Metal Nanoparticles-Mediated Changes on Gene Expressions and Physiological Parameters of Capsicum annuum L.

Hülya Akdemir1

1Department of Molecular Biology and Genetics, Faculty of Science, Gebze Technical University, Kocaeli, Turkey

ORCID ID: H.A. 0000-0001-7923-3031

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ABSTRACT

Objective: The uptake and accumulation of nanoparticles by plants create a potential threat for human health in cases where humans consume the plants. The aim of the study was to analyze the potential beneficial or inhibitory effects of nAl2O3 and nZnO on Capsicum annuum L. (pepper)’s germination, root growth, and expression levels of aquaporin and dehydrin genes.

Material and Method: Different concentrations (0.5, 2.5, or 5.0 mM) of nAl2O3 and nZnO were used for the germination of pepper seeds. ICP-MS analysis was performed to determine ion contents in nanoparticle-treated pepper plants. Levels of aquaporin and dehydrin gene expressions were analyzed by quantitative reverse-transcription polymerase chain reaction (qRT-PCR).

Results: The pepper germination was not affected by nanoparticle applications. While nAl2O3 treatments did not change root growth, higher concentrations of nZnO negatively affected root length and root number. In particular, the application of 0.5 mM nZnO significantly upregulated aquaporin and dehydrin gene expressions in roots. Downregulation of dehydrin gene expression occurred in stems and roots after exposure to nAl2O3 treatments.

Conclusion: The gene expression alterations and changes of growth parameters showed especially nZnO have potentially phytotoxic for pepper plants. Moreover, expression analysis suggested that the tested genes may play roles in response to the nanoparticle-based abiotic stress.

Keywords: Aquaporin, dehydrin, nanoparticles, nAl2O3, nZnO, germination, Capsicum annuum L., pepper

ÖZ

Amaç: Nanopartiküllelerin bitkiler tarafından alınması ve biriktirilmesi, bu bitkilerin insanlar tarafından tüketilmesi durumunda insan sağlığı için potansiyel bir tehdit oluşturmaktadır. Bu çalışmada, nAl2O3 ve nZnO’nun Capsicum annuum L. (biber)’in çimlenmesi, kök büyümeleri ile aquaporin ve dehidrin genlerinin ekspresyon seviyeleri üzerindeki potansiyelini yararlı veya inhibe edici etkilerinin analiz edilmesi amaçlanmıştır.

Gereç ve Yöntem: Biber tohumlarının çimlenmesi için farklı konsantrasyonlarda (0.5, 2.5 veya 5.0 mM) nAl2O3 ve nZnO kullanılmıştır. Nanopartikül uygulanmış biber bitkilerinde iyon içeriklerini belirlemek için, ICP-MS analizi yapılmıştır. Aquaporin ve dehidrin gen ekspresyonlarının seviyeleri ise kantitatif ters transkripsiyon polimeraz zincir reaksiyonu (qRT-PCR) ile analiz edilmiştir.

Bulgular: Nanopartikül uygulamalarının biber çimlenmesi üzerinde etkisi tespit edilmiştir. nAl2O3 uygulamaları kök gelişimini değiştirmezken, yüksek nZnO konsantrasyonları kök uzunluğunu ve kök sayısıını olumuz yolda etkilemiştir. Köklerdeki aquaporin ve dehidrin gen ekspresyonu, özellikle 0.5 mM nZnO uygulaması ile artmıştır. nAl2O3 uygulanan kök ve gövdelerde ise dehidrin gen ekspresyonu azalmıştır.

Sonuç: Gen ekspresyon ve büyümeyi parametrelerindeki değişiklikler, özellikle nZnO’nun biber bitkileri için potansiyel olarak fitotoksik olduğunu göstermiştir. Ayrıca, ekspresyon analizi, test edilen genlerin nanopartikül bazı abiotik strese yanıt olarak rol oynayabileceğini önermektedir.

Anahtar Kelimeler: Aquaporin, dehydrin, nanopartikül, nAl2O3, nZnO, çimlenme, Capsicum annuum L., biber
INTRODUCTION

Engineered nanomaterials with their unique technical properties have extensive use in various technology and industrial sectors including mechanical industries, energy applications, environmental remediation, biosensing applications and many others (1).

Metal/metal oxide nanoparticles as engineered nanomaterials are synthesized by the addition of reducing agents to produce metal nanoparticles or oxidizing/precipitating agents for metal oxide nanoparticles (2). Because of the wide variety of applications of these nanoparticles in various industries, their effects on environment and several organisms including plants have been extensively studied (1).

Since the nanoparticles can contaminate plants in different stages of their life cycle (3), detailed studies should have been performed at molecular, cellular, metabolic, and physiological levels to comprehend the actual effects of metal oxide nanoparticles on plants. Analysis of their effects on seed germination and root elongation as a phytotoxicity parameter of these nanoparticles (4) have been studied in many plant species.

Assessment of changes on gene expression levels are another important parameter to obtain nanoparticle-based changes in plant body. Plants contain a large number of aquaporins, which are proteins responsible for controlling water transport and facilitating the transport of uncharged solutes across membranes (5). The importance of aquaporins has been demonstrated in various abiotic stress conditions (5) and alterations in their gene expressions following nanoparticle treatments were also obtained in plants (6). Even though their roles are not yet clearly understood, dehydrins are considered as stress proteins, which play a role in dehydration stress, and it is also suggested that some dehydrins may have critical roles in plant growth (7). It was demonstrated that expression levels of several genes such as aquaporins, oxidative response genes and housekeeping genes were affected by nanoparticle treatments (8,9).

The uptake and accumulation of these nanoparticles by plants create a potential threat for human health in cases where humans consume the plants (10). Capsicum annuum L. (peppers) belong to the Solanaceae family and they are extensively consumed as both vegetables and spices across the world. Large areas of land in several countries such as Mexico, China, Korea, USA have been used for pepper cultivation because of their use in many cuisines (11). It suggests that there is possible nanoparticle contamination risk for these large land cultivation areas. In the study, pepper was used as a plant material because of its high consumption and production levels across the world and it was aimed to analyze potential beneficial or inhibitory effects of two metal oxide nanoparticles (nAl2O3 and nZnO) on its germination, root growth and expression levels of aquaporin (CaAqp) and dehydrin (CaDhn) genes.

MATERIAL AND METHOD

Plant Material
The pepper (Capsicum annuum L.) hybrid Bafra F1 seeds were used as a plant material. The seeds were gifted from Mehmet Yüksel, Yüksel Tohum (Antalya, Turkey) and kept in the dark at 4°C until use.

Characterization of Nanoparticles
The nAl2O3 (ca. 40 nm) and nZnO (<100 nm) nanopowders were purchased from PlasmaChem (Berlin, Germany) and Sigma-Aldrich (Saint Louis, MO, USA), respectively. Although commercial manufacturers characterized the nAl2O3 and nZnO in detail, scanning electron microscope (SEM) and transmission electron microscope (TEM) were also used to visualize shape and morphology of the nanoparticles.

For SEM analysis, the nanoparticles were placed on a carbon disc and coated with a few nm thick gold-layers by using a Baltec SDC 005 sputter-coater. SEM images were obtained using a Carl Zeiss Evo*40 instrument under high vacuum with an accelerating voltage of 10 kV.

For TEM analysis, FEI Tecnai G2 Spirit BioTwin CTEM instrument operating at an accelerating voltage of 120 kV were used.

Medium Preparation
Murashige and Skoog (MS) basal medium were purchased from Sigma Aldrich (Saint Louis, MO, USA). 0.5, 2.5 and 5 mM of nAl2O3 and nZnO were used to test their effects on pepper plants. Firstly, nanopowders were suspended in half strength MS medium (pH: 6.0) by sonication for 30 min in ultrasonic water bath at room temperature, and then the suspensions were homogenized by stirring them for 20 min before use.

Plant germination and seedling establishment
The pepper seeds were imbibed with distilled water for 16 hours before treatments. Then, the seeds were transferred to 900 cm3 vitrovents (Duchefa, The Netherlands) including 1 layer of sterile damp filter paper moistened with 5 mL of sterile one-half-MS medium (pH: 6.0) containing 0.5, 2.5 and 5 mM nAl2O3 or nZnO. For the control group, the seeds were transferred to the vitrovents including 1 layer of sterile damp filter paper moistened with 5 mL of sterile half strength MS medium without any of nanoparticles. Then, the vitrovents were incubated at 24°C for the first 7 days in the dark and then 8 days in the light. Each vitrovent containing 10 seeds per control group and nanoparticles treatments were used and each treatment was replicated three times.

Germination Data Analysis
The germination percentages, root number, length of roots and leaves of the control and nanoparticles-treated plants were obtained at the end of the 15th day of Capsicum seeds. SPSS version 25 (SPSS, USA) was used to perform statistical analysis. Germination percentages were analyzed with Chi-square statistics. One-way ANOVA (Analysis of variance) was performed to compare mean differences of leaf length. Root
length data were subjected to ANOVA, followed by Tukey post-hoc test to compare mean values. Because root number values do not have a normal distribution, the data were analyzed with the Kruskal-Wallis test, followed by the Mann-Whitney U test as a post hoc test. \( p < 0.05 \) was used as the level of significance for all the statistical analyses.

**Gene expression analysis of *Capsicum annuum* L. genes**

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was used to obtain impacts of nAl\(_2\)O\(_3\) and nZnO on the expression levels of genes, *CaDhn* (Accession no. AY225438.1) and *CaAqp2* (CaTIP1;1, Accession no. GU116569). Firstly, total RNA was extracted from the 15-day-old stems and 15-day-old roots of the peppers using the Plant/Fungi Total RNA purification kit (Norgen Biotek) according to the manufacturer’s instructions, with DNaseI treatment (DNase I, RNase-free, NEB). The RNA quantification was performed using Nano-drop equipment (Thermo Scientific Nanodrop™ 2000) and RNA samples were visualized by running them in 1.5% agarose gel with EtBr to check their integrity. Secondly, cDNA templates were reverse transcribed from purified RNA samples (3 µg per treatment) using SuperScript™ IV First-Strand Synthesis System (Invitrogen™) according to manufacturer’s instructions. The obtained cDNAs were kept at -20°C until use in qRT-PCR. The NCBI database (https://www.ncbi.nlm.nih.gov/) and its BLAST tool (blast.ncbi.nlm.nih.gov) were used to get full-length sequences and verification of mRNA sequences of *CaDhn* and *CaAqp2* genes, respectively. All gene-specific qRT-PCR primers from mRNA sequences were designed by Primer3 v4.1.0 (12).

The housekeeping genes, *Actin* mRNA, *Gapdh* (glyceraldehyde 3-phosphate dehydrogenase *GapCp*) and *EIF5A2* (Eukaryotic translation initiation factor 5A2) were used for normalization and their primer sequences were obtained from Wan et al. (13).

The primers used in the present study are listed below (5’ - 3’):

- **Actin mRNA (F; TGGTTATGGTAGGGATGGGTC, R; TTCTCTCTATTTCGCTGGG),**
- **Gapdh (F; ATGATGATGTGAAAGCAGCG, R; TTTCAACTGGTGGCTGCTAC),**
- **EIF5A2 (F; CCTGTTATGTCGTACCTTTG, R; GTTCCATGGCCTGCGACAGT),**
- **CaAqp2 (F; CGATGGCGTCACTACTCCTC, R; CACCAACGAAAGCCAGGA),**
- **CaDhn (F; GGAGAAATTGCCAGGGTATCACTC, R; CAGAACACCACAATCATAACATACC),**

qRT-PCR analysis was carried as three replicates. Reactions (10 µL) contain template cDNA (1 µL), Biorad iTaq Universal SYBR Green Supermix (5 µL), reverse (4 µM) and forward (4 µM) primers and nuclease-free water. CFX96 Touch Real Time PCR instrument (Biorad, France) was used for qRT-PCR analysis and the analysis conditions were 3 min at 95°C followed by 40 cycles of 5 sec at 95°C and 30 sec at 60°C.

Melt curve analysis was performed following each qRT-PCR amplification by heating the product from 65°C to 95°C, 0.5°C in 5 s increments. The optimal cycle threshold (Ct) was determined using Biorad CFX Manager 3.1 software. The above-men- tioned housekeeping genes were used for normalization using GeNorm V3 algorithm (14). The obtained normalized \( c_q \) values were processed according to the \( \Delta \Delta c_q \) method and presented as relative expression values for each gene.

**Determination of ion content**

Inductively coupled plasma mass spectrometry (ICP-MS, Thermo ICP MS X Series 2) was used to determine the level of aluminum and zinc ion contents of nAl\(_2\)O\(_3\) or nZnO treated pepper plants. The control and nanoparticles-treated plant samples (ca. 0.1 g) were weighted and digested with a 6 mL 65% (v/v) nitric acid. Following incubation in an ultrasonic water bath for 15 min, the samples were kept at room temperature for 24 h to complete the extraction. One-way ANOVA was performed to compare mean differences of ion contents in nanoparticles-treated plants.

Dissolution of Al\(^{3+}\) and Zn\(^{2+}\) ions in the exposure medium was obtained according to Leclerc and Wilkinson (15):

1. Centrifugation of the medium at 2500×g using centrifugation tubes containing 3 kDa centrifugal ultrafiltration unit (Merck Millipore, Germany) for 15 min at 0., 1., and 7. day.
2. Repetition of the 1st step four times and collecting only the filtrate at each centrifugation step.
3. Addition of 2% nitric acid to the 1 mL of filtrate.
4. ICP-MS analysis was evaluated to measure the release rates of Al\(^{3+}\) and Zn\(^{2+}\) from nAl\(_2\)O\(_3\) or nZnO.

For all ICP-MS analysis, multi-element ICP QC Standard solution (Chem-Lab, Belgium) was used as ICP-MS standard.

**RESULTS**

**Characterization of Nanoparticles**

SEM (Figure 1A-1B) and TEM (Figure 1C-1D) analysis were performed to visualize the shape and size of the tested nanoparticles.
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Particles. The images demonstrated the presence of uneven size with irregular, elongated, spherical shaped nanoparticles. While TEM analysis clearly showed that nZnO has different sizes from 40 nm to 100 nm, the nAl2O3 images were very low-resolution so it was not possible to obtain their mean sizes.

Effects of nAl2O3 and nZnO on the germination parameters of pepper

The effects of different nAl2O3 and nZnO concentrations (0.5, 2.5 and 5 mM) on pepper germination were analyzed and the germination percentages are presented in Figure 2. The results showed that the germination percentages of pepper seeds germinated in the MS medium at those nanoparticles concentrations were not statistically different in comparison to the control group seeds, which were germinated in the medium without nanoparticles.

The average length of root and leaf of pepper plants were recorded and the results are presented in Table 1. Even though the longest leaves (60.8 mm) were obtained in the control and 2.5 mM nAl2O3-treated pepper plants, there were no statistically significant differences among the tested plants.

While the control and nAl2O3-treated pepper plants had statistically similar root lengths, the application of nZnO at different concentrations affected root length compared to control plants. The longest roots (36.2 mm) and the shortest roots (14.7 mm) were obtained with the application of the lowest and highest nZnO concentrations, respectively.

Regarding number of roots, similar effects were observed with the application of nZnO at the end of the 15th day (Table 2). Even though a statistically similar number of roots were obtained in the control and nAl2O3-treated pepper plants, higher nZnO concentrations had adverse effects on the number of roots. The decreased root numbers (1.11 and 1.00) were obtained with the application of 0.25 and 0.5 mM nZnO concentrations (Table 2).

Determination of ion content

To understand the detailed impacts of applied metal nanoparticles on pepper plants, ICP-MS analysis was performed to obtain Al3+ and Zn2+ ions in pepper stems and roots following nAl2O3 and nZnO treatments. Interestingly, Al3+ ions were not detected in nAl2O3-treated pepper stems and roots with ICP-MS analysis. ICP-MS analysis showed the presence of Zn2+ ions in both pepper stems and roots with the application of nZnO in MS medium (Table 3). An accumulation of Zn2+ ions [44.3 and 70.4 mg/kg FW (fresh weight)] was found in 0.5 mM and 2.5 mM nZnO applied stems, respectively. The higher levels of Zn2+ ions were determined in the 0.5 mM (312.4 mg/kg FW) and 2.5 mM nZnO treatments (Table 2).

| Nanoparticles | Concentration (mM) | Average root length (mm)* | Average leaf length (mm)* |
|---------------|--------------------|----------------------------|--------------------------|
| Control       | 0                  | 29.1±1.59b                | 60.8±1.57a               |
| Al2O3         | 0.5                | 26.8±1.48b                | 59.4±3.03a               |
|               | 2.5                | 27.5±1.89b                | 60.8±1.66a               |
|               | 5.0                | 29.4±1.78ab               | 59.4±2.01a               |
| ZnO           | 0.5                | 36.2±1.97a                | 55.5±2.56a               |
|               | 2.5                | 22.7±1.31bc               | 57.0±2.27a               |
|               | 5.0                | 14.7±0.88d                | 55.2±2.06a               |

The different letters following the means in each column show statistical differences at p≤0.05 according to ANOVA Test. The mean differences were analyzed vertically.
mM (933.5 mg/kg FW) nZnO-applied pepper roots. However, no statistical differences were detected in the content of Zn$^{2+}$ ions between control and 5 mM nZnO-applied pepper stems and roots (Table 3).

ICP-MS analysis was performed to obtain the release rates of dissolved Al$^{3+}$ and Zn$^{2+}$ ions from nAl$_2$O$_3$ and nZnO containing MS media in 1st and 7th day. The results showed that the dissolution of Al$^{3+}$ and Zn$^{2+}$ ions from the applied nanoparticles was very low (Table 4). While Al$^{3+}$ release from nAl$_2$O$_3$ was only 0.053%, Zn$^{2+}$ release from nZnO was 0.153% even after 7 days (Table 4).

Table 2. The impacts of nAl$_2$O$_3$ and nZnO on root numbers of pepper plants$^a$.

| Nanoparticles | Concentration (mM) | Number of roots$^b$ | Mean rank | Significance test |
|---------------|-------------------|---------------------|-----------|------------------|
| Control       | 0                 | 1.84±0.27           | 78.53     |                  |
| Al$_2$O$_3$   | 0.5               | 1.85±0.24           | 79.83     |                  |
|               | 2.5               | 2.15±0.33           | 82.93     |                  |
|               | 5.0               | 1.37±0.17           | 63.61     |                  |
| ZnO           | 0.5               | 1.58±0.25           | 68.50     |                  |
|               | 2.5               | 1.11±0.07$^*$       | 55.74     |                  |
|               | 5.0               | 1.00±0.00$^*$       | 50.00     |                  |

$^a$ Root number values of the tested groups were individually compared with each other by using the Mann-Whitney U non-parametric test.

$^b$ Values were represented as mean±SE.

$^*$ Values were significantly different (p≤0.05)

Table 3. Zn$^{2+}$ content of pepper stems and roots treated with nZnO.

| Concentration (mM) | Zn$^{2+}$ content (mg/kg FW) |
|--------------------|------------------------------|
|                    | Stem$^*$ | Root$^*$ |
| 0 (Control)        | 2.21±0.06c | 4.27±0.07c |
| 0.5                | 44.32±0.19b | 312.44±1.16b |
| 2.5                | 70.39±0.60a | 933.49±3.61a |
| 5.0                | 2.81±0.13c | 4.68±0.02c |

$^*$ Values were represented as mean±SE.

The different letters following the means in each column show statistical differences at p≤ 0.05 according to ANOVA Test.

Table 4. The percentages (%) of Al$^{3+}$ and Zn$^{2+}$ released from 2.5 mM nAl$_2$O$_3$ or nZnO containing culture medium.

| Duration (days) | Al$^{3+}$ release (%) | Zn$^{2+}$ release (%) |
|----------------|-----------------------|-----------------------|
| 0              | -                     | -                     |
| 1              | 0.028                 | 0.069                 |
| 7              | 0.053                 | 0.153                 |

qRT-PCR analysis of CaAqp2 and CaDhn genes

To understand the effects of the tested metal nanoparticles at the molecular level, relative expression levels of two pepper genes (CaAqp2 and CaDhn) were analyzed by qRT-PCR (Figure 3 and

Figure 3. Relative expression levels of CaAqp2 and CaDhn in nAl$_2$O$_3$-treated pepper plants.
with respect to the control group (Figure 4B).

creased CaDhn group (Figure 4A).

similar gene expression levels when compared to the control in stems, the highest nZnO concentration (5 mM) resulted in slight decrease in lower concentrations of nZnO applications expression in roots. While CaAqp2 concentrations of nZnO applications resulted in inhibition of nZnO-treated pepper plants (Figure 4A). Increased concentration than that of control) was obtained in the roots of 0.5 mM CaDhn pepper plants had lower gene expression of highest strongly affected by nZnO treatments (Figure 4A and 4B). The expression was three-times higher than that of the control roots. However, higher nZnO treatments dramatically decreased CaDhn gene expression in stems compared to the other applied concentrations.

Figure 4. Relative expression levels of CaAqp2 and CaDhn in nZnO-treated pepper plants.

The CaAqp2 and CaDhn expression levels of pepper plants were strongly affected by nZnO treatments (Figure 4A and 4B). The highest CaAqp2 expression (almost five times higher expression than that of control) was obtained in the roots of 0.5 mM nZnO-treated pepper plants (Figure 4A). Increased concentrations of nZnO applications resulted in inhibition of CaAqp2 expression in roots. While CaAqp2 expression levels showed a slight decrease in lower concentrations of nZnO applications in stems, the highest nZnO concentration (5 mM) resulted in similar gene expression levels when compared to the control group (Figure 4A). CaDhn gene expression in roots was significantly affected with the application of 0.5 mM nZnO (Figure 4B). Its expression was three-times higher than that of the control roots. However, higher nZnO treatments dramatically decreased CaDhn gene expression levels in roots. nZnO-applied pepper plants had lower gene expression of CaDhn in stems with respect to the control group (Figure 4B).

DISCUSSION

An increase in the worldwide use of engineered metal nanoparticles is predicted to result in an elevated transfer of these particles to the environment. The studies and modeling analysis showed that thousands of tons of nanoparticles including Al, Zn, Ag, Fe etc. have been released into the environment due to their extensive production (16). The studies demonstrated their toxic and sometimes promoting effects in various plant growth parameters. Being one of the most consumed vegetables, the contamination of pepper production areas with these nanoparticles is highly possible. Among these nanoparticles, nZnO and nAl2O3 are the metal oxides and they have a wide application from electronics to biomedical applications and many others (17,18). In the present study, the impacts of nAl2O3 (ca. 40 nm) and nZnO (<100 nm) metal oxide nanoparticles were tested on pepper germination and seedling establishment parameters. The results showed that germination of pepper seeds was not affected with treatments of different concentrations of nZnO and nAl2O3. Similarly, Cucurbita pepo (zucchini) germination was unaffected by the treatment of 1000 mg/L nZnO (<5 nm and <10 nm) (19). Kumar et al. (20) tested the impacts of nZnO (≤50 nm) on several plant species and reported its inhibitory effects on cucumber germination and on shoot and root growth of wheat, green gram and cucumber. However, germination of rice was not significantly inhibited by the nanoparticles. Lee et al. (21) tested the effects of various metal oxide nanoparticles including nAl2O3, nSiO2, nFe3O4 and nZnO on seed germination and root elongation of Arabidopsis thaliana. The results showed that while nZnO was the most phytotoxic, nAl2O3 was not toxic for these plants. Regarding root length and number, none of the concentrations of nAl2O3 used affected root growth of peppers. While the lowest concentration of nZnO treatments increased average root length, the highest levels of nZnO decreased root length compared to the control plants in this study. Higher nZnO concentrations also negatively affected the number of roots. Similarly, an increased root length in rice was obtained when the seeds were soaked in <100 mg/L nZnO solution for 1-2 days, but the root growth was dramatically inhibited at concentrations of 500 and 1000 mg/L ZnO (22). Similar to the shortest roots resulting from high nZnO concentrations, the application of higher nZnO concentrations also resulted in reduced root length in barley (23).

The studies show that the size, concentration, composition, physical and chemical properties determine the fate of these nanoparticles on plants (24). The plant cell walls have a size exclusion limit (~5-20 nm) (25). However, some nanoparticles such as silver nanoparticles in Chlamydomonas reinhardtii (26) led to the formation of bigger pores in the cell walls and thereby resulted in the entrance of larger particles. Root junction, wounding, endocytosis, and symplastic transport could be other ways to accumulate or transport the nanoparticles in the
that the decrease in expression of certain barley aquaporin genes was stronger with lower Zn treatments than higher Zn treatments. It is concluded that plants would have limited Zn transport to the shoot to prevent major toxicity (30).

CONCLUSION

The extensive use of metal nanoparticles worldwide is predicted to result in an elevated transfer of these particles to the environment. In the present study, the impacts of nAl2O3 and nZnO on pepper germination, root growth and gene expression levels were analyzed. The results showed that pepper germination was not affected by nanoparticles applications. While nAl2O3 treatments did not significantly change root growth, higher concentrations of nZnO negatively affected root length and root number. In particular, the application of 0.5 mM nZnO upregulated aquaporin and dehydrin gene expressions in roots, significantly. Downregulation of dehydrin gene expression was obtained in stems and roots after exposure to nAl2O3 treatments. These gene expression alterations and changes of growth parameters showed that ZnNO especially is potentially phytotoxic for pepper plants. Moreover, expression analysis suggested that the tested genes may play roles in response to the nanoparticles based abiotic stress.

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