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RESEARCH ARTICLE

Effects of TiO_2 nanoparticles on wheat (*Triticum aestivum L.*) seedlings cultivated under super-elevated and normal CO_2 conditions

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Abstract

Concerns over the potential risks of nanomaterials to ecosystem have been raised, as it is highly possible that nanomaterials could be released to the environment and result in adverse effects on living organisms. Carbon dioxide (CO_2) is one of the main greenhouse gases. The level of CO_2 keeps increasing and subsequently causes a series of environmental problems, especially for agricultural crops. In the present study, we investigated the effects of TiO_2 NPs on wheat seedlings cultivated under super-elevated CO_2 conditions (5000 mg/L CO_2) and under normal CO_2 conditions (400 mg/L CO_2). Compared to the normal CO_2 condition, wheat grown under the elevated CO_2 condition showed increases of root biomass and large numbers of lateral roots. Under both CO_2 cultivation conditions, the abscisic acid (ABA) content in wheat seedlings increased with increasing concentrations of TiO_2 NPs. The indolepropioponic acid (IPA) and jasmonic acid (JA) content notably decreased in plants grown under super-elevated CO_2 conditions, while the JA content increased with increasing concentrations of TiO_2 NPs. Ti accumulation showed a dose-response manner in both wheat shoots and roots as TiO_2 NPs concentrations increased. Additionally, the presence of elevated CO_2 significantly promoted Ti accumulation and translocation in wheat treated with certain concentrations of TiO_2 NPs. This study will be of benefit to the understanding of the joint effects and physiological mechanism of high-CO_2 and nanoparticle to terrestrial plants.

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Introduction

Nanotechnology is one of the revolutionary fields in science and technology and it is expected to contribute to advances in sustainability, including energy generation, conservation, storage, and conversion [1]. Nanoparticles (NPs), which are defined as particles in which at least one of the dimensions does not exceed 100 nm, are being applied in diverse industries including cosmetics, medicine, food and food packaging, bioremediation, and paints and coatings [1–4]. Sales of nanomaterial products are expected to reach 3 trillion dollars by 2020 [1]. With the increasing use and types of nano-products, the risks of NPs are receiving considerable attention. However, there is still a lack of information about interactions at the molecular level between NPs and biological systems [5–7]. At present, the NPs most commonly released into the environment include carbonaceous nanoparticles, metal oxides, quantum dots, zero-valent metals, and nanopolymers [8].

Higher plants are a major component of the food chain, and play an important role in ecosystem. Therefore, studying the toxic effects of NPs on plants will help us to understand the uptake, transportation, transformation, and degradation of NPs in the environment. Although the uptake, transport, and toxicity of NPs into plants are still not fully understood, it is thought that all of these factors are affected by the composition, size, and shape of NPs [8,9]. Previous studies have shown that NPs can enter the vascular system of plants and be transported to other plant tissues, and movements over short distances are favored [10]. Kurepa et al. [11] demonstrated that nanoconjugates could traverse cell walls to enter plant cells, and accumulate in specific subcellular locations.

To date, NPs have been demonstrated to have positive, negative, or no effects on plants, and the effect depends on the type of NPs and the plant species [12–15]. Nanoscale Zero Valent Iron (nZVI) was shown to inhibit seed germination and shoot growth of ryegrass, barley, and flax both in aqueous suspensions and soil [16]. In another study, FeO NPs inhibited plant growth by adversely affecting arbuscular mycorrhizal fungi [17]. Nanoparticles of ZnO, Fe_3O_4, and SiO_2 were shown to have toxic effects on *Arabidopsis thaliana*, while Al_2O_3 NPs did not [18]. Recently, Fe_2O_3 NPs with superb adsorption capacity were successfully used as fertilizer to replace traditional Fe fertilizer [19].

Poorly soluble TiO_2 NPs are used widely in paints, plastics, cosmetics, and catalysts [20,21]. Interactions between TiO_2 NPs and plants have raised concern, and several studies have explored the effects of these NPs on plants [21–27]. Compared with bulk TiO_2, nano-anatase TiO_2 resulted in significant increases in biomass, total nitrogen, oxygen, chlorophyll, and protein contents of spinach leaves [28]. Also, nano-anatase TiO_2 promoted spectral responses, which led to increased primary electron separation, electron transfer, and light energy conversion of the D1/D2/Cyt b559 complex by binding to this complex [29]. However, TiO_2 NPs have also been shown to negatively affect plants. For example, at higher concentrations, TiO_2 NPs were shown to delay germination, reduce the mitotic index, and inhibit root elongation of *Vicia narbonensis* L. and *Zea mays* L. [30].

Concerns over the potential risks of nanomaterials to ecosystem have been raised, as it is highly possible that nanomaterials could be released to the environment and result in adverse effects to living organisms. Carbon dioxide (CO_2) is one of the main greenhouse gases. The level of CO_2 keeps increasing and subsequently causes a series of environmental problems, especially for agricultural crops[31,32]. In the present study, we investigated the effects of TiO_2 NPs on wheat seedlings cultivated under super-elevated CO_2 conditions (5000 mg/L CO_2) and under normal CO_2 conditions (400 mg/L CO_2). Representative parameters such as biomass, root length, phytohormone were determined to understand plant's defense and response to abiotic stress caused by TiO_2 NPs. Additionally, TiO_2 NPs uptake was studied using ICP-MS.

Materials and methods

Characterization of TiO_2 NPs

The TiO_2 NPs (purity, ≥99.5%) were purchased from Sigma-Aldrich Inc. (3050 Spruce Street, Saint Louis, MO 63103, USA). These NPs were anatase and in a fine white powder form. The size and morphology of TiO_2 NPs were determined by transmission electronic microscopy (TEM, JEM-200, Japan). Wheat seeds (Zhongmai 11) were purchased from the Chinese Academy of Agricultural Sciences. All of the chemicals were of analytical grade and were purchased from Signofarm Chemical Research Co., Ltd (Shanghai, China).

Hydroponic culture

Wheat seeds were germinated after surface sterilization and were immersed in deionized water. The seeds were first incubated in a growth chamber (GZP-250B, Hengyu, China) at 24˚C in the dark for 48 h. Then, the germinated seedlings were cultivated in the same growth chamber at 24˚C under a 12-h light (light intensity of 15000 Lux)/12-h dark photoperiod for an additional 48 h. After germination, ten uniform seedlings were selected and transferred into plastic tubes containing 50 mL Hoagland's solution [composition (mmol/L): $Ca(NO_3)_2 \cdot 4H_2O$, 2; KH_2PO_4, 0.1; $MgSO_4 \cdot 7H_2O$, 0.5; 0.1 mM KCl, 0.1; 0.7 mM K_2SO_4, 0.7; 10 μM H_3BO_3, 10×10^{-3}; $MnSO_4 \cdot H_2O$, 0.5×10^{-3}; $ZnSO_4 \cdot 7H_2O$,1×10^{-3}; $CuSO_4 \cdot 5H_2O$, 0.2×10^{-3}; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.01×10^{-3}; 100 μM Fe-EDTA, 100×10^{-3}]. The hydroponic assay was conducted using the CELSS (controlled ecological life support system) Integration Experiment Platform (CIEP) [33,34] in plant growth chamber in January, 2016. Healthy wheat seedlings were selected for TiO_2 exposure in the CELSS under controlled conditions [25˚C, 55% relative humidity, 12-h light (light intensity of 15000 Lux)/12-h dark photoperiod]. The CELSS was supplied with treated air with atmospheric pressure and 5000 mg/L CO_2, and the plant growth chamber was supplied with fresh air in which the concentration of CO_2 was 400 mg/L. The concentration of CO_2 in the CELSS was more than 11 folds higher than in the plant growth chamber and all the other components in the air were the same. Under the two different CO_2 concentrations, four TiO_2 NPs treatment groups were applied: 10 mg/L, 100 mg/L, and 1000 mg/L, and a control without TiO_2 NPs. There were three replicates in each treatment under 400 mg/L CO_2 concentrations and four replicates under 5000 mg/L CO_2 concentrations. TiO_2 NPs were dispersed in Hoagland's solution by sonication for 30 min. During the 14-day exposure, deionized water was added every day to compensate for the evaporation losses. The TiO_2 NPs amended Hoagland's solution was replaced completely at day 5 and day 10.

Biomass measurement

At harvest, the treated seedlings were firstly washed with tap water for five times and then thoroughly washed with deionized water to remove impurities adsorbed on the surface of tissues. For each treatment, four seedlings were selected randomly to measure number of lateral root, root length and shoot height. And three of seedlings were selected to determined fresh weight of roots and shoots, separately.

Quantification of Ti content by inductively coupled plasma mass spectroscopy

Dried shoots were ground to a fine powder, and then digested with a mixture of concentrated plasma-pure HNO_3 and H_2O_2 (v/v, 6:1) in microwave digestion system (Ultra WAVE, Milestone, Italy). The obtained residual solutions were then diluted with deionized water and analyzed using inductively coupled plasma mass spectroscopy (ICP-MS).

Phytohormone determination

According to previous studies [7,35], the concentrations of indole acetic acid (IAA), gibberellins (GA_s), abscisic acid (ABA), jasmonic acid (JA), brassinosteroid (BR), zeatin riboside (ZR), dihydrozeatin riboside (DHZR), and indolepropioponic acid (IPA) were determined by ELISA methods.

Data analysis

All results are presented as mean±standard deviation (SD). Data were analyzed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by LSD test and independent samples t-test. A confidence of 95% ($P<0.05$) was considered significant in all cases.

Results and discussion

Characterization of TiO_2 NPs

The morphology of NPs is presented in Fig 1 and S1 Fig. The NPs were easily agglomerated. The NPs were not uniform and had a wide size distribution with diameter ranging from 32 nm to 171 nm. Such aggregation was also evident in previous studies [3,20,23].

Growth of wheat seedlings

As shown in Fig 2, the seedlings grown under super-elevated CO_2 turned to yellow or light brown, whereas those in normal CO_2 conditions were green and healthy. This result indicated that the elevated CO_2 adversely notably impacted on the wheat seedlings.

Effects of TiO_2 NPs on seedling biomass, root elongation, and shoot height

As shown in Fig 3I, shoot biomass decreased slightly with the concentration of TiO_2 NPs increasing in seedlings grown under super-elevated CO_2 conditions, while no significant difference of shoot biomass in all three TiO_2 NPs treatments was found under normal CO_2 conditions. Compared with super-elevated CO_2, shoot height (Fig 3IV) was a little higher under normal CO_2 condition. Upon exposure to the same concentration of TiO_2 NPs, super-elevated

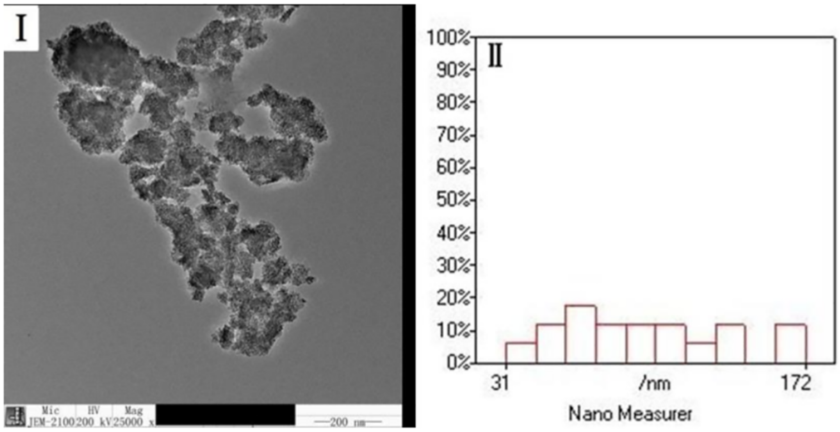

Fig 1. TEM image and particle size distribution of TiO_2 NPs.

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Fig 2. Phenotypic images of wheat seedlings in different concentrations of TiO2 NPs treatments with or without super elevated CO_2. (I) Seedlings grown in different concentrations of TiO_2 NPs under normal CO_2 conditions in a plant growth chamber. (II) Seedlings grown in different concentrations of TiO_2 NPs under super-elevated CO_2 conditions.

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CO_2 did not significantly alter the shoot biomass (Fig 3I) or shoot height (Fig 3IV) and compared with those treated with normal CO_2.

Root biomass was higher under super-elevated CO_2 conditions than under normal CO_2 conditions (Fig 3II). The results aligned with a previous study which demonstrated that elevated CO_2 could stimulate root growth[36]. Additionally, compared with control groups, root fresh weight significantly decreased in 100 and 1000 mg/L TiO_2 NPs under normal CO_2 conditions, while no similar trends were observed among different TiO_2 NPs concentrations under super-elevated CO_2 conditions.

Plants grown under elevated CO_2 produced more lateral roots than those grown under normal CO_2 conditions. In the super-elevated CO_2 and normal CO_2 treatments, there was no significant difference in lateral root abundance among all three TiO_2 NPs concentrations (Fig 3III). As shown in Fig 3V, similar trend was evident in root biomass. However, super-elevated CO_2 significantly affected root length. According to the results, parameters except shoot fresh weight under super-elevated CO_2 conditions and root fresh weight under normal CO_2 conditions, there was no significant difference among CK, 10, 100 and 1000 mg/L TiO_2 NPs treatments under same CO_2 circumstance. Several previous studies also agreed with the findings that TiO_2 NPs exhibited no toxic effects on plants regardless of exposure concentrations. [3,24,37] Additionally, it can't be excluded that the sample sizes might be too low to detect to actual effects.

In order to further reveal the effects of different levels of CO_2 on wheat growth, we set up the individual treatments without TiO_2 NPs additions. As shown in Fig 4, different concentrations of CO_2 did not change shoot fresh biomass (Fig 4I) and shoot height (Fig 4III). Root fresh weight (Fig 4II) and number of lateral roots (Fig 4V) was significantly higher in super-elevated CO_2 than in normal CO_2. However, The root length treated with super concentration of CO_2 was 1.5 times as long as the ones treated with normal level of CO_2 (Fig 4VI).

Effects of TiO_2 NPs on phytohormone contents

Phytohormones are of importance in plant growth and development [38]. The contents of different phytohormones are determined using ELISA methods in Fig 5. The ABA contents in seedlings exposed to elevated CO_2 increased with TiO_2 NPs concentrations increasing (Fig 5I). For plants grown under normal-CO_2 condition, the highest ABA content (119.4±9.41 ng/g FW) was in the 100 mg/L TiO_2 NPs treatment. Within each of the TiO_2 NPs treatments, there was no significant difference in ABA content between the treatments with elevated level of CO_2 and normal level of CO_2.

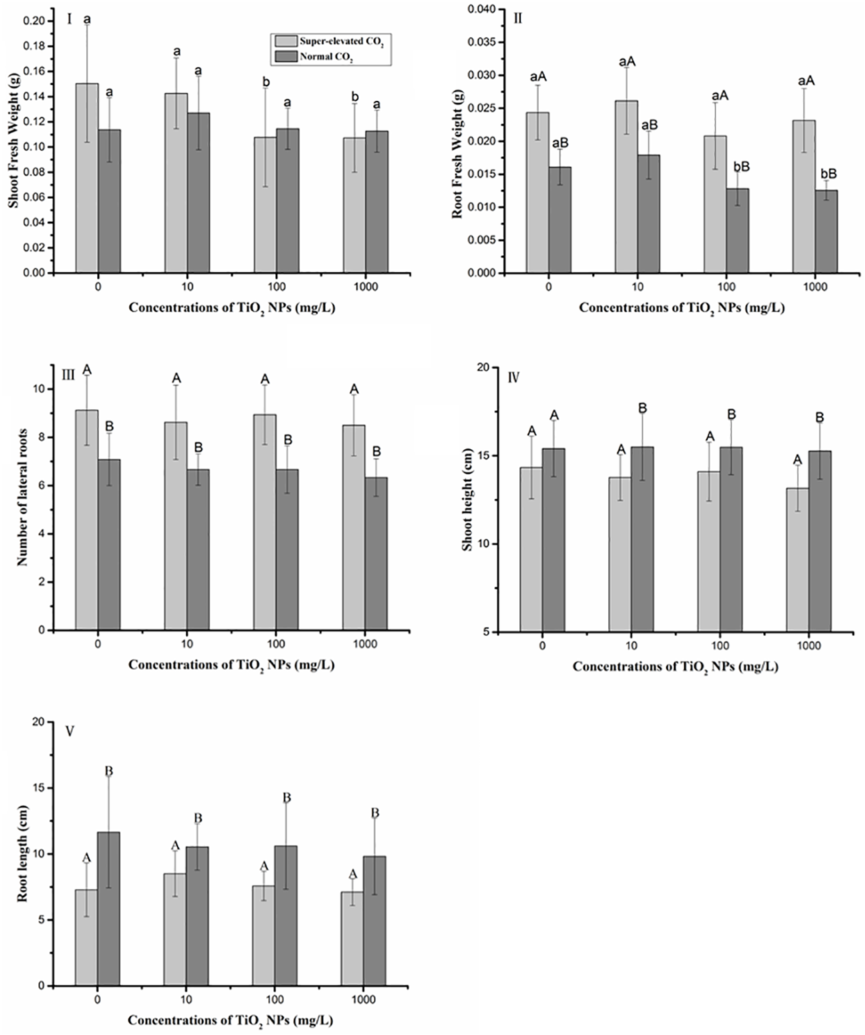

Fig 3. Effects of TiO_2 NPs on seedling biomass and number of lateral roots. Values are mean±SD, error bars represent standard deviation (sample size, n = 12 for I and II, n = 16 for III, IV and V). Lower letters represent significant difference at $p<0.05$ among TiO_2 NPs treatments under the same CO_2 conditions; Upper letters represent significant difference at $p<0.05$ between super-elevated CO_2 and normal CO_2 conditions at the same TiO_2 NPs concentration.

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BR is responsible for stem elongation and cell division in plants [39,40]. The BR contents were not changed upon exposure to the concentrations of 10 and 100 mg/L TiO_2 NPs, regardless of the levels of CO_2 (Fig 3II). However, when exposing to 1000 mg/L TiO_2 NPs, the BR content in wheat seedlings treated with super-elevated CO_2 was 42% lower than the one treated with normal CO_2.

ZR and DHZR are two cytokines, which can regulate cell growth and inhibit senescence [38,41]. As shown in Fig 5III, the normal level of CO_2 significantly increased the ZR contents in wheat seedlings treated with 1000 mg/L TiO_2 NPs by 50% relative to the elevated level of CO_2 treatment. However, neither the CO_2 concentrations nor the TiO_2 NPs concentrations affected the DHZR contents (Fig 3IV).

Gibberellins is another important phytohormone that can regulate cell elongation and plant growth [42]. To date, more than 100 different types of GA have been identified, although

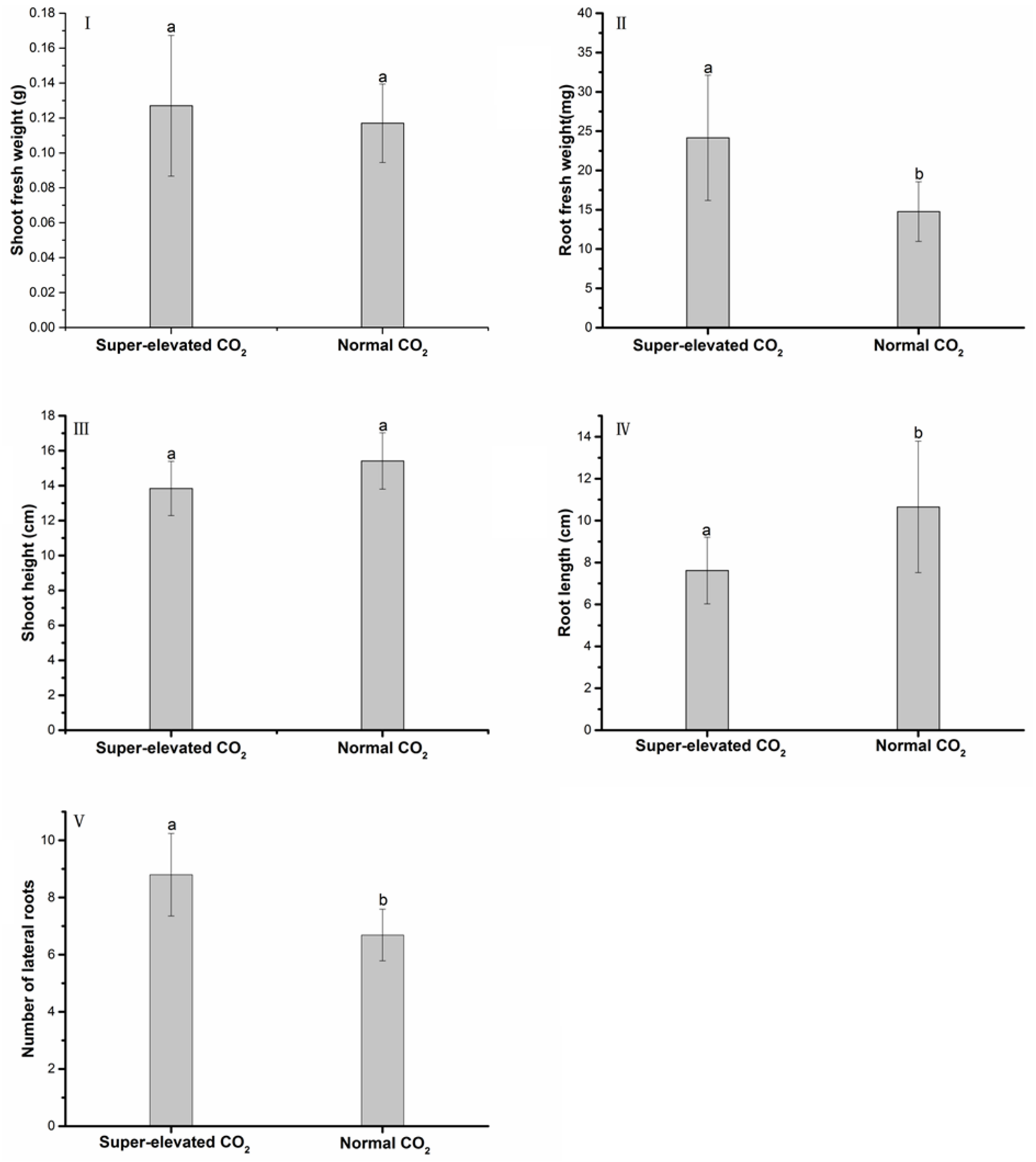

Fig 4. Physiological responses of wheat seedlings upon exposure to different levels of CO_2. Values are presented as mean ±SD, error bars represent standard deviation (sample size, n = 64 under super-elevated CO_2 condition and n = 48 under normal CO_2 condition). Lower letters represent significant difference at $p<0.05$ between super-elevated and normal CO_2 treatments.

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only a few such as GA_3 and GA_4 are bioactive. In this study, the GA_3 content in seedlings neither differed between elevated and normal CO_2 conditions nor among all three treatments with different concentrations of TiO_2 NPs (Fig 5V). However, in the 1000 mg/L TiO_2 NPs treatment, the GA_4 content in normal CO_2 treatment was approximately 1.6-fold of the one treated super-elevated CO_2 (Fig 3VI).

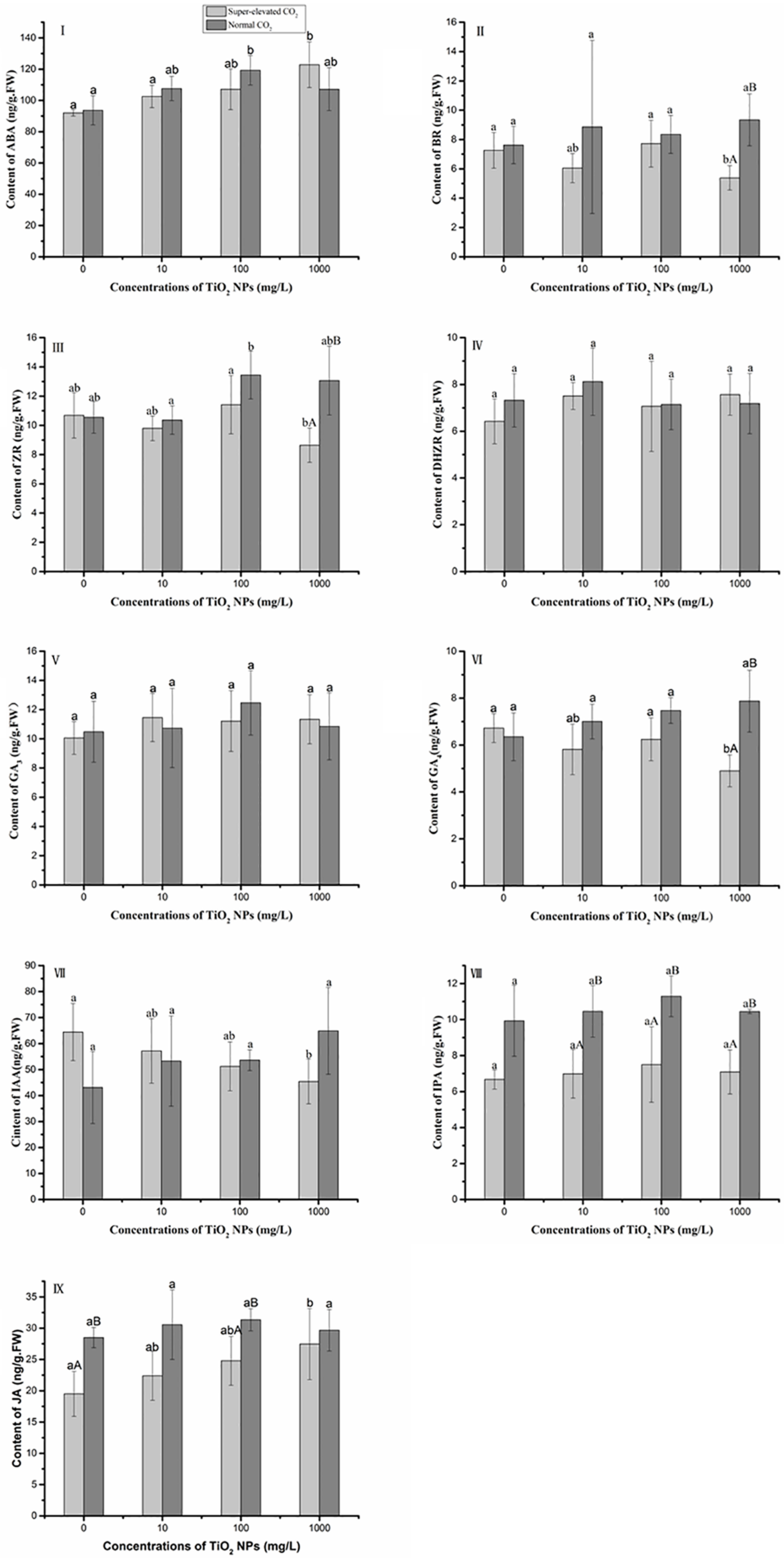

Fig 5. Effects of TiO_2 NPs on phytohormone contents in wheat seedlings grown under elevated-and normal CO_2 conditions. Data are mean±SD, error bars represent standard deviation (sample size, n = 16 for

treatments under super-elevated CO_2 condition and n = 12 for treatments under normal CO_2 condition). Lower letters represent significant difference at $p<0.05$ among TiO_2 NPs treatments under the same CO_2 conditions; Upper letters represent significant difference at $p<0.05$ between elevated CO_2 and normal CO_2 conditions at the same TiO_2 NPs concentration.

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IAA, one of the earliest discovered phytohormones, involves many physiological and biochemical processes, including cell elongation, growth, and division, and vascular tissue differentiation [43]. Under the condition of elevated CO_2, the IAA contents decreased with TiO_2 NPs concentrations increasing (Fig 5VII). However, the normal level of CO_2 did not significantly alter the IAA contents among TiO_2 NPs treatments. Another auxin, IPA, is important in stimulating root growth [44]. Similar to IAA, the super-elevated concentration of CO_2 significantly decreased the IPA contents than the normal concentration of CO_2, regardless of TiO_2 NPs concentrations (Fig 5VIII).

Jasmonic acid (JA) is a lipid-derived signaling molecular that is important for plant development and responds to biotic and abiotic stresses [45,46]. The results show that under the conditions of super-elevated CO_2, the JA contents exhibited a dose-response manner with TiO_2 NPs concentrations increasing (Fig 5IX). However, under normal CO_2 conditions, different concentrations of TiO_2 NPs did not significantly change the JA contents. The JA content was much lower in seedlings treated with elevated CO_2 than the ones with normal CO_2, regardless of NPs concentrations.

Also, as shown in Fig 6, we analysed effects of CO_2 on phytohormone contents. Most of the hormones showed no significant difference under different CO_2 concentrations. However, compared to normal CO_2, the contents of GA_4 (Fig 6VI) and IPA(Fig 6VIII) decreased under super-elevated CO_2.

Ti Content in wheat shoots and roots

As shown in Fig 7I, a dose-response fashion of Ti accumulation in wheat roots was evident under both super-elevated and normal CO_2 conditions. As TiO_2 NPs concentration increased to 1000 mg/L, the Ti content in the normal CO_2 treatment was significantly lower than in the super-elevated CO_2 treatment, implying that the excess amounts of CO_2 promoted Ti uptake. The pattern of Ti distribution in wheat shoots was similar to the roots (Fig 7II). At the 100 mg/L TiO_2 NPs, the elevated level of CO_2 resulted in more Ti translocation to shoots from roots as compared to the normal level of CO_2.

Conclusions

When seedlings were exposed to NPs, most of NPs aggregated on the surface of roots [47,48], which led to reduction of hydraulic conductivity and water availability to plants, and subsequently lower transpiration rate and inhibit plant development. Compared to the normal CO_2 conditions, wheat seedlings treated with the elevated level of CO_2 exhibited higher root biomass and formed more lateral roots. Under both elevated and normal CO_2 conditions, the ABA content increased with the concentrations of TiO_2 NPs increasing, but the CO_2 levels did not alter the ABA content in NPs treated wheat seedlings. The combined effects of elevated CO_2 and high TiO_2 NPs concentrations caused decreases of BR, ZR, and GA_3 contents, while neither CO_2 nor TiO_2 NPs negatively affected the phytohormone level. The IPA and JA contents were lower in plants grown under super-elevated CO_2 conditions, and the JA content increased with increasing TiO_2 NPs concentrations. The Ti contents showed a dose-response manner in both shoots and roots, and the levels of CO_2 could alter Ti accumulation and

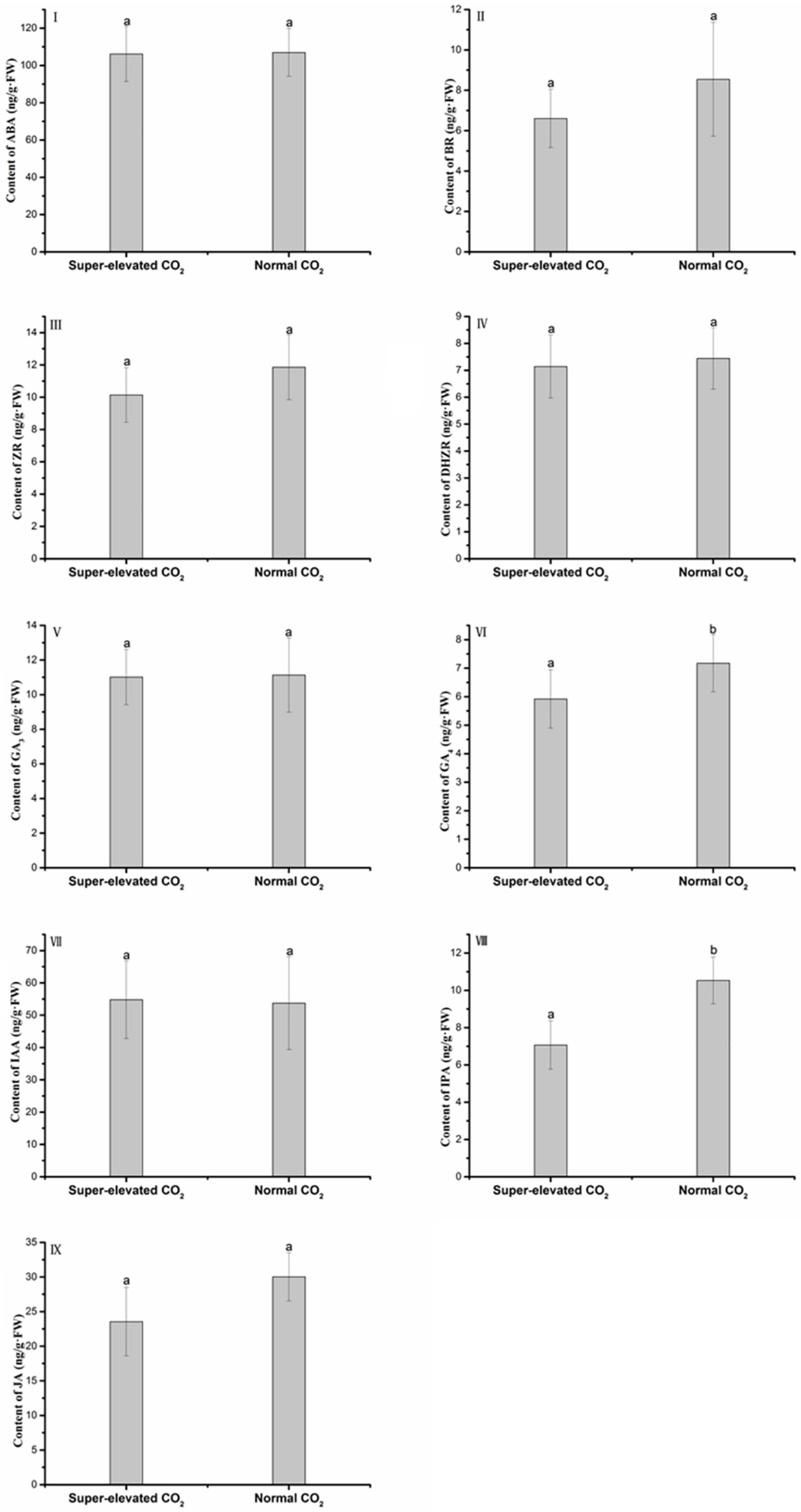

Fig 6. Phytohormone contents of wheat seedlings exposure to different levels of CO_2. Values are presented as mean±SD, error bars represent standard deviation (sample size, n = 64 under super-elevated

CO_2 condition and n = 48 under normal CO_2 condition). Lower letters represent significant difference at $p<0.05$ between super-elevated and normal CO_2 treatments.

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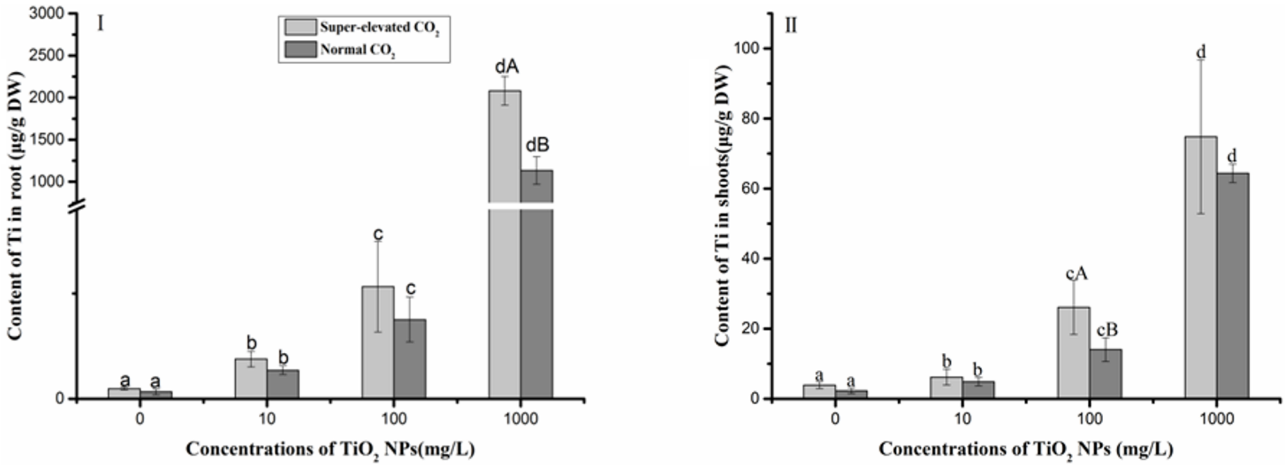

Fig 7. Ti contents in wheat roots and shoots. Data are mean±SD, error bars represent standard deviation (sample size, n = 3 in all treatments). Lower letters represent significant difference at $p<0.05$ among TiO_2 NPs treatments under the same CO_2 conditions; Upper letters represent significant difference at $p<0.05$ between super-elevated CO_2 and normal CO_2 conditions at the same TiO_2 NPs concentration.

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distribution at certain exposure concentrations of TiO_2 NPs. The study is helpful in understanding effects of TiO_2 NPs on plants under different CO_2 conditions.

Supporting information

S1 Fig. TEM images of TiO_2 NPs.
(TIF)

S1 Table. Shoot fresh biomass. Values are mean ± SD ($n\geq3$). Letters represent significant difference ($p<0.05$) among TiO_2 NPs treatments under the same growth conditions; * represents significant difference ($p<0.05$) between super-elevated CO_2 and normal CO_2 conditions at each TiO_2 NPs concentration.
(PDF)

S2 Table. Root fresh biomass. Values are mean ± SD ($n\geq3$). Letters represent significant difference ($p<0.05$) among TiO_2 NPs treatments under the same growth conditions; * represents significant difference ($p<0.05$) between super-elevated CO_2 and normal CO_2 conditions at each TiO_2 NPs concentration.
(PDF)

S3 Table. Number of lateral roots. Values are mean ± SD ($n\geq3$). Letters represent significant difference ($p<0.05$) among TiO_2 NPs treatments under the same growth conditions; * represents significant difference ($p<0.05$) between super-elevated CO_2 and normal CO_2 conditions at each TiO_2 NPs concentration.
(PDF)

S4 Table. Shoot height. Values are mean ± SD ($n\geq3$). Letters represent significant difference ($p<0.05$) among TiO_2 NPs treatments under the same growth conditions; * represents

significant difference ($p<0.05$) between super-elevated CO_2 and normal CO_2 conditions at each TiO_2 NPs concentration.
(PDF)

S5 Table. Root length. Values are mean ± SD ($n\geq3$). Letters represent significant difference ($p<0.05$) among TiO_2 NPs treatments under the same growth conditions; * represents significant difference ($p<0.05$) between super-elevated CO_2 and normal CO_2 conditions at each TiO_2 NPs concentration.
(PDF)

S6 Table. Effects of TiO_2 NPs on phytohormone contents. Values are mean ± SD (n = 3). Lowercase letters represent significant difference ($p<0.05$) among TiO_2 NPs treatments under the same growth conditions; Uppercase letters represent significant difference ($p<0.05$) between super-elevated CO_2 and normal CO_2 conditions at each TiO_2 NPs concentration.
(PDF)

S7 Table. Content of Ti in shoots. Content of Ti in roots. Values are mean ± SD ($n\geq3$). Letters represent significant difference ($p<0.05$) among TiO_2 NPs treatments under the same growth conditions; * represents significant difference ($p<0.05$) between super-elevated CO_2 and normal CO_2 conditions at each TiO_2 NPs concentration.
(PDF)

S8 Table. Content of Ti in roots. Values are mean ± SD ($n\geq3$). Letters represent significant difference ($p<0.05$) among TiO_2 NPs treatments under the same growth conditions; * represents significant difference ($p<0.05$) between super-elevated CO_2 and normal CO_2 conditions at each TiO_2 NPs concentration.
(PDF)

S1 Data. The data for all of the experiments.
(PDF)

Author Contributions

Investigation: FJ YS XZ WC.

Methodology: YR FJ.

Writing – original draft: FJ.

Writing – review & editing: YR CM.

References

1. Roco MC, Mirkin CA, Hersam MC (2011) Nanotechnology research directions for societal needs in 2020: summary of international study. Journal of Nanoparticle Research 13: 897–919.
2. Zhang Z, He X, Zhang H, Ma Y, Zhang P, Ding Y, et al. (2011) Uptake and distribution of ceria nanoparticles in cucumber plants. Metallomics 3: 816–822. https://doi.org/10.1039/c1mt00049g PMID: 21731965
3. Ji Y, Zhou Y, Ma C, Feng Y, Hao Y, Rui Y, et al. (2016) Jointed toxicity of TiO_2 NPs and Cd to rice seedlings: NPs alleviated Cd toxicity and Cd promoted NPs uptake. Plant Physiology and Biochemistry 110: 82–93. https://doi.org/10.1016/j.plaphy.2016.05.010 PMID: 27193349
4. Gui X, Zhang Z, Liu S, Ma Y, Zhang P, He X, et al. (2015) Fate and Phytotoxicity of CeO_2 Nanoparticles on Lettuce Cultured in the Potting Soil Environment. PLoS One 10: e0134261. https://doi.org/10.1371/journal.pone.0134261 PMID: 26317617
5. Barrena R, Casals E, Colon J, Font X, Sanchez A, Puntes V (2009) Evaluation of the ecotoxicity of model nanoparticles. Chemosphere 75: 850–857. https://doi.org/10.1016/j.chemosphere.2009.01.078 PMID: 19264345

6. Rui Y, Zhang P, Zhang Y, Ma Y, He X, Gui X, et al. (2015) Transformation of ceria nanoparticles in cucumber plants is influenced by phosphate. Environmental Pollution 198: 8–14. https://doi.org/10.1016/j.envpol.2014.12.017 PMID: 25549862
7. Nhan le V, Ma C, Rui Y, Liu S, Li X, Xing B, et al. (2015) Phytotoxic Mechanism of Nanoparticles: Destruction of Chloroplasts and Vascular Bundles and Alteration of Nutrient Absorption. Scientific Reports 5: 11618. https://doi.org/10.1038/srep11618 PMID: 26108166
8. Ma X, Geisler-Lee J, Deng Y, Kolmakov A (2010) Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation. Science of the Total Environment 408: 3053–3061. https://doi.org/10.1016/j.scitotenv.2010.03.031 PMID: 20435342
9. Schwab F, Zhai G, Kern M, Turner A, Schnoor JL, Wiesner MR (2015) Barriers, pathways and processes for uptake, translocation and accumulation of nanomaterials in plants–Critical review. Nanotoxicology 10: 257–278. https://doi.org/10.3109/17435390.2015.1048326 PMID: 26067571
10. Corredor E, Testillano PS, Coronado MJ, Gonzalez-Melendi P, Fernandez-Pacheco R, Marquina C, et al. (2009) Nanoparticle penetration and transport in living pumpkin plants: in situ subcellular identification. BMC Plant Biology 9: 1–11.
11. Kurepa J, Paunesku T, Vogt S, Arora H, Rabatic BM, Lu J, et al. (2010) Uptake and distribution of ultrasmall anatase TiO_2 Alizarin red S nanoconjugates in Arabidopsis thaliana. Nano Letter 10: 2296–2302.
12. Ma Y, Kuang L, He X, Bai W, Ding Y, Zhang Z, et al. (2010) Effects of rare earth oxide nanoparticles on root elongation of plants. Chemosphere 78: 273–279. https://doi.org/10.1016/j.chemosphere.2009.10.050 PMID: 19897228
13. Lin D, Xing B (2007) Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. Environmental Pollution 150: 243–250. https://doi.org/10.1016/j.envpol.2007.01.016 PMID: 17374428
14. Lee WM, Kwak JI, An YJ (2012) Effect of silver nanoparticles in crop plants Phaseolus radiatus and Sorghum bicolor: media effect on phytotoxicity. Chemosphere 86: 491–499. https://doi.org/10.1016/j.chemosphere.2011.10.013 PMID: 22075051
15. Musante C, White JC (2012) Toxicity of silver and copper to Cucurbita pepo: differential effects of nano and bulk-size particles. Environmental Toxicology 27: 510–517. https://doi.org/10.1002/tox.20667 PMID: 22887766
16. El-Temsah YS, Joner EJ (2012) Impact of Fe and Ag nanoparticles on seed germination and differences in bioavailability during exposure in aqueous suspension and soil. Environmental Toxicology 27: 42–49. https://doi.org/10.1002/tox.20610 PMID: 20549639
17. Feng Y, Cui X, He S, Dong G, Chen M, Wang J, et al. (2013) The role of metal nanoparticles in influencing arbuscular mycorrhizal fungi effects on plant growth. Environmental Science &Technology 47: 9496–9504.
18. Lee CW, Mahendra S, Zodrow K, Li D, Tsai YC, Braam J, et al. (2010) Developmental phytotoxicity of metal oxide nanoparticles to Arabidopsis thaliana. Environmental Toxicology and Chemistry 29: 669–675. https://doi.org/10.1002/etc.58 PMID: 20821493
19. Rui M, Ma C, Hao Y, Guo J, Rui Y, Tang X, et al. (2016) Iron Oxide Nanoparticles as a Potential Iron Fertilizer for Peanut (Arachis hypogaea). Frontiers in Plant Science 7: 815–824. https://doi.org/10.3389/fpls.2016.00815 PMID: 27375665
20. Chen X, Kuo D-H, Lu D (2016) N-doped mesoporous TiO_2 nanoparticles synthesized by using biological renewable nanocrystalline cellulose as template for the degradation of pollutants under visible and sun light. Chemical Engineering Journal 295: 192–200.
21. Wang JJ, Sanderson BJ, Wang H (2007) Cyto- and genotoxicity of ultrafine TiO_2 particles in cultured human lymphoblastoid cells. Mutation Research 628: 99–106. https://doi.org/10.1016/j.mrgentox.2006.12.003 PMID: 17223607
22. Josko I, Oleszczuk P (2013) Influence of soil type and environmental conditions on ZnO, TiO(2) and Ni nanoparticles phytotoxicity. Chemosphere 92: 91–99. https://doi.org/10.1016/j.chemosphere.2013.02.048 PMID: 23541360
23. Du W, Sun Y, Ji R, Zhu J, Wu J, Guo H (2011) TiO_2 and ZnO nanoparticles negatively affect wheat growth and soil enzyme activities in agricultural soil. Journal of Environmental Monitoring 13: 822–828. https://doi.org/10.1039/c0em00611d PMID: 21267473
24. Mattiello A, Filippi A, Pošćić F, Musetti R, Salvatici MC, Giordano C, et al. (2015) Evidence of Phytotoxicity and Genotoxicity in Hordeum vulgare L. Exposed to CeO_2 and TiO_2 Nanoparticles. Frontiers in Plant Science 6: 1043–1055. https://doi.org/10.3389/fpls.2015.01043 PMID: 26635858
25. Warheit DB, Hoke RA, Finlay C, Donner EM, Reed KL, Sayes CM (2007) Development of a base set of toxicity tests using ultrafine TiO_2 particles as a component of nanoparticle risk management. Toxicology Letters 171: 99–110. https://doi.org/10.1016/j.toxlet.2007.04.008 PMID: 17566673

26. Warheit DB, Sayes CM, Reed KL, Swain KA (2008) Health effects related to nanoparticle exposures: environmental, health and safety considerations for assessing hazards and risks. Pharmacology & Therapeutics 120: 35–42.

27. Warheit DB, Webb TR, Reed KL, Frerichs S, Sayes CM (2007) Pulmonary toxicity study in rats with three forms of ultrafine-TiO2 particles: differential responses related to surface properties. Toxicology 230: 90–104. https://doi.org/10.1016/j.tox.2006.11.002 PMID: 17196727

28. Yang F, Liu C, F, Su M, Wu X, Zheng L, Hong F, et al. (2007) The improvement of spinach growth by nano-anatase TiO_2 treatment is related to nitrogen photoreduction. Biological Trace Element Research 119: 77–88. https://doi.org/10.1007/s12011-007-0046-4 PMID: 17914222

29. Su M, Liu H, Liu C, Qu C, Zheng L, Hong F (2009) Promotion of nano-anatase TiO_2 on the spectral responses and photochemical activities of D1/D2/Cyt b559 complex of spinach. Spectrochimica Acta Part A: Molecular & Biomolecular Spectroscopy 72: 1112–1116.

30. Ruffini Castiglione M, Giorgetti L, Geri C, Cremonini R (2010) The effects of nano-TiO2 on seed germination, development and mitosis of root tip cells of Vicia narbonensis L. and Zea mays L. Journal of Nanoparticle Research 13: 2443–2449.

31. Yadav RC (2013) Interaction of iron and zinc nanoparticles in the rice rhizosphere at elevated CO_2. Master of Science in Soil Science and Agricultural Chemistry: 1–139.

32. Yadav RC, Patra AK, Purakayastha TJ, Bhattacharyya R, Singh R (2014) Response of Rice Plant to Application of Nanoparticles of Fe and Zn at Elevated CO_2: A Hydroponic Experiment under Phytotron. International Journal of Bio-Resource & Stress Management 5: 207–212.

33. Guo S, Dong W, Ai W, Feng H, Tang Y, Huang Z, et al. (2014) Research on regulating technique of material flow for 2-person and 30-day integrated CELSS test. Acta Astronautica 100: 140–146.

34. Shen Y, Guo S (2014) Effects of photoperiod on wheat growth, development and yield in CELSS. Acta Astronautica 105: 24–29.

35. He P, Osaki M, Takebe M, Shinano T, Wasaki J (2005) Endogenous hormones and expression of senescence-related genes in different senescent types of maize. Journal of Experimental Botany 56: 1117–1128. https://doi.org/10.1093/jxb/eri103 PMID: 15723826

36. Wong SC, Osmond CB (1991) Elevated Atmosphere Partial Pressure of CO_2 and Plant Growth. III. Interactions Between Triticum aestivum (C_3) and Echinochloa frumentacea (C_4) During Growth in Mixed Culture Under Different CO_2, N Nutrition and Irradiance Treatments, With Emphasis on Below-Ground Responses Estimated Using the $\delta^{13}C$ Value of Root Biomass Australian Journal of Plant Physiology 18: 137–152.

37. Song U, Shin M, Lee G, Roh J, Kim Y, Lee EJ (2013) Functional analysis of TiO_2 nanoparticle toxicity in three plant species. Biological Trace Element Research 155: 93–103. https://doi.org/10.1007/s12011-013-9765-x PMID: 23900645

38. Bari R, Jones JDG (2009) Role of plant hormones in plant defence responses. Plant Molecular Biology 69: 473–488. https://doi.org/10.1007/s11103-008-9435-0 PMID: 19083153

39. Shigeta T, Yoshimitsu Y, Nakamura Y, Okamoto S, Matsuo T (2011) Does brassinosteroid function require chromatin remodeling? Plant Signaling & Behavior 6: 1824–1827.

40. Adam G, Marquardt V (1986) Brassinosteroid. Phytochemistry 25.

41. Kende H, Zeevaart J (1997) The Five "Classical" Plant Hormones. Plant Cell 9: 1197–1210. https://doi.org/10.1105/tpc.9.7.1197 PMID: 12237383

42. Hedden P, Thomas SG (2016) 1.Signal achievements in gibberellin research: the second half-century: John Wiley & Sons, Ltd.

43. Gordon SA, Weber RP (1951) Colorimetric estimation of indoleacetic acid. Plant Physiology 26: 192–195. PMID: 16654351

44. Johnson HE, Crosby DG (1960) 3-Indolepropionic Acid. Journal of Organic Chemistry 25: 569–570.

45. Creelman RA, Mullet JE (1995) Jasmonic acid distribution and action in plants regulation during development and response to biotic and abiotic stress. Proceedings of the National Academy of Sciences 92: 4114–4119.

46. Wasternack C, Stenzel I, Hause B, Hause G, Kutter C, Maucher H, et al. (2006) The wound response in tomato—role of jasmonic acid. Journal of Plant Physiology 163: 297–306. https://doi.org/10.1016/j.jplph.2005.10.014 PMID: 16368162

47. Miralles P, Church TL, Harris AT (2012) Toxicity, Uptake, and Translocation of Engineered Nanomaterials in Vascular plants. Environmental Science & Technology 46: 9224–9239.

48. Asli S, Neumann PM (2009) Colloidal suspensions of clay or titanium dioxide nanoparticles can inhibit leaf growth and transpiration via physical effects on root water transport. Plant, Cell & Environment 32: 577–584.