Science of the Total Environment 593–594 (2017) 535–542

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

ZnO nanoparticle effects on hormonal pools in *Arabidopsis thaliana*

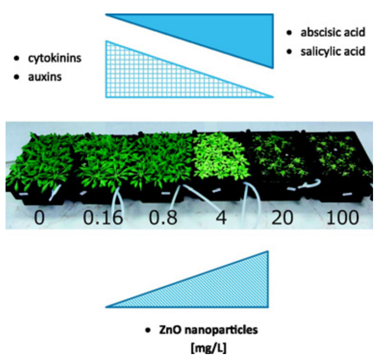
Radomira Vankova [a], Premysl Landa [b], Radka Podlipna [b], Petre I. Dobrev [a], Sylva Prerostova [a,c], Lenka Langhansova [b], Alena Gaudinova [a], Katerina Motkova [b], Vojtech Knirsch [a], Tomas Vanek [b,*]

[a] *Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany AS CR, Rozvojová 263, 165 02 Prague 6, Czech Republic*
[b] *Laboratory of Plant Biotechnologies, Institute of Experimental Botany AS CR, Rozvojová 263, 165 02 Prague 6, Czech Republic*
[c] *Department of Experimental Biology, Faculty of Science, Charles University, Viničná 7, 128 43 Prague 2, Czech Republic*

HIGHLIGHTS

- Growth suppression of Arabidopsis plants correlates with ZnO nanoparticle content.
- Growth inhibition is associated with decrease of cytokinins and auxins in apices.
- Nanoparticle stress effects are indicated by abscisic and salicylic acids elevation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 December 2016
Received in revised form 16 March 2017
Accepted 17 March 2017
Available online 27 March 2017

Keywords:
Abscisic acid
Cytokinin
Plant hormone
ZnO nanoparticle

ABSTRACT

At present, nanoparticles have been more and more used in a wide range of areas. However, very little is known about the mechanisms of their impact on plants, as both positive and negative effects have been reported. As plant interactions with the environment are mediated by plant hormones, complex phytohormone analysis has been performed in order to characterize the effect of ZnO nanoparticles (mean size 30 nm, concentration range 0.16–100 mg L^{-1}) on *Arabidopsis thaliana* plants. Taking into account that plant hormones exhibit high tissue-specificity as well as an intensive cross-talk in the regulation of growth and development as well as defense, plant responses were followed by determination of the content of five main phytohormones (cytokinins, auxins, abscisic acid, salicylic acid and jasmonic acid) in apices, leaves and roots. Increasing nanoparticle concentration was associated with gradually suppressed biosynthesis of the growth promoting hormones cytokinins and auxins in shoot apical meristems (apices). In contrast, *cis*-zeatin, a cytokinin associated with stress responses, was elevated by 280% and 590% upon exposure to nanoparticle concentrations 20 and 100 mg L^{-1}, respectively, in roots. Higher ZnO nanoparticle doses resulted in up-regulation of the stress hormone abscisic acid, mainly in apices and leaves. In case of salicylic acid, stimulation was found in leaves and roots. The other stress hormone jasmonic acid (as well as its active metabolite jasmonate isoleucine) was suppressed at the presence of nanoparticles.
The earliest response to nanoparticles, associated with down-regulation of growth as well as of cytokinins and auxins, was observed in apices. At higher dose, up-regulation of abscisic acid, was detected. This increase, together with elevation of the other stress hormone - salicylic acid, indicates that plants sense nanoparticles as severe stress. Gradual accumulation of *cis*-zeatin in roots may contribute to relatively higher stress resistance of this tissue.

* Corresponding author.
E-mail address: vanek@ueb.cas.cz (T. Vanek).

http://dx.doi.org/10.1016/j.scitotenv.2017.03.160

1. Introduction

The rapid development of nanotechnology has resulted in production and utilization of a number of nanoparticles of varying composition, size and shape characteristics (Nair and Chung, 2014a). The nanoparticles have been applied in multiple industrial, agricultural as well as medicinal areas. Their wide-spread use is inevitably associated with their release into the environment, creating a potential risk, as the knowledge of their interactions with biological systems is mostly lacking. Plants, as the main source of food and feed, represent an important component of the food chain. Thus, an understanding of the fate of nanoparticles in plants and of their impact on plant growth, metabolism and yield is highly desirable.

Both positive and negative effects of nanoparticles on plants have been reported (for review, see Arruda et al., 2015). Seed germination and root growth were found promoted, e.g., in case of zinc oxide nanoparticles (Prasad et al., 2012; Adhikari et al., 2016), silver nanoparticles (Syu et al., 2014) or carbon nanotubes (Martinez-Ballesta et al., 2016). Toxic effects were reported as well, e.g., in case of zinc oxide nanoparticles (Zhang et al., 2015a, 2015b). Thus, not only the particle composition, but many other factors like their concentration, size, shape, charge, exposure time, plant species and age, as well as type of the substrate should be taken into consideration.

Generally, nanoparticle application leads to an increase of the level of reactive oxygen species (e.g., H_2O_2) with subsequent activation of the antioxidant system (Zhao et al., 2012). The oxidative stress may be accompanied with DNA damage, as indicated by transient stimulation of the expression of DNA mismatch repair genes (Nair and Chung, 2014b). Transcriptomic studies revealed upon nanoparticle application also up-regulation of the expression of genes related to abiotic and biotic stress responses (Thiruvengadam et al., 2015; Landa et al., 2015). Nanoparticle exposure may result in the change of transcription of genes involved in biosynthesis or signal transduction of plant hormones, e.g., of auxin repressor or auxin response genes, abscisic acid (ABA) biosynthetic genes or ethylene signaling components (Kaveh et al., 2013; Syu et al., 2014).

Zinc oxide nanoparticles (ZnO NP) are produced in high amounts. At present their production reaches approximately 550 tons per year (Piccinno et al., 2012). Due to a wide application of zinc oxide, e.g., in cosmetics (UV-protection in sunscreens), in paints or as anticorrosive, antibacterial and antifungal agents, further increase of ZnO NP use can be anticipated. Therefore we focused on evaluation of the impact of ZnO NP on *Arabidopsis thaliana* plants. As plant growth and development as well as plant interactions with the environment are regulated by plant hormones, we followed the response of Arabidopsis to ZnO NP at the level of cytokinins (CKs), auxin, ABA, salicylic acid (SA) and jasmonic acid (JA). The changes in hormone pools were correlated with the physiological performance of the plants.

2. Materials and methods

2.1. Plant material and growth conditions

Arabidopsis thaliana Columbia 0 plants were cultivated in hydroponic conditions in cultivation containers (Araponics SA, Belgium) using 25% Hoagland solution as a cultivation medium. The pH of the medium was adjusted to 6.2–6.3 prior to autoclaving. Plants were cultivated at 21 °C with 8/16 h light/dark cycle, at a light intensity of 130 μmol m^{-2} s^{-1} in a growth cabinet (MLR-350, Sanyo Electric Co., Japan) with aeration of cultivation media every 3 h for 15 min.

2.2. Treatment of plants with ZnO NPs

Four-week old plants were exposed to ZnO NPs (average particle size 30 nm, specific surface area 70 m^2 g^{-1}, purity 99.9%; MKnano, Canada). The nanoparticles were added to the cultivation media to reach final concentrations of 0.16, 0.8, 4, 20, and 100 mg L^{-1}. After two-week exposure to ZnO NPs, the weight of rosettes and length of roots were determined. Samples of apices, leaves and roots were quickly cut, weighed, flash frozen in liquid nitrogen and stored at −80 °C until further analysis.

2.3. Hormone analysis

Samples were purified and analyzed as described earlier (Dobrev and Kaminek, 2002; Dobrev and Vankova, 2012). Samples (ca 20 mg fresh weight) were homogenized with a ball mill and extracted with cold (−20 °C) methanol/water/formic acid (15/4/1 v/v/v). The following stable isotope-labeled internal standards (10 pmol/sample) were added: $^{13}C_6$-IAA (indole-3-acetic acid, Cambridge Isotope Laboratories); $^{2}H_4$-SA (Sigma-Aldrich); $^{2}H_3$-PA (phaseic acid), $^{2}H_3$-DPA (dihydrophaseic acid, NRC-PBI), $^{2}H_6$-ABA, $^{2}H_5$-JA, $^{2}H_5$-transZ (*trans*-zeatin), $^{2}H_5$-transZR (*trans*-zeatin riboside), $^{2}H_5$-transZ7G (*trans*-zeatin N7-glucoside), $^{2}H_5$-transZ9G (*trans*-zeatin N9-glucoside), $^{2}H_5$-transZOG (*trans*-zeatin O-glucoside), $^{2}H_5$-transZROG (*trans*-zeatin riboside O-glucoside), $^{2}H_5$-transZRMP (*trans*-zeatin riboside phosphate), $^{2}H_3$-DHZ (dihydrozeatin), $^{2}H_3$-DHZR (dihydrozeatin riboside), $^{2}H_3$-DHZ9G (dihydrozeatin N9-glucoside), $^{2}H_6$-iP (isopentenyladenine), $^{2}H_6$-iPR (isopentenyladenosine), $^{2}H_6$-iP7G (isopentenyladenine N7-glucoside), $^{2}H_6$- iP9G (isopentenyladenine N9-glucoside), $^{2}H_6$-iPRMP (isopentenyladenosine phosphate) (Olchemim). Extract was applied to a mixed mode reverse phase–cation exchange SPE column (Oasis-MCX, Waters). Two hormone fractions were sequentially eluted: (1) fraction A, eluted with methanol (auxins, ABA, SA, JA); and (2) fraction B, eluted with 0.35 M NH_4OH in 60% methanol (CKs). Fractions were evaporated to dryness, dissolved in 10% methanol and analyzed using high-performance liquid chromatograph (HPLC) (Ultimate 3000, Dionex) coupled to a hybrid triple quadrupole/linear ion trap mass spectrometer (3200 Q TRAP, Applied Biosystems), column Luna C18(2) (100 × 2 mm, 3 μm, Phenomenex), flow rate 0.25 mL min^{-1}. Quantification of hormones was done by isotope dilution with multilevel calibration curves ($r^2 > 0.99$) (Ljung et al., 2005). Data processing was carried out with the Analyst 1.5 software (Applied Biosystems).

2.4. Statistical analysis

Data on the hormone values ($n = 6$; two independent biological experiments, in each three biological replicates) were analyzed using a multi-factorial ANOVA. The significance of the differences among the measured hormone values was assessed using a *t*-test.

3. Results

3.1. Effect of ZnO NPs on growth of Arabidopsis plants

Exposure to different concentrations of ZnO NPs in the range 0.16–100 mg L^{-1} resulted in substantial phenotypic changes in Arabidopsis plants (Fig. 1). The lowest ZnO NP concentration of 0.16 mg L^{-1} had an only negligible influence on shoot growth in comparison to control plants. Application of 0.8 mg L^{-1} had already a negative effect, which, however, did not reach statistical significance (Table 1). Substantial negative impact was observed at 4 mg L^{-1} ZnO NP, very strong growth inhibition being found in case of 20 and 100 mg L^{-1}. The two highest ZnO NP concentrations resulted in substantial elevation of anthocyanin content in leaves

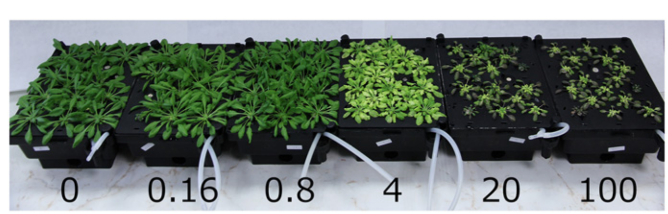


Fig. 1. *Arabidopsis thaliana* plants exposed for two-weeks to ZnO nanoparticles in concentration range 0–100 mg L^{-1}.