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ARTICLE

Phenotype, Physiology, and Gene Expression of Barley Seedlings in Response to Nano Zinc Oxide Stress

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ABSTRACT

In recent years, zinc oxide nanoparticles (ZnO NPs) have been widely used as zinc fertilizers and pesticides. The use of ZnO NPs in this way can provide benefits to humans, but also has potential risks. ZnO NPs inevitably enter the environment during their production and use, which affects the ecological environment and crop growth. In order to investigate the phenotype, physiology, and gene expression of barley (*Hordeum vulgare* L.) seedlings under ZnO NPs stress, the barely cultivars ZJU3 (P21), Golden Promise (GP) and L23 were chosen for study. Different ZnO NPs concentrations were applied to compare the physiological and biochemical indexes of the barley seedlings and the responses of six stress-related genes, when seedlings were cultured to the two-leaf stage through hydroponics. The results showed that the density of brown spots on the leaf surface increased with increasing ZnO NPs concentration. ZnO NPs stress inhibited the root growth of barley seedlings, and P21 was the most sensitive. Furthermore, ZnO NPs stress could stimulate plants to produce a large number of reactive oxygen species (ROS), resulting in an imbalance between the production and removal of ROS and membrane lipid peroxidation in plants. This imbalance inhibited the growth and development of the barley seedlings. With increasing ZnO NPs concentration, the activity of superoxide dismutase was gradually increased, the activity of catalase was progressively decreased, and the contents of malondialdehyde and proline were increased. Compared with the control, among six stress-related genes, the expression levels of five genes were downregulated and one gene was upregulated in the experimental group. This study preliminarily revealed the toxic effect of ZnO NPs on seedlings and the effect on the expression of stress-related resistance genes in different barley varieties.

KEYWORDS

Antioxidant enzymes; barley seedlings; reactive oxygen species; gene expression; zinc oxide nanoparticles

1 Introduction

As a new type of material with desirable properties, artificial nanomaterials are widely used in the biomedical, cosmetic and energy industries, among others, thus promoting a new round of industrial change [1]. It is estimated that the nano-material industry will reach 773 billion dollars by 2021 [2]. With the mass production and application of nano-products, nanoparticles (NPs) have inevitably entered the water and atmosphere, and have been transferred and enriched in the soil through atmospheric

precipitation and the infiltration of surface runoff. This pervasion of NPs has an impact on plant growth and crop production.

The mechanisms underlying the toxic effect of NPs on plants are not clear. However, recent studies have shown that NPs stimulate plants to produce reactive oxygen species (ROS). The induction of oxidative stress is a common toxic mechanism [3]. ZnO NPs are widely used in the cosmetics, rubber and electronic chemical fiber industries, among others. ZnO NPs are among the earliest commercially used and most widely used nanomaterials. While ZnO NPs provide benefits to humans, the potential soil pollution and risks to the ecological environment caused by the production and use of ZnO NPs have gradually attracted public attention. Through model predictions, Zhang et al. [4] found that the concentration of ZnO NPs was much higher in the soil than in the air and water. Compared with ordinary ZnO, ZnO NPs have a smaller particle size and larger specific surface area. These properties can provide ZnO NPs with greater surface reactivity than ZnO, which enables easier production of free radicals [5]. Therefore, ZnO NPs may have strong toxic effects on plants.

In many studies, the toxic effects of ZnO NPs are often manifested in seed germination, root and bud growth. For example, Zeynep et al. [6] found that the germination rates of bread wheat and rye seeds were decreased under a treatment of 10 $mg \cdot L^{-1}$ ZnO NPs. Lin [7] found that ZnO NPs were adsorbed on the root surface of ryegrass at the concentration of 2000 $mg \cdot L^{-1}$, which inhibited the absorption of root substances and thus had a toxic effect on ryegrass. Watson et al. [8] took wheat roots as the research object and found that the root growth was significantly inhibited under the treatment of 500 $mg \cdot L^{-1}$ ZnO NPs. Different plant seeds have different resistance to ZnO NPs, such as Xiang et al. [9] discovered that ZnO NPs concentrations of 1–80 $mg \cdot L^{-1}$ had no significant effect on the germination rate of cabbage, but had a significant inhibitory effect on root and shoot growth. Further, they found that 30 nm spherical ZnO NPs showed greater toxicity to cabbage. Overall, these findings imply that ZnO NPs have toxic effects on the growth and development of wheat, rye, and other plants. However, the effects of ZnO NPs on the growth and development of barley have not been reported. Studies on the toxic effects of metal oxide nanomaterials on plants have mainly focused on phenotypic changes; there have been few studies on the physiological and molecular effects.

Barley (*Hordeum vulgare* L.) is one of the oldest crops in the world. It is widely planted because of its ability to adapt to various environmental conditions. Globally, barley is the fourth most important food crop, and its planting area and total yield only follow those of wheat, rice, and maize [10]. However, barley is susceptible to various abiotic stresses that can lead to reduction in yield. In recent years, zinc pollution in farmland soil has rapidly spread to different scales [11]. Some studies have shown that soil zinc can affect barley root growth, material transportation, and ion exchange [12]. Thus, soil zinc can influence the growth and production of barley. In this study, the main aims were to explore the physiological effects of ZnO NPs on the growth and development of barley and the expression level of barley stress resistance genes. The results of this study initially reveal the toxic effects of ZnO NPs on barley seedlings and provide theoretical support for performing ecological risk assessments of ZnO NPs.

2 Materials and Methods

2.1 Experimental Materials

The barley cultivars ZJU3 (P21), Golden Promise (GP), and L23 were selected as the experimental materials. ZnO NPs were used to simulate abiotic stress. The ZnO NPs sample purity was ≥99% and the average particle size was 30 ± 10 nm.

2.2 Experimental Treatments

Entire barley seeds of uniform size were disinfected with 2.5% sodium hypochlorite for 10 min, and washed with distilled water four times. The seeds were spread on a Petri dish with wet filter paper, and

then germinated at 28°C under dark conditions. After 48 h, the seedlings with a root length of approximately 0.5 cm were selected and transferred to a 96-well polymerase chain reaction (PCR) plate for hydroponic culture. Attention was paid not to damage the roots of the seedlings. The seedlings were cultured in a constant temperature culture chamber (day/night temperatures of 26°C/24°C, relative humidity 50%–60% and light/dark cycle of 14 h/10 h) [13].

The seedlings were treated with 1/4 Hoagland nutrient solution when they reached the one-leaf stage. Subsequently, 100 mg and 300 mg of ZnO NPs were added to 1 L of nutrient solution for ultrasonic dispersion (100 W, 40 kHz) for 1.5 h to prepare the ZnO NPs suspension, respectively. When seedlings were at two-leaf stage, they were transferred to the 100 mg $\cdot$ L^{-1} and 300 mg $\cdot$ L^{-1} ZnO NPs suspensions and 1/4 Hoagland nutrient solution for 5 days. After the 5 days of treatment, the barley seedlings were sampled, and 20 seedlings were selected for each sample. The obtained materials were quickly frozen in liquid nitrogen and stored at −80°C. The experiment was performed in triplicate. The Hoagland nutrient solution formula was as described by Zhang et al. [14].

2.3 Phenotypic Observation and Root Growth Status

To determine the relative root elongation, 20 barley plants were randomly selected from the CK, 100 mg $\cdot$ L^{-1} ZnO NPs and 300 mg $\cdot$ L^{-1} ZnO NPs treatment groups. Then, the root length (based on the longest root) was measured after the 5 days of treatment. The average (n = 20) root length and the relative root length of the seedlings were then calculated. Relative root length (RRL) = experimental group root length/control group root length × 100%.

2.4 Determination of Physiological and Biochemical Indexes

The superoxide dismutase (SOD) and catalase (CAT) activities, malondialdehyde (MDA) and proline (Pro) contents were determined using the corresponding kits (Suzhou Geruisi Biotechnology Co., Ltd., China) according to the manufacturer's instructions [14].

2.5 Selection of Genes and Design of Specific Primers

Six genes related to barley and other plants stress resistance in Tab. 1 were selected, and the target cDNA sequences were downloaded from the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/). The homologous barley gene sequences were compared and searched using IPK (https://galaxy-web.ipk-gatersleben.de/). Primer Premier V5.0 software was used to design quantitative real-time PCR (qRT-PCR) primers. The primer sequences with melting temperature (Tm) values of 58–62°C and GC values of 40%–60% were selected in Ensembl Plants (http://plants.ensembl.org/Oryza_sativa/Tools/Blast?db=core) for specific comparison.

2.6 Total RNA Extraction, cDNA Synthesis, and qRT-PCR Analysis

The total RNA was extracted from barley leaves using an RNA extraction kit, and reverse transcribed into cDNA using a reverse transcription kit (Beijing Gold Biotechnology Co., Ltd., China). Specific primers for real-time fluorescent quantitative PCR were designed according to the stress resistance-related genes found in the literatures. Actin was used as the internal reference and the CFX96 Real-Time PCR Detection System (Bio-Rad, USA) was used for qRT-PCR amplification. The reaction steps were as described by the manufacturers of the real-time fluorescent quantitative PCR kit (Beijing Tiangen Biotech Co., Ltd., China). The relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method after three repeated experiments.

Table 1: Primer sequences used to amplify barley stress resistance-related genes

Gene	Gene ID	Name	Primer sequence (5′-3′)
HvUXE	*HORVU1Hr1G095430.9*	*HvNM1*	F: CCCGACTGGAAGATCATACTGC R: GCGACTTGCTGGACATAGGG
HvGRF4	*HORVU2Hr1G054270.3*	*HvNM2*	F: TCTGTCCAGTGATGTGGCTTCT R: CCAATCACATGGATCATCGC
HvPAA1	*HORVU7Hr1G048770.2*	*HvNM3*	F: CAGCCCTAGCTTTAGCTGACG R: ATACGTCACTAGCTGCACCAAC
OsZIP4	*HORVU1Hr1G070450.3*	*HvNM4*	F: CAACGGAAGGCTACAGGTCAT R: ATACGGCGAGCATTGACATG
AtHMA2	*HORVU7Hr1G097240.1*	*HvNM5*	F: ACAAAGAGCCGAGCAACCTG R: CTTGCTTGTGACCATCTGGCT
AtMHX	*HORVU5Hr1G055060.6*	*HvNM6*	F: AGGCGTTCTCGATGACGTATC R: GACGATTTGCTGGATACTCAGG
HvActin	*HORVU1Hr1G002840*	*Actin*	F: TGGATCGGAGGGTCCATCCT R: GCACTTCCTGTGGACGATCGCTG

2.7 Data Processing

Excel was used for data statistics, and SigmaPlot v10.0 was applied for drawing graphs. IBM SPSS Statistics 20 was used for significance analysis (*, ** and *** indicate significant, highly significant, and very significant differences at the $p < 0.05$, $p < 0.01$ and $p < 0.001$ levels, respectively).

3 Results and Analysis

3.1 Effects of ZnO NPs on the Growth of Barley Seedlings

The phenotypic differences between barley seedlings treated with different ZnO NPs concentrations for 5 days are shown in Fig. 1. With increasing ZnO NPs concentration, brown spots appeared on the surface of the barley seedling leaves, and the density of brown spots gradually increased. At the same time, after ZnO NPs stress, the leaves turned yellow, spreading from the main leaf vein to both sides of the leaves. The anterior segment of the leaves in the 300 $mg \cdot L^{-1}$ treatment group almost turned completely yellow, and brown spots gathered into patches.

The root length of the barley seedlings was measured after the fifth day of stress treatment and the relative root length was calculated. The results showed that the root length of barley seedlings was generally shorter in the experimental group than in the control group (Fig. 2), especially in the P21 cultivar, the difference was very significant, which indicated that ZnO NPs stress could inhibit the root growth of barley seedlings, and P21 was the most sensitive to the stress.

3.2 Effects of ZnO NPs on the Physiology and Biochemistry of Barley Seedlings

SOD is an antioxidant enzyme that scavenges ROS in plants. As shown in Fig. 3A, the SOD activity increased with increasing ZnO NPs treatment concentration. This clearly showed that the ZnO NPs had a toxic effect on the barley seedlings. The SOD activity was significantly higher in the treated P21, GP, and L23 cultivars than in the control group. The SOD activity of L23 was only 3.95% higher in the 300 $mg \cdot L^{-1}$ ZnO NPs treatment than in the 100 $mg \cdot L^{-1}$ treatment.

Figure 1: Phenotype of P21 seedlings treated with different concentrations of zinc oxide nanoparticles (ZnO NPs) for 5 days. A: overall phenotypic changes; B: control (CK); C: 100 mg · L^{-1} ZnO NPs; D: 300 mg · L^{-1} ZnO NPs

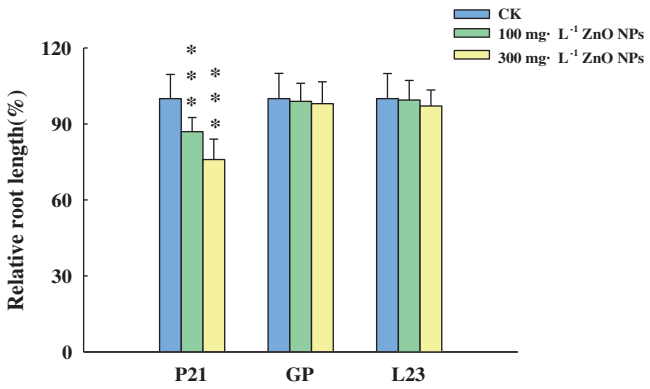

Figure 2: Relative root length of barley seedlings treated with different concentrations of zinc oxide nanoparticles (ZnO NPs) for 5 days. The seedlings of three barley cultivars (P21, GP, and L23) were subjected to a control (CK) treatment and two ZnO NPs treatments (100 mg · L^{-1} and 300 mg · L^{-1}), *** indicates significant difference at $p < 0.001$

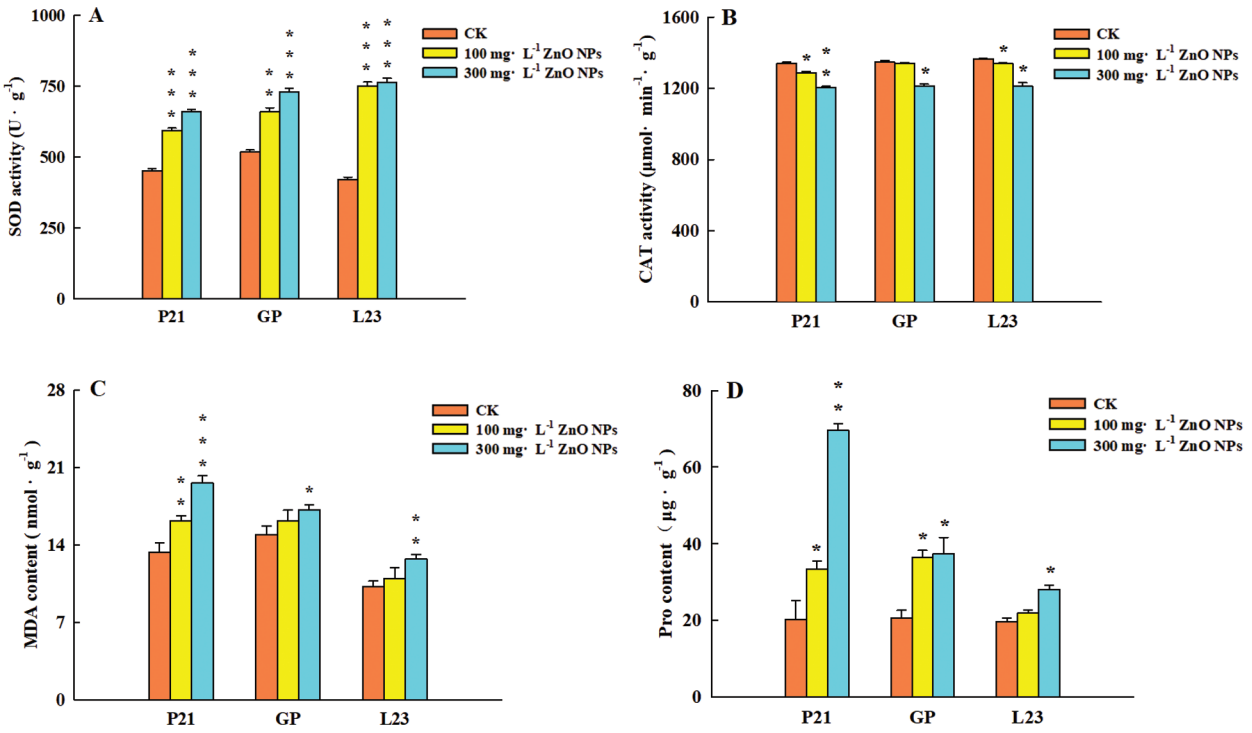

Figure 3: The effects of zinc oxide nanoparticles (ZnO NPs) on physiological and biochemical indexes of barley seedlings. A: SOD activity; B: CAT activity; C: MDA content; D: Pro content; *, ** and *** indicate significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively

As shown in Fig. 3B, the CAT activity of each barley variety showed a decreasing trend with increasing ZnO NPs concentration. Under 100 mg · L^{-1} ZnO NPs stress, the CAT activity of P21 and L23 was 3.78% and 1.91% lower, respectively. Further, under 300 mg · L^{-1} ZnO NPs stress, the CAT activity of P21 and L23 was 9.91% and 11.25% lower, respectively, than in the control group. In addition, the CAT activity of GP was obviously lower than in the control, only in the 300 mg · L^{-1} treatment. These results showed that the ZnO NPs were strongly toxic for P21 and L23.

The MDA is a physiological index that can reflect the degree of cell membrane damage. As shown in Fig. 3C, the MDA content in the barley seedling leaves was positively correlated with ZnO NPs concentration. The MDA content of P21, GP, and L23 was 21.16%, 6.04%, and 16.21% higher, respectively, in the 300 mg · L^{-1} treatment group than in the 100 mg · L^{-1} treatment group. This shows that the toxic effect of ZnO NPs on the barley seedlings was most significant under the 300 mg · L^{-1} treatment and that there were differences between varieties.

Pro can scavenge ROS in plants, and the accumulation of Pro indicates that plants are under stress. The Pro content increased with increasing ZnO NPs concentration. Especially, the Pro content of P21 under the 300 mg · L^{-1} treatment was up to 243.62%, which was significantly higher than the other treatments (Fig. 3D). However in the other two varieties, Pro content changed slightly. The result shows that the 300 mg · L^{-1} had a stronger toxic effect on P21.

3.3 Effect of ZnO NPs on the Expression of Stress Resistance Genes in Barley Seedlings

According to the physiological characteristics of three cultivars, P21 was selected for subsequent research. The expression levels of six stress resistance-related genes were detected under ZnO NPs stress,

and the results (Fig. 4) showed that the expression levels were downregulated for all genes except *HvNM1*. Among the five downregulated genes, the expression of *HvNM4* was decreased most significantly, and thus displayed the strongest response to the ZnO NPs. Compared to the control, the expression levels of all six genes were significantly different under the 100 mg · L^{-1} ZnO NPs treatment. However, only four genes were significantly different from the control under the 300 mg · L^{-1} ZnO NPs treatment. As the ZnO NPs concentration increased, the *HvNM1* gene expression first increased and then decreased. *HvNM4* expression was 77.7% and 78.5% lower under the 100 and 300 mg · L^{-1} ZnO NPs stress, respectively, than in the control. The expression levels of *HvNM2*, *HvNM3*, *HvNM5,* and *HvNM6* genes were similar under the two stress treatments; the relative expression of each gene was slightly higher under the high concentration stress than under the low concentration stress.

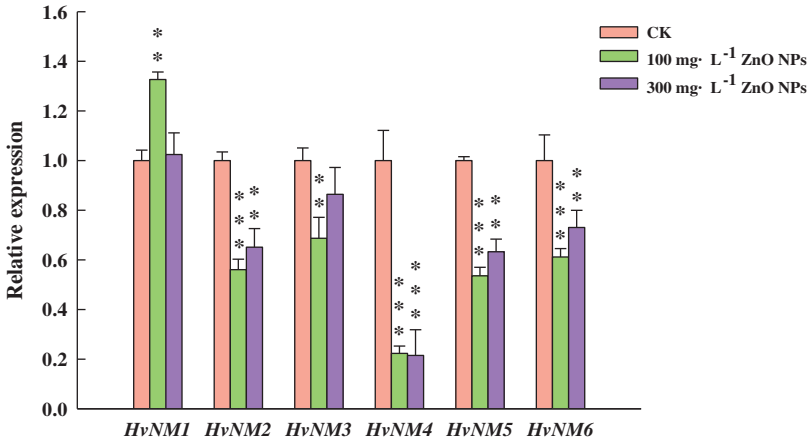

Figure 4: Relative expression levels of stress-related genes in the barley cultivar P21 under zinc oxide nanoparticle (ZnO NPs) stress. The barley P21 seedlings were subjected to a control (CK) treatment and two ZnO NPs treatments (100 mg · L^{-1} and 300 mg · L^{-1}). ** and *** indicate significant differences at $p < 0.01$, and $p < 0.001$, respectively

4 Discussion

At present, ZnO NPs have useful applications in industrial production and animal husbandry. However, safety problems associated with ZnO NPs entering the environment or even the food chain have also been repeatedly found. ZnO NPs can inhibit the germination and growth of a variety of plants to a certain extent. Pramod et al. [15] found that the growth of mung bean and chickpea seedlings was impeded when the concentration of ZnO NPs was greater than 20 mg · L^{-1}. In the present study, it was revealed that under ZnO NPs stress, brown spots appeared on the leaf surface of the barley seedlings and the root growth was somewhat inhibited. These results provided further evidence for the toxic effect of ZnO NPs on plants.

MDA is one of the final decomposition products of membrane lipid peroxidation, and its content reflects the degree of damage caused to plant cells [16,17]. In this study, the MDA content increased with increasing ZnO NPs concentration, indicating that the cell membrane system of the barley seedlings was damaged by the ZnO NPs treatments. Furthermore, the degree of damage was positively correlated with the ZnO NPs concentration, which was consistent with the results obtained by Yang et al. [18]. The result confirmed that exposure to nano-metal oxide particles increased the MDA content in crop seedlings, and thus causes membrane lipid peroxidation and certain damage to cells [19,20].

ZnO NPs induce plants to produce ROS such as superoxide and H_2O_2 [21], and excessive ROS can attack unsaturated fatty acids in cell membranes, induce membrane peroxidation, enhance membrane

permeability, and accelerate cell senescence and death. SOD and CAT constitute an active oxygen-scavenging system that maintains the dynamic ROS balance. Meanwhile, Pro plays a role in assisting in the scavenging of ROS and maintains the stability of cell membranes to some degree [22]. In this study, under different ZnO NPs concentrations, the physiological and biochemical indexes of the different barley genotypes varied, but the overall trends were similar.

SOD is an essential component of the ROS-scavenging system, and Pro also plays an important role in the process of plant adaptation to stress [23]. In this study, the SOD activity and Pro content both showed an increasing trend with increasing ZnO NPs concentration. This indicated that the toxic effect of ZnO NPs on barley was mainly due to oxidative stress. In the different varieties of barley, the increasing SOD activity demonstrated that there was increasing ROS accumulation within the plant with increasing stress. This excessive ROS would cause plasma membrane peroxidation, and thus these SOD activity results are consistent with the MDA content results. In addition, in the P21, the increase in Pro content was significantly higher than the increase in SOD activity, but the opposite was true for GP and L23. It was thus speculated that SOD and Pro exhibited an element of synergy and complementarity in the scavenging of ROS. The CAT activity showed a downward trend with increasing stress, and varied only slightly. This indicated that the plant antioxidant system may be characterized by different reaction mechanisms, and that CAT responded differently under ZnO NPs stress, which may be related to the species of ROS produced in the leaves.

The expression levels of stress-related genes in plants are positively correlated with their stress resistance. Among the six genes selected in this experiment, five genes showed a downward trend under increasing ZnO NPs stress. The *HvNM2* belongs to the *HvGRF* transcription factor family, and its promoter region has *cis* elements related to the regulation of the stress response [24]. *HvNM3* and *HvNM5* belong to the *HMA* gene family and have the ability to transport zinc [25]. The *HvNM4* encodes a zinc transporter, which is involved in the homeostatic distribution of Zn^{2+}. The *HvNM6* encodes the reverse exchange Mg^{2+}/H^{+} transporter, which also has a function in Zn^{2+} transport [26]. In this study, the expression levels of these five genes were decreased in response to ZnO NPs stress, which illustrated that in the barley leaves, the absorption and transportation of ZnO NPs was reduced to resist zinc toxicity and improve stress resistance. This result was consistent with the phenotypic variation observed in the leaves. ZnO NPs can be absorbed and transported from the root to the leaf, which leads to the leaves gradually turning yellow from the middle of the veins toward both sides (Fig. 1). Under different concentrations of stress, the *HvNM4* was downregulated most significantly, which implied that the transport of ZnO NPs in barley was mainly regulated by the zinc transporter. The results suggested that the expression of different genes may be activated to inhibit the transport of ZnO NPs in barley seedling leaves according to pathway requirements under stress. The *HvNM1* encodes a Golgi membrane binding protein that acts as a donor of arabinose in cell wall biosynthesis, and thus can directly affect cell wall biosynthesis [27]. With respect to the control, the *HvNM1* gene was significantly upregulated under 100 $mg \cdot L^{-1}$ ZnO NPs stress, but the expression only was increased by 2.5% under the 300 $mg \cdot L^{-1}$ treatment. It was speculated that when a small amount of ZnO NPs were adsorbed on the root surface, ZnO NPs might pass through the cell wall in the form of vesicles, causing damage to the cell wall and stimulating the upregulation of *HvNM1* gene expression [28].

In this study, the effects of ZnO NPs on physiological and biochemical indexes and stress resistance genes of barley were explored. This research provides a preliminarily evaluation of the harm caused by ZnO NPs to plant and environmental safety. In addition, the findings provide a basis for the rational development and safe use of nanotechnology. Future research should focus on the transformation and transport of ZnO NPs in plants, and on the interaction between ZnO NPs and plant organs, which will help to reveal the molecular mechanisms underlying the toxic effects of ZnO NPs on plants.

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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. Cao, J. L., Feng, Y. Z., Lin, X. G. (2016). A review: The influences of engineered nanomaterials on plant-microorganisms. *Acta Pedologica Sinica, 53(1),* 1–11. DOI 10.11766/trxb201506110191.
2. Andre, N., Tian, X., Lutz, M., Ning, L. (2006). Toxic potential of materials at the nanolevel. *Science, 311(5761),* 622–627. DOI 10.1126/science.1114397.
3. Jiao, J., Du, P. (2019). Research on the development trend of international nanotechnology industry. *Industrial Innovation, (10),* 36–37.
4. Zhang, J. Y., Song, W. H. (2010). Review of health hazards and ecological safety of Nano-znO. *Asian Journal of Ecotoxicology, 5(4),* 457–468.
5. 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.
6. Doğaroğlu, Z. G., Eren, A., Baran, M. F. (2019). Effects of ZnO nanoparticles and ethylenediamine-n, N′-Disuccinic acid on seed germination of four different plants. *Global Challenges,* 1–5. DOI 10.1002/gch2.201800111.
7. Lin, D. H., Xing, B. S. (2007). Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. *Environmental Pollution, 150(2),* 243–250. DOI 10.1016/j.envpol.2007.01.016.
8. Watson, J. L., Fang, T., Dimkpa, C. O., Britt, D. W., McLean, J. E. et al. (2015). The phytotoxicity of ZnO nanoparticles on wheat varies with soil properties. *BioMetals, 28(1),* 101–112. DOI 10.1007/s10534-014-9806-8.
9. Xiang, L., Zhao, H. M., Li, Y. W., Huang, X. P., Wu, X. L. et al. (2015). Effects of the size and morphology of zinc oxide nanoparticles on the germination of Chinese cabbage seeds. *Environmental Science and Pollution Research International, 22(14),* 10452–10462. DOI 10.1007/s11356-015-4172-9.
10. Schulte, D., Close, T. J., Graner, A., Langridge, P., Matsumoto, T. et al. (2009). The international barley sequencing consortium–At the threshold of efficient access to the barley genome. *Plant Physiology, 149(1),* 142–147. DOI 10.1104/pp.108.128967.
11. Song, W., Chen, B. M., Liu, L. (2013). Soil heavy metal pollution of cultivated land in China. *Research of Soil and Water Conservation, 4(15),* 922–930.
12. He, J., Tian, X. Z., Wang, X. D., Liu, B., Li, N. et al. (2017). Zn-toxicity thresholds as determined by micro morphological endpoints of barley roots in polluted soils and its prediction models. *Scientia Agricultura Sinica, 50(7),* 1263–1270. DOI 10.3864/j.issn.0578-1752.2017.07.009.
13. Zhang, X. Q., Chen, H. N., Jiang, H., Lu, W. Y., Pan, J. J. et al. (2015). Measuring the damage of heavy metal cadmium in rice seedlings by SRAP analysis combined with physiological and biochemical parameters. *Journal of the Science of Food and Agriculture, 95(11),* 2292–2298. DOI 10.1002/jsfa.6949.
14. Zhang, X. Q., Tong, T., Tian, B., Fang, Y. X., Xue, D. W. (2019). Physiological, biochemical and molecular responses of barley seedlings to aluminum stress. *Phyton-International Journal of Experimental Botany, 88(3),* 253–260. DOI 10.32604/phyton.2019.06143.

15. Pramod, M., Dhoke, S. K., Khanna, A. S., Yoke, K. Y. (2011). Effect of nano-znO particle suspension on growth of mung (*Vigna radiata*) and gram (*Cicer arietinum*) seedlings using plant agar method. *Journal of Nanotechnology,* DOI 10.1155/2011/696535.
16. Fan, W. H., Wang, X. L., Cui, M. M., Zhang, D. F., Zhang, Y. et al. (2012). Differential oxidative stress of octahedral and cubic Cu_2O micro/nanocrystals to daphnia magna. *Environmental Science & Technology, 46(18),* 10255–10262. DOI 10.1021/es3011578.
17. Xu, Y. J., Zhao, L. F., Xing, H. F., Luo, Y. X., Wei, Z. X. (2020). Effects of endophytic bacteria on proline and malondialdehyde of wheat seedlings under salt stress. *Acta Ecologica Sinica, 40(11),* 3726–3737. DOI 10.5846/stxb201802060309.
18. Yang, Z. Z. (2016). *The phytotoxicity assessment of metal oxide nanoparticles on maize (Zea mays L.) and rice (Oryza sativa L.)*. (*M.S. Thesis*). Northeast Normal University, China.
19. Li, H. B., Xing, B. S. (2019). Study on physiological toxicity of engineered nanoparticles on crop seedlings. *Journal of Jilin Agricultural Science and Technology University, 28(2),* 1–7+117. DOI 10.3969/j.issn.1674-7852.2019.02.001.
20. Yang, Z., Fan, S., Wei, X., Zhang, Z., Gui, X. et al. (2020). Effects of nano-cerium oxide on seed germination and seedling growth of vitex negundo. *Phyton-International Journal of Experimental Botany, 89(4),* 893–903. DOI 10.32604/phyton.2020.013644.
21. Xia, X. J., Zhou, Y. H., Shi, K., Zhou, J., Christine, H. F. et al. (2015). Interplay between reactive oxygen species and hormones in the control of plant development and stress tolerance. *Journal of Experimental Botany, 66(10),* 2839–2856. DOI 10.1093/jxb/erv089.
22. Song, M., Xu, W. J., Peng, X. Y., Kong, F. H. (2013). Effects of exogenous proline on the growth of wheat seedlings under cadmium stress. *Chinese Journal of Applied Ecology, 24(1),* 129–134. DOI 10.13287/j.1001-9332.2013.0134.
23. Zhao, F. G., Liu, Y. L., Zhang, W. H. (2002). Proline metabolism in the leaves of barley seedlings and its relation to salt tolerance. *Journal of Nanjing Agricultural University, 25(2),* 7–10.
24. Xue, Z. G., Wang, S. J., Yang, Y. Q., Gao, Z. N., Feng, H. et al. (2021). Genome-wide identification and bioinformatic analysis of growth regulating factor (GRF) family in barley. *Molecular Plant Breeding, 19(6),* 1750–1757. DOI 10.13271/j.mpb.019.001750.
25. Wang, X. K. (2019). *Identification of QTLs associated with Cd accumulation and tolerance, and functional characterization of a candidate gene HvPAA1 in barley* (*M.S. Thesis*). Zhejiang University, China.
26. Wang, H., Jin, J. Y. (2009). The physiological and molecular mechanisms of zinc uptake, transport, and hyperaccumulation in plants: A review. *Journal of Plant Nutrition and Fertilizers, 15(1),* 225–235. DOI 10.3321/j.issn:1008-505X.2009.01.033.
27. Cai, N. (2009). *Cloning and prokaryotic expression of the truncate UXE gene of hordeum vulgare* (*M.S. Thesis*). Northwest A&F University, China.
28. Zhou, L. S. (2014). *The uptake, accumulation and phytotoxicitv of ZnO nanoparticles to maize (Zea mays L.)* (*M.S. Thesis*). Zhejiang University, China.