM image revealed that, particles are homogeneous shaped particles with an average size of 54 nm. Additionally, the image shows that, the synthesized particles have a tendency to form uniform aggregations, therefore, uniform particle dimension (shape and size). UV-VIS analysis (Figure 1b) displays that, NiO nanoparticles exhibit the characteristic absorption edge in the range of 280-350 nm. From FTIR spectrum of fabricated NiO nanoparticles (Figure 1c), the peak at 3418.1 cm^{-1} is ascribed to O-H group stretching vibrational mode and the peak at 1625.48 cm^{-1} is attributed to the bending vibrational mode of O-H group. The appearance of these peaks indicated the presence of water molecules trapped on the surface of NiO particles from surrounding atmosphere. Additionally, there is band absorption in the range of 424.38- 542.18 cm^{-1}, corresponding to the Ni-O bond stretching vibration, while, absorption bond at 636 cm^{-1} is assigned to Ni-O-H stretching bond.

3.2. Ni accumulation and biomass production

plants grown under eCO_2 have significantly higher biomass than those grown in aCO_2 (Table 1). NiO-NPs reduced biomass production under both levels of CO_2. However, the NiO-NPs-induced biomasses reduction was much lower under eCO_2 than under. Root growth was more affected by NiO-NPs than shoot, whereas about 52% and 41% inhibition in dry masses were observed for root and shoot, respectively. On the other hand, NiO-NPs increased Ni ions accumulation in root and shoot at both CO_2 levels. Meanwhile, the concentration of Ni ions in both organs was not significantly affected by CO_2 enrichment.

3.3. Photosynthesis and stomatal conductance

Elevated CO_2 alone did not significantly affect chlorophyll and carotenoids contents, chlorophyll fluorescence, rubisco activity or stomatal conductance; however it significantly improved the net photosynthetic rate and consequently increased starch level (Table 2). On the other hand, NiO-NPs significantly decreased all photosynthesis related parameters, except for the content of carotenoids that was significantly increased. Interestingly, the synchronous treatment with eCO_2 and NiO-NPs significantly mitigated NiO-NPs-induced inhibition in photosynthesis related parameters . The values of photosynthesis related parameters, except for carotenoids, under the combined treatment (eCO_2+NiO-NPs) were significantly higher than those in NiO-NPs alone treatment (Table 2).

3.4. Photorespiration

As compared with the control plants, eCO_2 had no significant impact on photorespiratory key enzymes (GO or HPR) as well as the glycine/serine ratio; while NiO-NPs significantly induced them (Table 2). Interestingly, the co-application of eCO_2 with NiO-NPs significantly reduced the impact of NiO-NPs on photorespiration related parameters.

3.5. Stress markers, TAC and TTC-DHA

Atmospheric enrichment with eCO_2 had no significant effect on the levels of H_2O_2, MDA, NO or protein carbonyls in either root or shoot tissues (Figure 2). On contrary, NiO-NPs treatment significantly increased H_2O_2 and MDA levels in root and shoot and protein carbonyls and NO level in root of aCO_2 treated plants. The coexistence of eCO_2 and NiO-NPs significantly reduced the levels of the measured oxidative markers to values comparable to those detected in the control plant.

Although, TAC or TTC-DHA levels were not altered in eCO_2 treated plants, they were scientifically reduced in shoot and root after NiO-NPs treatment (Figure 2). Meanwhile, the incorporation of eCO_2 mitigated the NiO-NPs-induced inhibition in TTC-DHA, which was in consistence with the improvement in TAC (FRAP) in root and to less extent in shoot.

3.6. Antioxidant metabolites

Phenolics, flavonoids and tocopherols levels were not significantly affected by eCO_2 treatment (Figure 2). NiO-NPs treatment alone improved the accumulation of these antioxidants in root but not in shoot tissues. CO_2 enrichment further improved the levels of tocopherols in root and that for phenolics in shoot of NiO-NPs treated plant, while it had no significant impact on the content of flavonoids.

3.7. SOD, CAT, POD and GST activities

CO_2 enrichment had no significant impact on SOD, CAT and POD activities (Figure 2). Plant treated with NiO-NPs showed increase in POD activity in both organs, but not in SOD activity, relative to the control plant. On the other hand, CAT activity significantly increased only in root-treated with NiO-NPs and eCO_2. No change in GST activity was observed for shoot after eCO_2 and/or NiO-NPs treatments, while it was significantly improved in root of NiO-NPs treated plant regardless of CO_2 level, whereas the improvement was more evident in the combined treatment.

3.8. ASC-GSH cycle

GSH/ASC cycle related enzymes and metabolites were not altered by eCO_2 treatment in root (Figure 2). However, eCO_2 treatment caused significant improvement in the MDAR activity

and in the ratios of ASC/DHA and GSH/GSSG in shoot, which was in consequence with reduced levels of DHA and GSSG, respectively, rather than the increase in the concentrations of their reduced forms, compared to control plant.

Regardless of the plant organ, NiO-NPs treatment improved GPX and GR activities, but not APX, MDAR or DHAR activities. Moreover, NiO-NPs treatment increased the contents of DHA in root and GSSG in both organs resulting in lower ASC/DHA and GSH/GSSG ratios in plant grown under aCO_2. On the other hand, the synchronous existence of NiO-NPs and eCO_2 resulted in significant enhancement in GR and DHAR activities in root and shoot and that for GPX and APX in root only, as compared with NiO-NPs alone treatment. Moreover, the combined treatment (NiO-NPs+eCO_2) significantly recovered the decrease in ASC/DHA and GSH/GSSG ratios in both organs. Such improvement in ASC/DHA and GSH/GSSG ratios was mainly in consistence with increased levels of the reduced forms, ASC and GSH.

4. Discussion

The current study was conducted to assess, for the first time, how the future climate (eCO_2) could alter the growth retardation and oxidative damage induced by NiO-NPs in wheat. Although few studies have addressed the concurrent effect of eCO_2 and nano-sized heavy metals on plants (Guo et al., 2011; Li et al., 2010), none of them had investigated the physiological and biochemical mechanisms underlying the mitigating effect of eCO_2 on heavy metal-induced oxidative stress in plants. Therefore, the current results have been discussed in view of the previous studies addressing the combined effect of eCO_2 and other types of oxidative stress inducing agents such as the bulk counterpart of heavy metals.

4.1. eCO_2 did not affect Ni accumulation, but reduced NiO-NPs induced growth inhibition in wheat plants.

As one of the essential micronutrient for plants, Ni is normally taken up by roots and then translocated to shoot in extremely small amounts, not exceeding few micrograms per gram dry mass in nonaccumulators (Yusuf et al., 2011). However, increasing the level of Ni in the growth medium is known to increase the uptake and translocation of Ni in plants (Pandey and Gopal, 2010; Rodrigues et al., 2017). Compared to their bulk counterpart NiO-NPs are reported to provide higher levels of dissolved Ni ions, and consequently could exhibit higher uptake and accumulation in the plant tissues (Faisal et al., 2013). In the current study, application of NiO-NPs into the soil caused manifold increases in the concentration of Ni in both root and shoot tissues, about 410 and 78 fold respectively under aCO_2 environment (Table 1). This result suggests that Ni is preferentially accumulated in root tissues, a mechanism whereby plants act to tolerate Ni toxicity by limiting the translocation of Ni to the photosynthetic organs (Soares et al.,

2018). In line with the present result, Soares et al. (2018) reported that root of barley grown under NiO-NPs had accumulated much higher Ni than shoot did. Similar behavior was recorded for wheat and other plants treated with bulk Ni (Pandey and Gopal, 2010; Rodrigues et al., 2017; Soares et al., 2016; Uruç Parlak, 2016).

The comparable levels of Ni that detected in plants grown under both levels of CO_2 suggest that eCO_2 had no impact on the processes of uptake and translocation of Ni in wheat. In fact, no studies have discussed the accumulation of Ni in eCO_2 treated-plants, however the uptake and accumulation of other heavy metals such as Cd, Cu, Pb, Ti, Zn, and Fe from their nano-sized or bulk forms under eCO_2 have been previously reported (Du et al., 2017; Guo et al., 2015, 2011, Jia et al., 2016b, 2010; Jiang et al., 2017; Yadav et al., 2014). Moreover, heavy metal accumulation in eCO_2 treated-plants seems to be dependent on the plant species and the metal studied (Guo et al., 2015, 2011; Jia et al., 2010). For instance, Cd accumulation increased in wheat and rice plants (Guo et al., 2011), but not in poplars and willows (Guo et al., 2015) under CO_2 enriched environment.

Despite of the considerable difference in their physical properties, nano-sized heavy metals share the same phytotoxicity mechanisms as their bulk counterpart (Faisal et al., 2013; Soares et al., 2018; Yusuf et al., 2011). It is well known that Ni has deleterious effect on various critical processes for plant growth and development such as cell division, nutrient utilization and photosynthesis (Seregin and Kozhevnikova, 2006; Srivastava et al., 2012). The accumulation of Ni reported herein was concomitant with a great reduction in wheat growth (Table 1). Such growth retardation could be attributed to the adverse impact of NiO-NPs on photosynthesis related parameters (chlorophyll and starch contents, chlorophyll fluorescence, rubisco activity and the net photosynthetic rate). In agreement, parallel reductions in growth and chlorophyll

concentration and fluorescence in plants exposed to NiO-NPs (Soares et al., 2018) or bulk Ni (Pandey and Gopal, 2010; Pandolfini et al., 1992; Rodrigues et al., 2017; Soares et al., 2016; Yusuf et al., 2011) were reported.

Synchronous application of eCO_2 significantly recovered the inhibitory impact of Ni on photosynthesis related parameters (Table 2) and consequently improved wheat growth (Table 1). This stress mitigation role could be explained by the bio-fertilization activity of eCO_2, particularly in C_3 plants such as wheat. In this context, increasing the CO_2 concentration, within a physiological limit, could improve C fixation and thus lead to higher availability of non-structural carbohydrates that are needed for normal plant growth and metabolism (Al Jaouni et al., 2018). In fact, there is no literature about the combined effect of eCO_2 and Ni stress on plants, however there are evidence that eCO_2 could reduce the deleterious impact of other heavy metals, especially Cd, on growth and photosynthesis of hyperaccumulator and nonaccumulator plants (Jia et al., 2016a; Li et al., 2010). The protective effect of eCO_2 on plants suffering Cd and/or Pb stress was attributed to enhanced chlorophyll content, intracellular CO_2 concentration and net assimilation rate (Guo et al., 2015; Jia et al., 2016b, 2010). Oppositely, eCO_2 further strengthen the inhibitory effect of TiO_2-NPs on growth of rice and wheat (Du et al., 2017; Jiang et al., 2017).

4.2. eCO_2 mitigated NiO-NPs-induced oxidative damage in wheat via reducing H_2O_2 production

As the current results revealed, NiO-NPs treatment had increased the endogenous Ni levels in wheat. Although Ni is not considered as a redox active metal, but at phytotoxic levels Ni is known to cause oxidative stress by impairing redox homeostasis leading to accumulation of ROS (Gajewska and Skłodowska, 2007). Such over accumulation of ROS could trigger damage

of cell compartments by oxidation of lipids and proteins (Baccouch et al., 2006; Pandey and Gopal, 2010). Oxidative damage due to increased uptake and accumulation of Ni from both nano-sized and bulk materials was reported in wheat and other plant species (Baccouch et al., 2006; Faisal et al., 2013; Pandolfini et al., 1992; Soares et al., 2018; Yusuf et al., 2011). In agreement, we found that NiO-NPs induced sever oxidative stress i.e., higher levels of H_2O_2, oxidation products of lipids and proteins (MDA and protein carbonyls, respectively) and NO (a signal molecule in stress response), and reduced values of TTC-DHA (indication for cell metabolic activity, (Berridge et al., 2005)). Similar to our results, Faisal et al. (2013) and Soares et al. (2018) reported increases in the levels of ROS and MDA in tomato and barley after treatment with NiO-NPs.

Interestingly, the coexistence of eCO_2 with NiO-NPs significantly mitigates the Ni-induced oxidative stress, whereas treated plants grown under eCO_2 showed less tissue damage e.g., reduced H_2O_2, MDA, protein carbonyls and NO levels, and increased TTC-DHA. In this context, eCO_2 treatment has been reported to ameliorate the oxidative damage imposed by Cd on poplar, willow and *lolium* sp. as indicated by lower levels of MDA as compared with Cd-stressed plants grown under aCO_2 (Guo et al., 2015; Jia et al., 2010). Moreover, eCO_2 was found to to decrease the production of H_2O_2, MDA and protein carbonyls in plants suffering several types of oxidative stress-inducing agents such as heat, drought, salinity and ozone (Abdelgawad et al., 2016, 2015; Zinta et al., 2014).

The mitigating effect of eCO_2 can be partly explained by downregulation of the stress induced-H_2O_2 generating process such as photorespiration as indicated by decreased Gly/Ser ratio and GO and HPR activities. Although H_2O_2 is produced by several cellular activities such as NADH oxidase, lipid oxidation and mitochondrial electron transport chain, however

375 photorespiration is considered as the major and fastest pathway for production of H_2O_2 (Costa et
376 al., 2010; Foryer and Noctor, 2000; Quan et al., 2008). The lower photorespiration and higher
377 photosynthesis rates as affected by eCO_2 could be attributed to the improvement in the
378 carboxylation rather than the oxygenation activity of rubisco (Al Jaouni et al., 2018). Decreased
379 GO and HPR and Gly/Ser ratio as indication for lower photorespiration were frequently seen in
380 eCO2 (Abdelgawad et al., 2015; Zinta et al., 2014). Taking together, these results suggest a role
381 for eCO_2 in maintaining redox homeostasis in plants grown under Ni stress by reducing the
382 production of ROS.

383 *4.3. eCO_2 improved ROS detoxification system and maintained GSH/GSSG and ASC/DHA redox*
384 *balances in NiO-NPs stressed wheat.*

385 Plants have evolved different strategies to maintain the levels of ROS under control.
386 These include molecular antioxidant scavengers, such as carotenoids, tocopherols, phenolics,
387 flavonoids, ASC and GSH, and the antioxidant enzymes SOD, CAT and various peroxidases
388 (Blokhina et al., 2003). Among these, the ASC/GSH pathway is of immense importance for
389 scavenging H_2O_2 (Foyer and Noctor, 2009). Overall, we found that eCO_2 improved TAC in root
390 and to less extent in shoot of NiO-NPs treated wheat. Such impact was in consistence with the
391 positive impact of eCO_2 on the accumulation of tocopherols in root and that for phenolics in both
392 root and shoot, under NiO-NPs stress. Similar increases in the contents of tocopherols and
393 phenolics were reported in *Arabidopsis thaliana* subjected to eCO_2 under combined heat and
394 drought stress (Zinta et al., 2014). The enhanced accumulation of phenolics and vitamins in
395 eCO_2 treated plants could be ascribed to the increased availability of C and N intermediates and
396 metabolic energy required for their biosynthesis (Herms and Mattson, 1992). In this regard, the

397 regulatory effect of eCO_2 on C and N metabolism is well known (Al Jaouni et al., 2018; Noguchi
398 et al., 2015; Nunes-Nesi et al., 2010).

399 The improved activity of peroxidases (APX, GPX and POX) reported herein under eCO_2
400 climate suggests a significant role for direct H_2O_2 scavenging enzymes in detoxification of H_2O_2
401 in wheat subjected to Ni stress. Similarly, Gajewska et al. (2006) recorded four-fold
402 improvement in POX activity in shoot of wheat treated with 200 μM Ni. Moreover, the activity
403 of APX were significantly improved by eCO_2 treatment in willow grown in high Cd
404 contamination (Guo et al., 2015). On the other hand, SOD activity was not affected by NiO-NPs
405 and or eCO_2 treatment in either root or shoot. In disagreement, SOD was improved in tomato and
406 barley subjected to NiO-NPs (Faisal et al., 2013; Soares et al., 2018). Thus, the response of SOD
407 to NiO-NPs seems to be species dependent.

408 Elevated CO_2 treated-plants possessed much higher GSH/GSSG and ASC/DHA ratios
409 during NiO-NPs stress. Such improved redox status could be attributed to the enhanced activities
410 of GSH and ASC recycling enzymes, GR and MDAR respectively, under CO_2 enriched
411 environment. This result points to a role for the GSH/ASC cycle, upregulated by eCO_2, in
412 confrontation of the oxidative stress imposed by Ni in wheat. GSH and ASC levels and
413 GSH/GSSG and ASC/DHA ratios are known to be constitutively elevated in plants adapted to
414 oxidative stress induced by heavy metals and other agents (Foyer, 1993; Seth et al., 2012; Yadav,
415 2010). Similar to our results, Jia et al. (2010) reported higher GSH levels in Cd-stressed *Lolium*
416 plants grown under eCO_2 compared to those grown in aCO_2. eCO_2 treatments also has been
417 found to enhance GR activity in willow plant exposed to Cd (Guo et al., 2015) and retrieve the
418 depletion in GSH/GSSG ratio caused by Cd in *Lemna minor* (Pietrini et al., 2016).

In fact, the enhanced GSH level is not only necessary for maintaining GSH/GSSG redox balance and regeneration of ASC, as substrate for DHAR, but it is also important for detoxification of heavy metals and xenobiotics, due to nucleophilic nature of its thiol group, in a reaction catalyzed by GST (Yadav, 2010). Interestingly, the current result revealed that the activity of GST was improved in wheat roots as affected by NiO-NPs, the effect that strengthened by the co-application of eCO_2. In line with this result, Gajewska et al. (2006) reported significant enhancement in GST activity in wheat in response to Ni treatment. Similar improvement in GST activity was reported in *Pisum sativum* subjected to high concentrations of Cd in hydroponic culture (Dixit et al., 2001).

4.4. Root and shoot respond differently to NiO-NPs under future eCO_2 climate

Hierarchical clustering of stress markers and antioxidant metabolites and enzymes in root and shoot of wheat revealed variations in their response to NiO-NPs and/or eCO_2 treatments (Figure 3). Overall, three major groups are distinguished: those were higher in shoot than in root under eCO_2 levels, and were mainly improved in shoot of NiO-NPs treated plants by co-application of eCO_2 (group 1); those were not affected by eCO_2 alone treatment in both organs, but were improved in shoot and to more extent in root as affected by the combined treatment (group 2); and those were improved in root only in response to NiO-NPs, regardless of CO_2 level (group 3). Stress markers and most of direct H_2O_2 scavenging enzymes , were confined to group 3 and antioxidant metabolites were mainly clustered within group 2, while ASC-GSH cycle related parameters were distributed all over the groups. The relatively higher accumulation of H_2O_2, MDA, protein carbonyls and H_2O_2 detoxifying enzymes in roots of NiO-NPs treated wheat indicates a severe oxidative damage in root than in shoot. Such effect could be ascribed to the preferential accumulation of Ni ions in root, a mechanism whereby the plant act to limit the

translocation of Ni to the photosynthetic organs. Similarly, Soares et al. (2018) reported that root of barley grown under NiO-NPs accumulated much higher Ni than shoot did. Moreover, Faisal et al. (2013) reported that the accumulation of Ni ions in the roots of tomato seedlings treated with NiO-NPs had resulted in sever oxidative damage as indicted by elevated levels of ROS and lipid peroxidation products.

5. Conclusion

This study provides the first report regarding the interactive effect of NiO-NPs and eCO_2 on growth, photosynthesis, photorespiration and redox homeostasis in plants. Compared to NiO-NPs alone, the co-application of eCO_2 improved growth and photosynthesis and mitigated Ni-induced oxidative stress in wheat. In view of the present results, the plausible strategies whereby eCO_2 mitigates the oxidative stress imposed by NiO-NPs in wheat include: (1) recovery of the deleterious effect of Ni on photosynthesis; (2) Inhibition of photorespiration and therefore decreasing the production of H_2O_2; (3) increasing ROS detoxification via improving antioxidant defense. Consequently, reduced cellular damage (oxidized proteins and membrane lipids) and proper GSH/GSSG and ASC/DHA redox balances (Figure 4) were observed in this study.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Figure Captions

Figure 1. Characterization of NiO-nanoparticles using scanning electron microscope (SEM) (a), UV-VIZ spectroscopy (b) and Fourier transform infrared spectroscopy (FTIR, c).

Figure 2. Effect of NiO-nanoparticles (NiO-NPs), elevated CO_2 (eCO_2) and their combination (NiO-NPs+eCO_2) on the accumulation of oxidative stress markers, levels of molecular antioxidants and the activity of antioxidant enzymes in shoot (A) and root (B) of wheat. Each value is the mean of 3 independent replicates and vertical bars represent the standard error. Same lower-case letters on bars, in the same chart, indicate non-significant difference at the 0.05 probability level. MDA, malondialdehyde; NO, nitric oxide; TTC-DHA, triphenyl tetrazolium chloride–dehydrogenase activity; TAC, total antioxidant capacity; SOD, superoxide dismutase; APX, ascorbate peroxidase; CAT, catalase; POD, peroxidase; GPX, glutathione peroxidase; GST, glutathione S-transferase; ASC, reduced ascorbate; DHA, oxidized ascorbate; GSH, reduced glutathione; GSSG, oxidized glutathione; DHAR, dehydroascorbate reductase; MDHAR, monodehydroascorbate reductase; GR, glutathione reductase. Same lower-case letters on bars indicate no significant difference at the 0.05 probability level.

Figure 3. Heatmap for oxidative stress markers and antioxidant metabolites and enzymes in root and shoot of wheat grown under NiO-nanoparticles (NiO-NPs), elevated CO_2 (eCO_2) and their combination (NiO-NPs+eCO_2). MDA, malondialdehyde; NO, nitric oxide; TTC-DHA, triphenyl tetrazolium chloride–dehydrogenase activity; TAC, total antioxidant capacity; SOD, superoxide dismutase; APX, ascorbate peroxidase; CAT, catalase; POD, peroxidase; GPX, glutathione peroxidase; GST, glutathione S-transferase; ASC, reduced ascorbate; DHA, oxidized ascorbate; GSH, reduced glutathione; GSSG, oxidized glutathione; DHAR, dehydroascorbate reductase; MDHAR, monodehydroascorbate reductase; GR, glutathione reductase. The relative accumulation patterns are shown in the heatmap based on the average value (n = 3) for each parameter. Red and blue colors indicate lower and higher concentrations, respectively.

Figure 4. An overview of the impact of NiO-nanoparticles (NiO-NPs) on growth, physiology and redox homeostasis of wheat under ambient CO_2 (aCO_2) or elevated CO_2 (eCO_2). As compared with the control plant, +, = and − indicate positive, tolerant and negative impact, respectively. PODs, peroxidases; ASC, reduced ascorbate; DHA, oxidized ascorbate; GSH, reduced glutathione; GSSG, oxidized glutathione.

Table 1: Effect of NiO nanoparticles (NiO-NPs), elevated CO_2 (eCO_2) and their combination (NiO-NPs+eCO_2) on biomass production and accumulation of Ni ions in shoot and root of wheat. Values are mean ± standard error of three independent replicates. Means followed by the same lower-case letter in a row do not differ significantly at the 0.05 probability level.

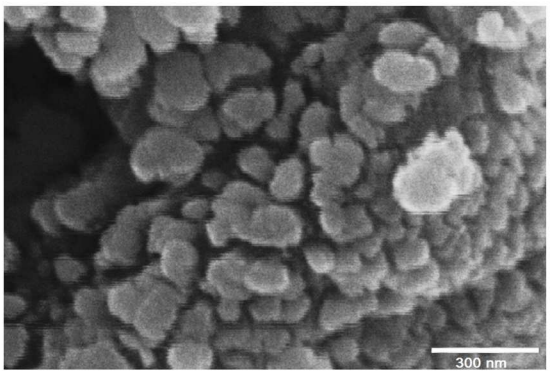
	Control	eCO_2	NiO-NPs	NiO-NPs+eCO_2
Fresh weight (g plant^{-1})				
Shoot	2.244 ± 0.162c	2.899 ± 0.109d	1.319 ± 0.036a	1.999 ± 0.101b
Root	0.365 ± 0.026cd	0.401 ± 0.018d	0.147 ± 0.006a	0.247 ± 0.012b
Total	2.610 ± 0.180c	3.300 ± 0.120d	1.465 ± 0.040a	2.240 ± 0.110b
Dry weight (g plant^{-1})				
Shoot	0.337 ± 0.015c	0.422 ± 0.010d	0.198 ± 0.007a	0.294 ± 0.015b
Root	0.050 ± 0.003c	0.069 ± 0.002d	0.024 ± 0.001a	0.040 ± 0.002b
Total	0.388 ± 0.020c	0.491 ± 0.010d	0.222 ± 0.010a	0.334 ± 0.020b
Ni accumulation (mg g^{-1} DW)				
Shoot	0.0039 ± 0.001a	0.0037 ± 0.001a	0.307 ± 0.083b	0.292 ± 0.039b
Root	0.0044 ± 0.000a	0.0041 ± 0.001a	1.806 ± 0.155b	1.688 ± 0.135b

Table 2:

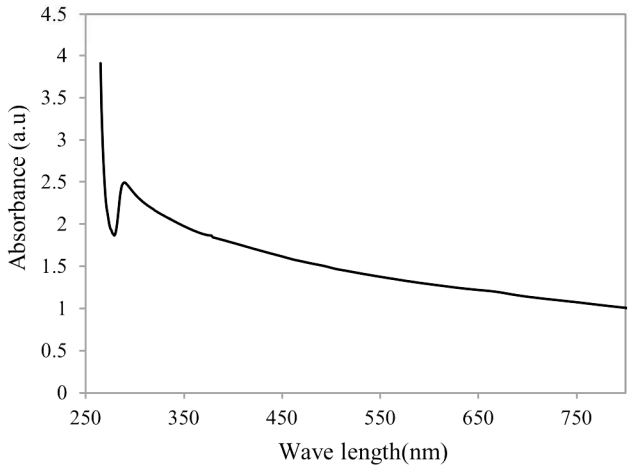
Effect of NiO nanoparticles (NiO-NPs), elevated CO_2 (eCO_2) and their combination (NiO-NPs+eCO_2) on photosynthesis and photorespiration related parameters in wheat. Values are mean ± standard error of three independent replicates. Means followed by the same lower-case letter in a row do not differ significantly at the 0.05 probability level**.**

	Control	eCO_2	NiO-NPs	NiO-NPs+eCO_2
Photosynthesis related parameters				
Chlorophyll (mg g^{-1} FW)	0.15 ± 0.02b	0.19 ± 0.03b	0.05 ± 0.00a	0.12 ± 0.01b
Carotenoids (mg g^{-1} FW)	0.021 ± 0.002a	0.018 ± 0.003a	0.038 ± 0.007b	0.027 ± 0.003a
Rate of photosynthesis (µmol CO_2 m^{-2} s^{-1})	7.29 ± 0.66c	9.45 ± 1.04d	3.24 ± 0.47a	5.94 ± 0.64b
Chlorophyll Fluorescence (F_v/F_m)	0.80 ± 0.00c	0.80 ± 0.01c	0.65 ± 0.01a	0.71 ± 0.01b
Rubisco activity (nmol 3-PGA mg $protein^{-1}$ min^{-1})	71.88 ± 9.25b	76.46 ± 3.33b	31.61 ± 2.77a	74.70 ± 11.43b
Stomatal conductance (mmol m^{-2} s^{-1})	129.98 ± 5.13b	119.72 ± 9.37b	101.23 ± 5.84a	113.77 ± 6.22ab
Starch (mg g^{-1} FW)	6.35 ± 0.73b	7.71 ± 0.69c	3.48 ± 0.41a	5.87 ± 0.24b
Photorespiration related parameters				
GO (unit/mg protein.min)	1.00 ± 0.12a	0.87 ± 0.05a	2.45 ± 0.12b	1.22 ± 0.08a
HPR (unit/mg protein.min)	2.06 ± 0.29a	1.96 ± 0.21a	4.01 ± 0.26b	2.89 ± 0.20a
GLY/SER	0.56 ± 0.02a	0.52 ± 0.05a	0.72 ± 0.04b	0.59 ± 0.05a

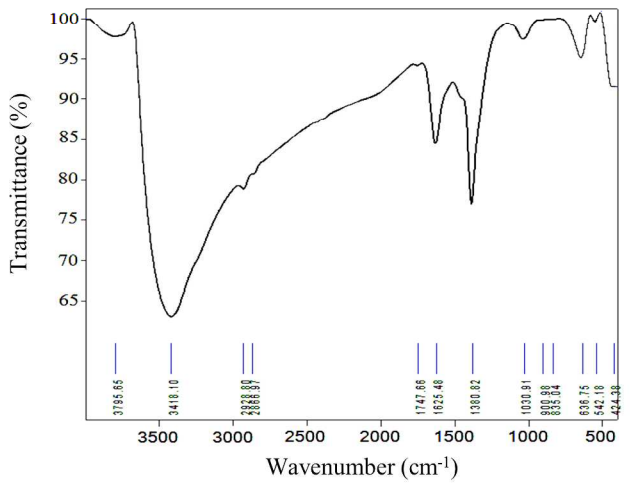
(a)

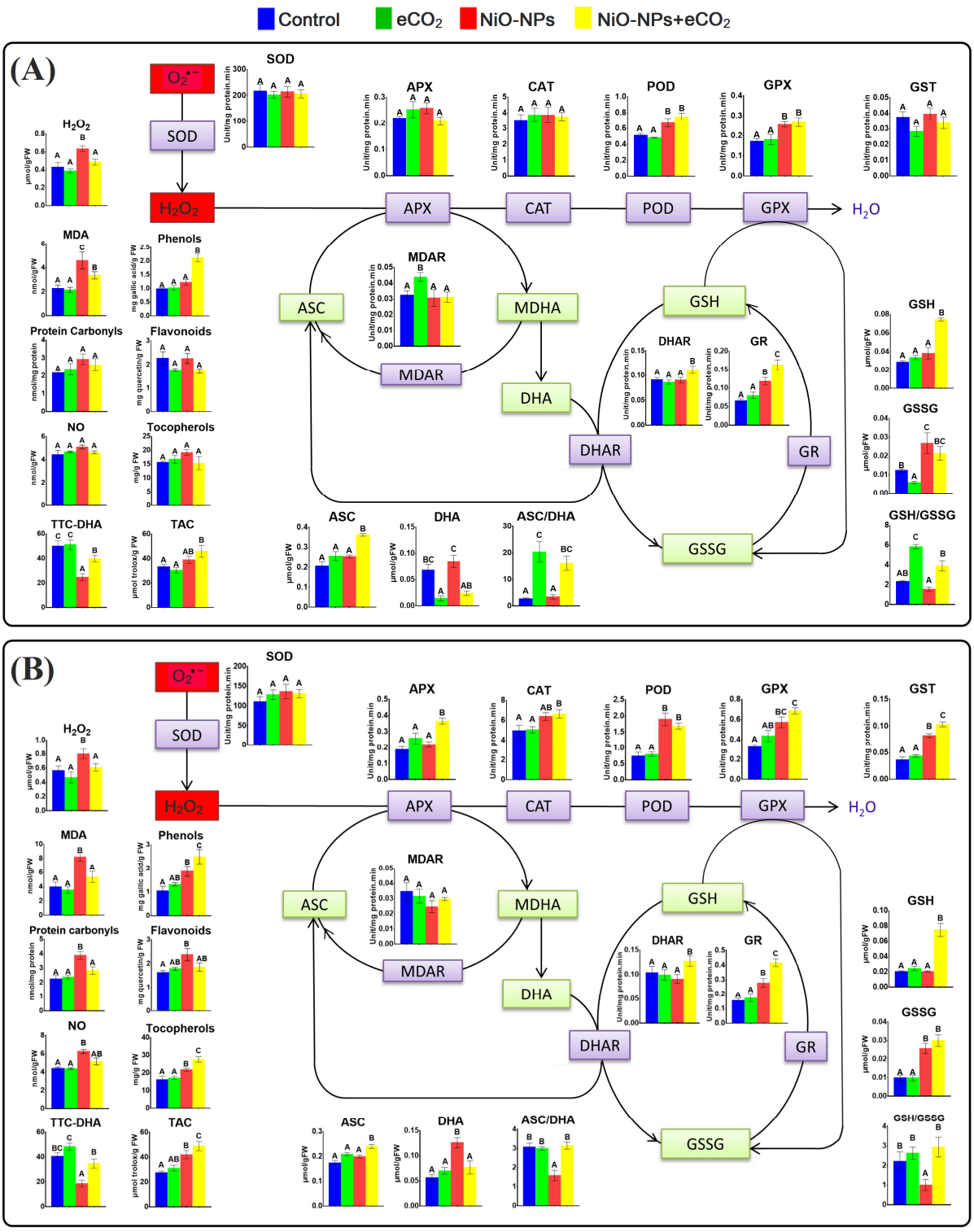

300 nm

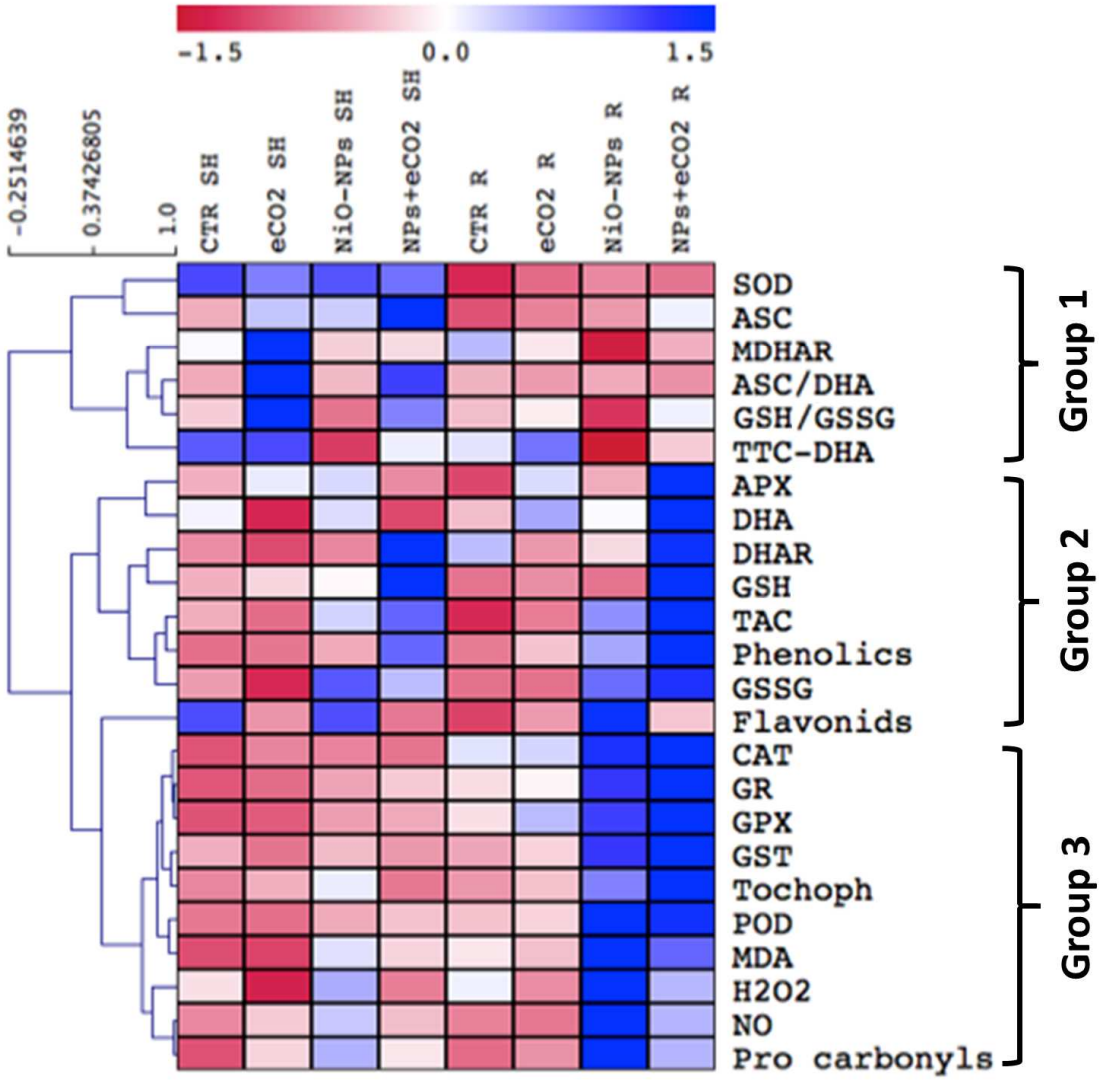
(b)

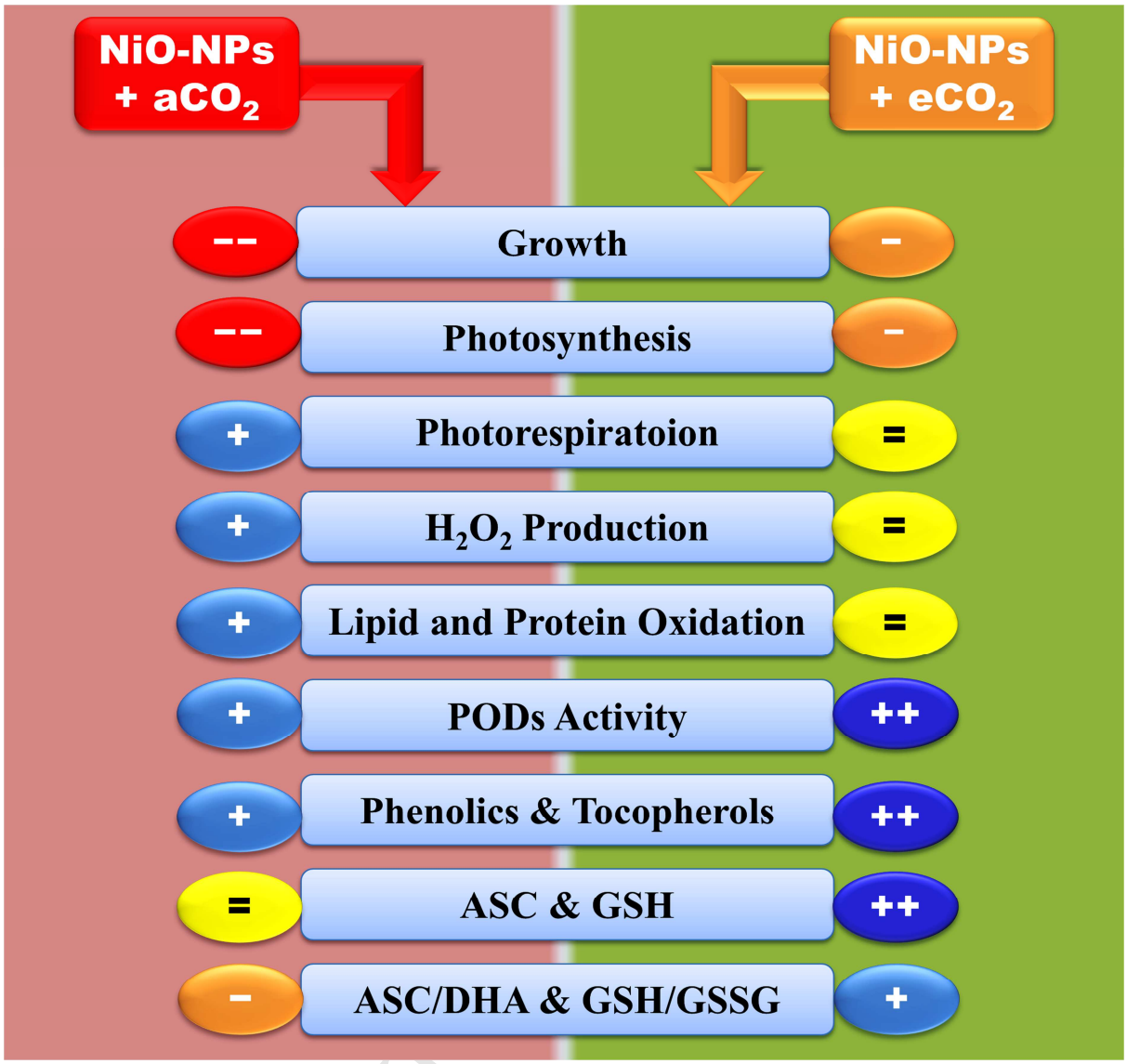

Absorbance (a.u)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
250
350
450
550
650
750
Wave length(nm)

(c)


Transmittance (%)
100
95
90
85
80
75
70
65
3795.65
3418.10
2928.80
2868.97
1747.66
1625.48
1380.82
1030.91
898.98
835.04
636.75
542.18
424.38
3500
3000
2500
2000
1500
1000
500
Wavenumber (cm-1)


Control
eCO2
NiO-NPs
NiO-NPs+eCO2
(A)
(B)
O2•−
SOD
H2O2
APX
CAT
POD
GPX
H2O
GST
MDA
Phenols
Protein Carbonyls
Flavonoids
NO
Tocopherols
TTC-DHA
TAC
MDAR
ASC
MDHA
DHA
DHAR
GR
GSH
GSSG
ASC/DHA
GSH/GSSG
Protein carbonyls


-1.5
0.0
1.5
-0.2514639
0.37426805
1.0
CTR SH
eCO2 SH
NiO-NPs SH
NPs+eCO2 SH
CTR R
eCO2 R
NiO-NPs R
NPs+eCO2 R
SOD
ASC
MDHAR
ASC/DHA
GSH/GSSG
TTC-DHA
APX
DHA
DHAR
GSH
TAC
Phenolics
GSSG
Flavonids
CAT
GR
GPX
GST
Tochoph
POD
MDA
H2O2
NO
Pro carbonyls
Group 1
Group 2
Group 3



Highlights

- NiO-NPs alone induced severe growth and oxidative damage in wheat.
- eCO_2 did not affect the accumulation and translocation of Ni in wheat, but antagonizes its phytotoxicity.
- eCO_2 promoted photosynthetic reactions and thus mitigated growth reduction in NiO-NPs treated wheat.
- eCO_2 reduced ROS induced cellular damage and maintained redox balance under NiO-NPs stress.
- ROS content were reduced at production level, via decreased photorespiration and at detoxification level by improved antioxidant defenses.
- Root was more responsive to imposed treatments and used several strategies to overcome stress impact.