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Titanium Dioxide Nanoparticles Promote Root Growth by Interfering with Auxin Pathways in *Arabidopsis thaliana*

Jiali Wei*, Ying Zou, Ping Li and Xiaojun Yuan

Shanghai Key Laboratory of Bio-Energy Crops, Research Center for Natural Products, Plant Science Center, School of Life Sciences, Shanghai University, Shanghai, 200444, China
*Corresponding Author: Jiali Wei. Email: jlwei3588@shu.edu.cn
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Abstract: TiO_2 nanoparticles (nano-TiO_2) are widely used in the world, and a considerable amount of nano-TiO_2 is released into the environment, with toxic effects on organisms. In the various species of higher plants, growth, including seed germination, root elongation, and biomass accumulation, is affected by nano-TiO_2. However, the underlying molecular mechanisms remain to be elucidated. In this study, we observed that nano-TiO_2 promoted root elongation in a dose-dependent manner. Furthermore, we found that nano-TiO_2 elevated auxin accumulation in the root tips of the auxin marker lines *DII-VENUS* and *DR5::GUS*, and, correspondingly, quantitative real-time PCR analysis revealed that nano-TiO_2 increased the expression levels of auxin biosynthesis- and transport-related genes. GFP fluorescence observation using transgenic *PIN2-GFP* indicated that nano-TiO_2 promoted root growth by inducing PIN2 accumulation. Thus, we propose that nano-TiO_2 promote root growth in *Arabidopsis thaliana* by altering the expression levels of auxin biosynthesis- and transport-related genes.

Keywords: Nanoparticles; nano-TiO_2; root length; auxin; PIN2; auxin-related genes

1 Introduction

With the development of nanotechnology, engineered nanoparticles are widely being applied, with inevitable increases in the release of nanoparticles into the environment and agricultural ecosystems [1,2]. Titanium dioxide nanoparticles (nano-TiO_2) are the most abundant engineered nanomaterials in the world and are used in such products as paints, household goods, cosmetics, and sunscreens [3–6]. The global production of TiO_2 has been reported to be approximately 88,000 t per year [7], and in Europe, the concentration of nano-TiO_2 in soils reached 0.13 $\mu g\ kg^{-1}\ yr^{-1}$ [7]. As research on plant growth in soil polluted by nano-TiO_2 has shown that plants can absorb and transport nano-TiO_2 from the root to the leaf [8,9], studies on the toxic effects of nano-TiO_2 in plants is very important. For example, nano-TiO_2 possess certain photocatalytic properties, causing damage to plants by inducing reactive oxygen species (ROS) production [10–14]. Moreover, accumulating evidence has verified the toxic effects of nano-TiO_2 on plants, causing DNA damage in *Allium cepa* roots and *Nicotiana tabacum* leaves, also with excessive lipid peroxidation products detected in *A. cepa* roots [15]. Soybean growth was also reportedly inhibited

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in soil mixed with nano-TiO_2 [16], and reductions in seed germination and seedling growth were observed in nano-TiO_2-treated *Solanum esculentum* L. [17]. In contrast, promoting effects on plants have also been reported for nano-TiO_2. For instance, crop yield was increased by treatment with a nano-TiO_2 foliar spray [18], and *Nigella sativa* L. germination was promoted after seeds were soaked in a nanoparticle solution [19]. Overall, the positive or negative effects of nanoparticles on plants depend on the particle type, size and concentration and on the plant species [20,21].

Though these studies have shown nano-TiO_2 regulate the growth of plant roots [12,22,23], though the molecular mechanism remains unclear. The phytohormone auxin plays an important role in root growth and development [24]. The auxin response is modulated by auxin biosynthesis and transport, as well as auxin perception to alter plant growth in response to environment conditions. IAA (indole-3-acetic acid) is the main auxin in plants. During IAA biosynthesis, Trp is converted to IAA in two steps by the tryptophan aminotransferase of *Arabidopsis*-1 (TAA1) family and YUC (YUCCA) family of flavin monooxygenases [25]. After biosynthesis, auxin is transported to generate IAA gradients for root growth and development, and AUX1 (AUXIN TRANSPORTER CARRIER1/AUXIN TRANS-PORTER-LIKE PROTEINS) influx carriers and PINFORMED (PIN) family efflux carriers co-regulate this process [3,26,27]. $SCF^{TIR1/AFB}$—Aux/IAA auxin receptor complexes play a central role in the perception of auxin. In auxin activation, TIR1/AFBs interact with Aux/IAAs, resulting in the degradation of Aux/IAAs, after which ARF (AUXIN RESPONSE FACTOR) proteins are released from repression by Aux/IAAs [28]. Recently, it was reported that AgNPs have an effect on auxin-efflux carriers and root gravitropism [5]. However, the effects of other types of nanoparticles on auxin pathways require further research.

In this study, we used *Arabidopsis thaliana* as a model plant to study the effects of nano-TiO_2 on root growth. We found that nano-TiO_2 promoted root elongation in a concentration-dependent manner. We also found the expression level of genes in auxin pathways to be significantly elevated. The results of this research indicate that TiO_2 have an effect on auxin-related gene expression, thereby affecting root growth.

2 Materials and Methods

2.1 Characterization of Nano-TiO_2

Titanium dioxide (IV) nanoparticles of anatase (>20 nm in diameter) were obtained from Macklin Co. (Macklin, China). The desired concentrations (100, 250, 500 and 1000 mg/L) were prepared by suspending the nanopowder in ultrapure sterile water. *Arabidopsis* seedlings were incubated overnight in the nano-TiO_2 suspensions, which had been sonicated in a heated ultrasonic bath for 1 h. After the nano-TiO_2 had been deposited on the root surface, the particles were measured by scanning electron microscopy (SEM) to determine their size and morphology.

2.2 Plant Materials and Growth Conditions

Wild-type *Arabidopsis* Col-0 and transgenic lines *DR5::GUS*, *DII-VENUS* and *mDII-VENUS* were used in this study. The seeds of transgenic lines were provided by the ABRC. Seeds were incubated at 4°C for 2 days, sterilized with 30% (v/v) bleach for 5 min, and rinsed four times with sterile deionized water. The sterile seeds were first sown onto 1/2 Murashige and Skoog (MS) agar medium and incubated for 3 days in a growth chamber at 22°C under a 16-h light/8-h dark photoperiod and then transferred to 1/2 MS agar medium with different nano-TiO_2 concentrations (100, 300, 500 and 1000 mg/L) for another 3 days.

2.3 Microscopy Images of Root Tips

Seedlings of the marker line *DR5::GUS* were incubated in GUS staining solution with the substrate 1 mM X-Gluc (5-bromo-4-chloro-3-indoxyl-beta-D-glucuronic acidcyclohexylammonium salt) at 37°C for 12 h, and chlorophyll was removed with 95% ethanol before microscopic observation (Zeiss, German). The root tips of the GFP marker line were imaged by laser-scanning confocal microscopy (Zeiss, Germany).

2.4 qRT-PCR Analysis

Three-day-old *Arabidopsis* Col-0 seedlings sown vertically on 1/2 MS medium were transferred onto 1/2 MS medium supplemented with nano-TiO_2 and incubated for 3 days. Total RNA was extracted from the seedlings using Trizol (invitrogen) according to the manufacturer's instructions. For analysis of gene expression, cDNA was synthesized by reverse transcription using PrimeScript™ 1st Strand cDNA Synthesis Kit (Takara, Japan). qRT-PCR was performed using SYBR Green Reagents (Takara, Japan) and a Real-Time PCR Detection System (Roche). Gene expression relative to PP2A was calculated by the 2-ΔΔCt method [29].

2.5 Statistical Analysis

Student's *t*-test was used to compare data for the two experimental groups. Differences were considered statistically significant at a p-value < 0.05. A p-value < 0.05 is indicated with *, < 0.01 with ** and < 0.001 with ***.

3 Results

3.1 Nanoparticle Characterization

Nano-TiO_2 was deposited on the surface of *Arabidopsis* root tips by incubating seedlings in nano-TiO_2 suspensions at various concentrations: 100, 250, 500, 1000 mg/L. We employed SEM to examine the effects of nano-TiO_2 deposited onto the root tip surface. More particles had attached to the root tip surface at the higher nano-TiO_2 concentrations than at the lower ones (Fig. 1a). In contrast, no nanoparticles were observed on roots in the H_2O control (Fig. 1a). To observe the characteristics of nano-TiO_2, microscopy at 50,000 times magnification was performed (Fig. 1b). Analysis of nano-TiO_2 showed that the size of the particles ranged from 20 to 100 nm, with approximately 50% being ~40 nm (Fig. 1c).

3.2 Root Growth of Arabidopsis was Enhanced by Nano-TiO_2

To research the effects of nano-TiO_2 on *Arabidopsis*, seeds were germinated in 1/2 MS medium containing nano-TiO_2 at four different concentrations (100 mg/L, 250 mg/L, 500 mg/L, 1000 mg/L); the control medium did not contain nano-TiO_2. The root length of six-day-old seedlings was markedly increased at the higher concentrations (500 and 1000 mg/L) in comparison to that in the control (Fig. 2). At the lower concentrations of nano-TiO_2 (100 and 250 mg/L), root length was only slightly increased (Fig. 2). This result was consistent with previous research showing that root elongation was enhanced in plants from seeds soaked in nano-TiO_2 suspensions [12]. These data indicate that root length in *Arabidopsis* increases with nano-TiO_2 treatment in a concentration-dependent manner.

3.3 Auxin Accumulation was Elevated in Root Tips Treated with Nano-TiO_2

The auxin marker line *DII-VENUS*, which expresses a GFP protein fused to the AUX/IAA auxin-interaction domain under the control of a constitutive promoter, was utilized to evaluate auxin accumulation in root tips. As shown in Fig. 3a, confocal imaging of the *DII-VENUS* line revealed much weaker fluorescence in roots grown in 1000 mg/L nano-TiO_2 medium compared with that of the control. Conversely, no difference in fluorescence was observed with or without nano-TiO_2 in *mDII-VENUS*, a marker line carrying a DII domain mutation.

In parallel, we utilized another auxin marker line, *DR5::GUS*, in which the *GUS* (glucuronidases) gene is driven by the promoter of *DR5*, an auxin response gene. After growing for six days in MS medium with different nanoparticle concentrations (100, 250, 500, 1000 mg/L), the roots of seedlings were incubated with the GUS substrate for 12 h at 37°C, and the stained roots were then observed under a light microscope. As shown in Fig. 3b, GUS activity was stronger at the higher nano-TiO_2 concentration and only slightly elevated

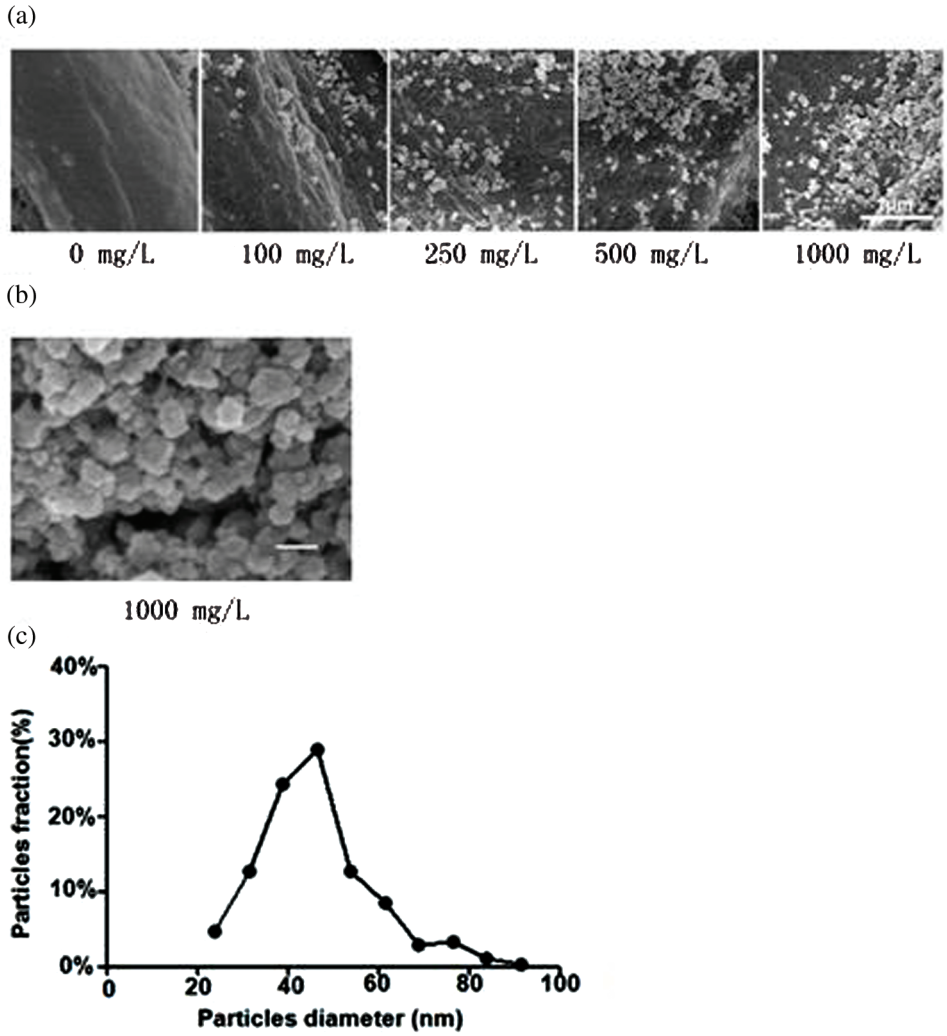

Figure 1: Nanoparticle characterization. (a) nano-TiO_2 on the surface of root tips exposed to different concentrations of nano-TiO_2. (b) the enlarged imagine of root after 1000 mg/L nano-TiO_2 treatment. (c) particle distribution curve of nano-TiO_2, n > 400

at the lower nano-TiO_2 concentration. Along with the results for the *DII-VENUS* marker line, the findings indicate that in comparison to the controls, more auxin accumulated in the root tips treated with nano-TiO_2.

3.4 Expression of Auxin-Related Genes in Nano-TiO_2-Treated Plants

To further verify the role of auxin in nano-TiO_2-mediated promotion of root elongation, we assessed the expression levels of a set of auxin-related genes, including auxin carrier genes *PIN1-3*, the auxin signaling pathway-related gene *IIR1*, and the auxin biosynthesis gene *YUC8*. Quantitative real-time RT-PCR showed that the *PIN2* gene was dramatically up-regulated at all tested nano-TiO_2 concentrations, and the expression levels of *TIR1* and *YUC8* were increased at 500 and 1000 mg/L nano-TiO_2, respectively. However, *PIN1* was down-regulated, and expression of *PIN3* showed no change (Fig. 4a). The *PIN2* expression pattern was examined after exposure to nano-TiO_2 with a line expressing a GFP reporter driven by the *PIN2* promoter. As expected, the GFP signal in the *PIN2-GFP* line was markedly increased after nano-TiO_2 treatment compared with the control (Fig. 4b). Moreover, the GFP signal at the higher concentration was

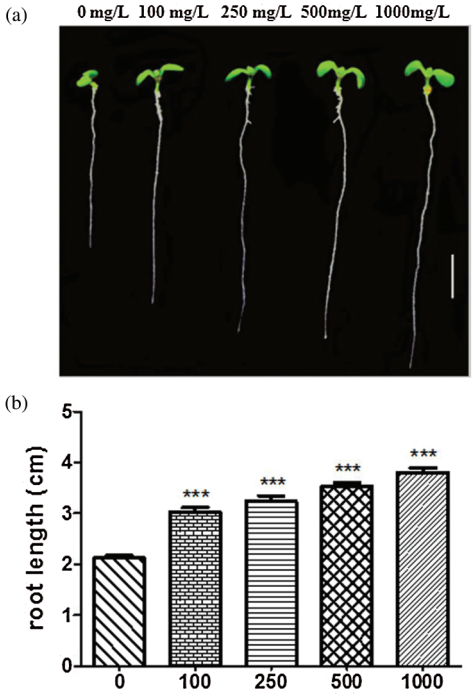

Figure 2: nano-TiO_2 promotes root growth in *Arabidopsis*. a. root length of six-day-old seedlings grown on 1/2 MS medium with different nano-TiO_2 concentrations. b. statistics of root lengths. Values are means ±SD. Error bars indicate SD of three biological repeated experiments. ***indicates $p < 0.001$. n = 30

stronger than that at the lower concentration, demonstrating that expression of PIN2-GFP depends on the concentration of nano-TiO_2.

3.5 Nano-TiO_2 Induced Root Elongation was Impaired in pin2 and tir1 Mutant

Our above RT-qPCR results showed that nano-TiO_2 treatment induced the expressions of PIN2 and TIR1, we want to know whether nano-TiO_2 treatment induced the root elongation through PIN2 or TIR1. Thus, we compared the effect of nano-TiO_2 on root elongation in Col, *pin2* or *tir1* mutant. As shown in Fig. 5, we found that nano-TiO_2 treatment activated the root elongation in Col seedlings, but such effect in the *pin2* or *tir1* mutant was not so obvious as that in wild-type Col line, thus, this data suggests that PIN2 or TIR1 is required for nano-TiO_2 induced root elongation.

4 Discussion

Ti is an essential micronutrient for plant growth and development [30]. However, nano-TiO_2 have some features that differ from those of TiO_2, which may affect plants in various ways. For example, nano-TiO_2 have photocatalytic properties, leading to excessive ROS production in plant cells. Nonetheless, nano-TiO_2 promote the growth of some plant species [30]. Thus, nano-TiO_2 have complex effects on plants. It has been reported that nanoparticles can affect plant growth by altering gene expression. For example, AgNPs inhibit root gravitropism by interfering with the expression of auxin-related genes [5]. Similarly, vitamin E gene expression is elevated at a high concentration (1000 mg/mL) [12].

In this study, we found that nano-TiO_2 promote root elongation in *Arabidopsis* in a dose-dependent manner. After exposure to nano-TiO_2, GUS expression in the *DR5::GUS* line was increased, whereas the fluorescent signal in *DII-VENUS* in root tips was reduced, indicating enhanced auxin accumulation. Expression of the auxin-related genes *PIN1-3*, *IIR1* and *YUC8* was analyzed by qPCR. We found the

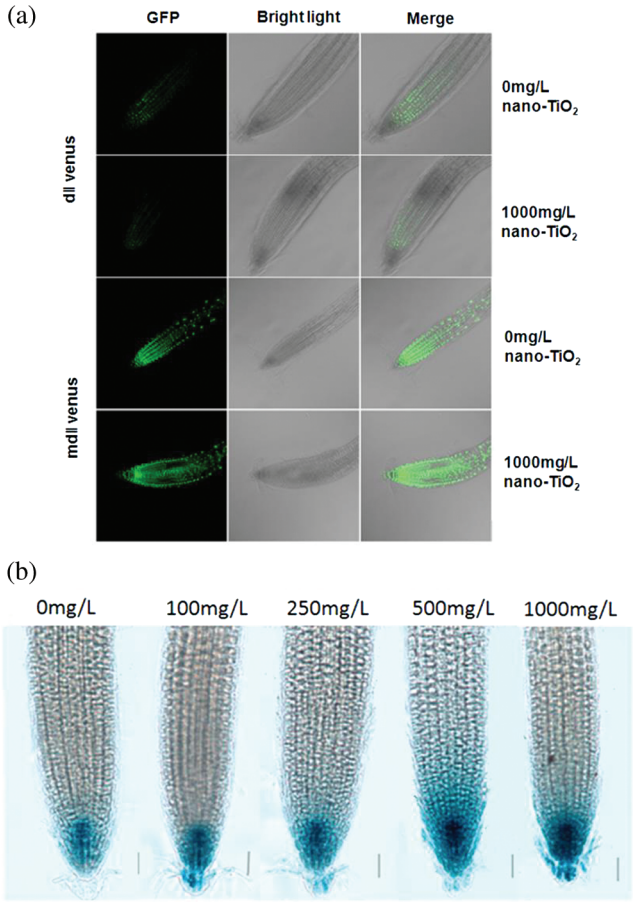

Figure 3: The effect of nano-TiO_2 on auxin-responsible DR5 reporter gene. (a) Fluorescent signal in the root tips of *DII-VENUS* and *mDII-VENUS* seedlings exposed to different concentration of nano-TiO_2 for 10 days. (b) images of GUS staining in root tips of *DR5::GUS* seedlings exposed to nano-TiO_2 for 6 days. Scale bars, 50 μm

transcription levels of *PIN2*, *YUC8* and *TIR1* to be notably unregulated, suggesting that nano-TiO_2 affect the transcription of genes in multiple pathways, including auxin transport (*PIN2*), biosynthesis (*YUC8*) and signaling (*TIR1*). Furthermore, the PIN2-GFP signal was enhanced with the increasing nano-TiO_2 concentration, consistent with the results of qPCR. Although the transcript level of *PIN1* was down-regulated at 250 and 500 mg/L nano-TiO_2, no significant difference was observed at 100 and 1000 mg/L nano-TiO_2, suggesting various effects of nano-TiO_2 on auxin-related gene expression.

Auxin accumulation in root tips is the result of auxin transport and biosynthesis [3,25–27], and based on our data, elevated expression of *PIN2* and *YUC8* accounts for the increases in auxin accumulation. As auxin accumulation is perceived by TIR1/AFB family F-box protein auxin receptors, activating downstream auxin responses, an increase in TIR1 should strengthen auxin responses in nano- TiO_2-treated root tips. Thus, it appears that PIN2 and YUC8 together with TIR1 co-regulate root elongation after treatment with nano-TiO_2. Nonetheless, it remains unclear how nano-TiO_2 activate expression of *PIN2, YUC8* and *TIR1*. Previous studies have reported that nano-TiO_2 induce ROS production [10–12,14], and auxin-related genes are regulated by H_2O_2 [31]. However, it remains to be further explored how nano-TiO_2 modulate the expression of auxin-related genes involved in root growth.

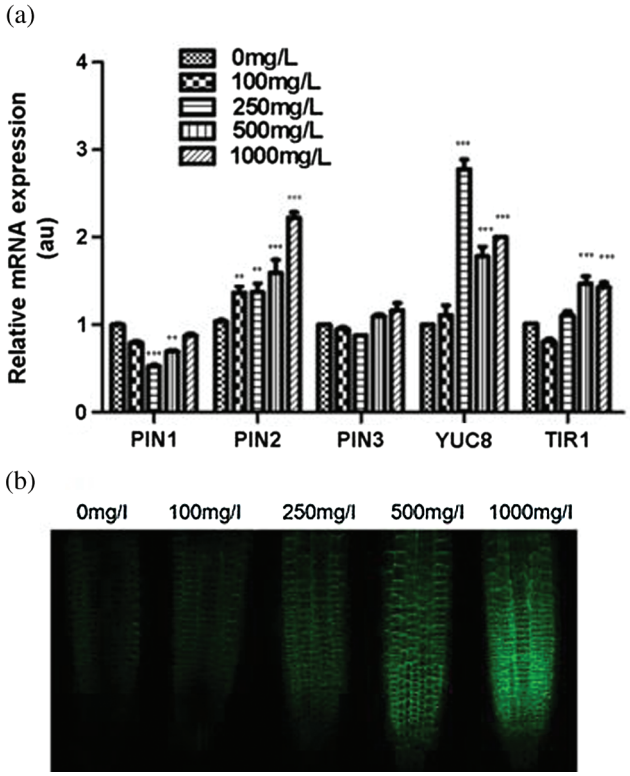

Figure 4: (a) qRT-PCR analysis of auxin-related gene expression in wild-type seedlings treated with or without nano-TiO_2. (b) the expression of *PIN2-GFP* in root tips treated with or without nano-TiO_2. Values are means ±SD. Error bars indicate SD of three biological repeated experiments. ***indicates $p <$ 0.001. n = 10

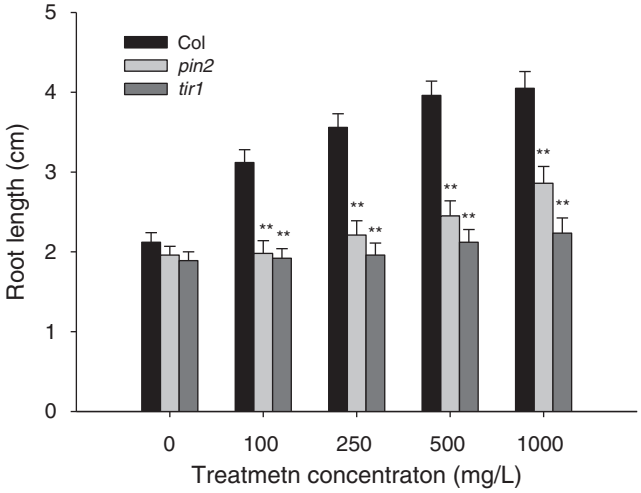

Figure 5: The effect of nano-TiO_2 on root elongation in Col, *pin2* and *tir1* mutant. The seeds of Col, *pin2* and *tir1* mutant was sowed on the 1/2 MS medium containing different concentration of nano-TiO_2 (0 mg/L, 100 mg/L, 250 mg/L, 500 mg/L and 1000 mg/L) for 6 days, and the root length was measured by rule. Values are means ± SD. Error bars indicate SD of three biological repeated experiments. ** indicates $p <$ 0.001. n = 10

5 Conclusion

Our data show that root elongation was promoted and was accompanied by increased auxin accumulation in root tips after exposure to nano-TiO_2. Expression of auxin-related genes, including *PIN1*, *PIN2*, *YUC8* and *TIR1*, was also altered, which was responsible for the observed auxin accumulation. The combined effect of these genes results in root growth promotion in *Arabidopsis*.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

1. 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(16),* 3053–3061. DOI 10.1016/j.scitotenv.2010.03.031.
2. Van Aken, B. (2015). Gene expression changes in plants and microorganisms exposed to nanomaterials. *Current Opinion in Biotechnology, 33,* 206–219. DOI 10.1016/j.copbio.2015.03.005.
3. Abas, L., Benjamins, R., Malenica, N., Paciorek, T., Wisniewska, J. et al. (2006). Intracellular trafficking and proteolysis of the Arabidopsis auxin-efflux facilitator PIN2 are involved in root gravitropism. *Nature Cell Biology, 8(3),* 249–256. DOI 10.1038/ncb1369.
4. Gottschalk, F., Sonderer, T., Scholz, R. W., Nowack, B. (2009). Modeled environmental concentrations of engineered nanomaterials (TiO_2 , ZnO, Ag, CNT, fullerenes) for different regions. *Environmental Science & Technology, 43(24),* 9216–9222. DOI 10.1021/es9015553.
5. Sun, J., Wang, L., Li, S., Yin, L., Huang, J. et al. (2017). Toxicity of silver nanoparticles to Arabidopsis: inhibition of root gravitropism by interfering with auxin pathway. *Environmental Toxicology and Chemistry, 36(10),* 2773–2780. DOI 10.1002/etc.3833.
6. Weir, A., Westerhoff, P., Fabricius, L., Hristovski, K., von Goetz, N. (2012). Titanium dioxide nanoparticles in food and personal care products. *Environmental Science & Technology, 46(4),* 2242–2250. DOI 10.1021/es204168d.
7. Keller, A. A., Lazareva, A. (2014). Predicted releases of engineered nanomaterials: from global to regional to local. *Environmental Science & Technology Letters, 1(1),* 65–70. DOI 10.1021/ez400106t.
8. Jośko, I., Oleszczuk, P. (2014). Phytotoxicity of nanoparticles—problems with bioassay choosing and sample preparation. *Environmental Science and Pollution Research International, 21(17),* 10215–10224. DOI 10.1007/s11356-014-2865-0.
9. Servin, A. D., Morales, M. I., Castillo-Michel, H., Hernandez-Viezcas, J. A., Munoz, B. et al. (2013). Synchrotron verification of TiO_2 accumulation in cucumber fruit: a possible pathway of TiO_2 nanoparticle transfer from soil into the food chain. *Environmental Science & Technology, 47(20),* 11592–11598. DOI 10.1021/es403368j.
10. Barbehenn, E. K., Wiggert, B., Lee, L., Kapoor, C. L., Zonnenberg, B. A. et al. (2002). Extracellular cGMP phosphodiesterase related to the rod outer segment phosphodiesterase isolated from bovine and monkey retinas. *Biochemistry, 24(6),* 1309–1316. DOI 10.1021/bi00327a007.
11. Shukla, R. K., Sharma, V., Pandey, A. K., Singh, S., Sultana, S. et al. (2011). ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells. *Toxicology in Vitro, 25(1),* 231–241. DOI 10.1016/j.tiv.2010.11.008.
12. Szymanska, R., Kolodziej, K., Slesak, I., Zimak-Piekarczyk, P., Orzechowska, A. et al. (2016). Titanium dioxide nanoparticles (100–1000 mg/l) can affect vitamin E response in Arabidopsis thaliana. *Environmental Pollution, 213,* 957–965. DOI 10.1016/j.envpol.2016.03.026.
13. Wang, Z. Y., Yu, X. L., Gao, D. M., Feng, W. Q., Xing, B. S. et al. (2010). Effect of nano-rutile TiO2 and multiwalled carbon nanotubes on the growth of maize (Zea mays L.) seedlings and the relevant antioxidant response. *Environmental Science, 31,* 480–487.

14. Xia, Y., Li, M., Charubin, K., Liu, Y., Heberle, F. A. et al. (2015). Effects of nanoparticle morphology and acyl chain length on spontaneous lipid transfer rates. *Langmuir, 31(47),* 12920–12928. DOI 10.1021/acs.langmuir.5b03291.
15. Ghosh, M., Bandyopadhyay, M., Mukherjee, A. (2010). Genotoxicity of titanium dioxide (TiO_2) nanoparticles at two trophic levels: plant and human lymphocytes. *Chemosphere, 81(10),* 1253–1262. DOI 10.1016/j.chemosphere.2010.09.022.
16. Burke, D. J., Pietrasiak, N., Situ, S. F., Abenojar, E. C., Porche, M. et al. (2015). Iron oxide and titanium dioxide nanoparticle effects on plant performance and root associated microbes. *International Journal of Molecular Sciences, 16(10),* 23630–23650. DOI 10.3390/ijms161023630.
17. Song, H., Lee, K. H., Jeong, H., Um, S. H., Han, G. S. et al. (2013). A simple self-assembly route to single crystalline SnO_2 nanorod growth by oriented attachment for dye sensitized solar cells. *Nanoscale, 5(3),* 1188–1194. DOI 10.1039/c2nr33114d.
18. Morteza, E., Moaveni, P., Farahani, H. A., Kiyani, M. (2013). Study of photosynthetic pigments changes of maize (Zea mays L.) under nano Tio_2 spraying at various growth stages. *Springerplus, 2(1),* 247. DOI 10.1186/2193-1801-2-247.
19. Hatami, M. G. M., Salehiarjomand, H. (2014). nano-anatase TiO_2 modulates the germination behavior and seedling vigority of some commercially important medicinal and aromatic plants. *Journal of Environmental Sciences, 8,* 53–59.
20. Tripathi, D. K., Shweta, S., Singh, S., Pandey, S. R. (2017). An overview on manufactured nanoparticles in plants: uptake, translocation, accumulation and phytotoxicity. *Plant Physiology and Biochemistry, 110,* 2–12. DOI 10.1016/j.plaphy.2016.07.030.
21. Zhu, Z. J., Wang, H. H., Yan, B., Zheng, H., Jiang, Y. et al. (2012). Effect of surface charge on the uptake and distribution of gold nanoparticles in four plant species. *Environmental Science & Technology, 46(22),* 12391–12398. DOI 10.1021/es301977w.
22. Andersen, C. P., King, G., Plocher, M., Storm, M., Pokhrel, L. R. et al. (2016). Germination and early plant development of ten plant species exposed to titanium dioxide and cerium oxide nanoparticles. *Environmental Toxicology and Chemistry, 35(9),* 2223–2229. DOI 10.1002/etc.3374.
23. Servin, A. D., Castillo-Michel, H., Hernandez-Viezcas, J. A., Diaz, B. C., Peralta-Videa, J. R. et al. (2012). Synchrotron micro-XRF and micro-XANES confirmation of the uptake and translocation of TiO(2) nanoparticles in cucumber (Cucumis sativus) plants. *Environmental Science & Technology, 46(14),* 7637–7643. DOI 10.1021/es300955b.
24. Sabatini, S., Beis, D., Wolkenfelt, H., Murfett, J., Guilfoyle, T. et al. (1999). An auxin-dependent distal organizer of pattern and polarity in the Arabidopsis root. *Cell, 99(5),* 463–472. DOI 10.1016/S0092-8674(00)81535-4.
25. Mashiguchi, K., Tanaka, K., Sakai, T., Sugawara, S., Kawaide, H. et al. (2011). The main auxin biosynthesis pathway in Arabidopsis. *Proceedings of the National Academy of Sciences, 108(45),* 18512–18517. DOI 10.1073/pnas.1108434108.
26. Galweiler, L., Guan, C. H., Muller, A., Wisman, E., Mendgen, K. et al. (1998). Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. *Science, 282(5397),* 2226–2230. DOI 10.1126/science.282.5397.2226.
27. Petrasek, J., Friml, J. (2009). Auxin transport routes in plant development. *Development, 136(16),* 2675–2688. DOI 10.1242/dev.030353.
28. Salehin, M., Bagchi, R., Estelle, M. (2015). SCFTIR1/AFB-based auxin perception: mechanism and role in plant growth and development. *Plant Cell, 27(1),* 9–19. DOI 10.1105/tpc.114.133744.
29. Chang, G., Yang, W., Zhang, Q., Huang, J., Yang, Y. et al. (2019). *ABI5-BINDING PROTIEN2 coordinates CONSTANS to delay flowering by recruiting the transcrptional corepressor TPR2*. *Plant Physiology, 179,* 477–490.
30. Lyu, S. H., Wei, X. Y., Chen, J. J., Wang, C., Wang, X. M. et al. (2017). Titanium as a beneficial element for crop production. *Frontiers in Plant Science, 8,* 139. DOI 10.3389/fpls.2017.00597.
31. Zhao, F. Y., Han, M. M., Zhang, S. Y., Wang, K., Zhang, C. R. et al. (2013). Hydrogen peroxide-mediated growth of the root system occurs via auxin signaling modification and variations in the expression of cell-cycle genes in rice seedlings exposed to cadmium stress. *Journal of Integrative Plant Biology, 55(5),* 484. DOI 10.1111/jipb.12029.