Morphological and biochemical responses of *Abelmoschus esculentus* (L.) Moench to zinc nanoparticles

I B Gokak and T C Taranath

P G Department of Botany, Karnatak University, Dharwad 580003, India

E-mail: tctaranath@rediffmail.com

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Abstract

The increasing application and use of nanoparticles are directly related to their release in the environment. There has arisen the necessity to study the interactions of nanoparticles with plants and other organisms. The present investigation is an attempt to evaluate the morphological and biochemical responses of *Abelmoschus esculentus* (L.) Moench to zinc nanoparticles treatment. Seeds were treated with zinc nanoparticles at concentrations of 50, 100, 200, 500 ppm. The morphological and biochemical responses recorded include germination percentage, root, shoot length, number of root hairs and number of leaves. Chlorophyll, protein, proline and carbohydrate contents in the leaves of 30-days-old treated plants were estimated. A greater amount of total dissolved solids (TDS) leached out from the seeds soaked in different concentrations of Zn nanoparticles compared to the control. Zinc nanoparticles executed a positive impact on the seed germination. The seed germination percentage increased in all the treatments compared to control. Increase in the chlorophyll and protein content was also observed in the treated plants. The proline content increased in treated plants indicating the stress. The carbohydrate content of leaves decreased drastically in response to the treatment.

Keywords: zinc nanoparticles, HR-SEM, TDS, germination, carbohydrate

Classification numbers: 4.02, 5.06, 5.07

1. Introduction

Nanoparticles (NPs) show unique physical and chemical properties and have attracted much attention for their distinct characteristics. Their uniqueness arises specifically due to higher surface-to-volume ratio representing an increased importance. The nanoparticles enter the environment unintentionally through atmospheric emissions, domestic wastewater, agriculture and accidental release during manufacture/transport; or through intentional releases such as during remediation efforts [1]. The interaction and the impact of nanomaterials with their unique physical and chemical properties, on living systems have been recently explored [2]. The use of nanoparticles may represent important toxicological risks to human beings and the environment, as it is difficult to evaluate the possible toxic effects of such materials.

Nanoparticles are predicted to have high efficiency of transport to collector surfaces due to Brownian diffusion, and their potential mobility may be predicted by knowing the exact surface properties. The surface properties of engineered nanoparticles are of essential importance for their aggregation behavior, mobility in aquatic and terrestrial systems and interactions with algae, plants, and fungi [3–5]. Terrestrial plants can be exposed to a multitude of nanomaterials through soil in many ways like potential leaching from nano-enabled products, intentional sub-surface release for environmental remediation, surface run-off and irrigation using contaminated surface water, land applications of contaminated biosolids or waste water effluent discharge. The nanoparticles exposure is of most concern as these materials have a half-life of months to possibly years. In terms of ecotoxicity, there has been significantly greater focus on aquatic species rather than terrestrial species.

The reports on the effects of metal oxide nanoparticles such as CuO, ZnO, TiO2 and silver on medicinal and crop plants are numerous, but there are meager reports on the effect...
of metallic zinc nanoparticles (Zn NPs) on crop plants. Hence, the present investigation was undertaken to assess the morphological and biochemical responses of *Abelmoschus esculentus* (L.) Moench to Zn NPs treatment. Zinc is an essential trace element for humans, animals, plants and for microorganisms. Zinc is found in nearly more than 100 specific enzymes, serves as structural ions in transcription factors and is stored and transferred in metallothioneins. It is ‘typically the second most abundant transition metal in organisms’ after iron and it is the only metal which appears in all enzyme classes. In proteins, Zn ions are often coordinated to the amino acid side chains of aspartic acid, glutamic acid, cysteine and histidine.

Zn NPs are one of the most commonly used and widely applied types of nanomaterials. These compounds have been shown to have high anticorrosion properties [6]. In addition, they are known to have antibiotic properties, which have encouraged the pharmaceutical preparation of these compounds as well as assessment of their potential use in pharmaceuticals and cosmetology [7]. Moreover, ZnO NPs are now used in personal care products such as sunscreens, as well as in coatings and paints due to their high levels of UV absorption efficiency and transparency [8].

### 2. Materials and methods

#### 2.1. Description of the materials

The seeds of *Abelmoschus esculentus* (L.) Moench var Arka anamika were obtained from the Seed Unit, Department of Seed Technology, University of Agricultural Science, Dharwad. The Zinc nanoparticles of size <50 nm and purity \( \geq 99\% \) trace metal basis were procured from Sigma Alderich Co. Further, the nanoparticles were subjected for high resolution scanning electron microscopy (HR-SEM) imaging (figure 1) and energy dispersive x-ray (EDAX) analysis (figure 2) at Sophisticated Analytical Instrument Facility, Indian Institute of Technology Madras, to confirm the shape and size of the nanoparticles. The EDAX analysis was performed to test the purity of the nanoparticles. HR-SEM imaging revealed the hexagonal shape of the nanoparticles and the EDAX analysis confirmed the purity of nanoparticles.

#### 2.2. Experimental design and data observation

##### 2.2.1. Seed treatment and germination experiments

In order to study the effect of different concentrations of zinc nanoparticles on germination of *Abelmoschus esculentus* seeds a complete randomized design with three replicates was employed. The experimental treatments included four concentrations (50, 100, 200 and 500 ppm) of zinc nanoparticles and a control (without zinc nanoparticles). The experiments were conducted in laboratory conditions at Environmental Biology Laboratory, P G Department of Botany, Karnataka University, Dharwad.

The seeds of uniform size were selected and surface sterilized using 0.2% mercurous chloride. The surface sterilized seeds were soaked in nanoparticles suspension and in distilled water (control) for 24 h. After 24 h of imbibitions the seed leachates (suspension) were subjected to measurement of electric conductivity and total dissolved solids (TDS) (figure 3). One milliliter of leachates was used to estimate the amount of soluble carbohydrates leached out from the seeds. The imbibed seeds were then transferred to the petriplates containing wet filter papers as three groups of 10 seeds in each petridish. 10 milliliters of treatment (Zn NPs suspension) was added to each petridish. For control, only distilled water was used. The germination test was conducted according to the rules of International Seed Testing Association (ISTA) [9]. The seeds were considered to be germinated when the radical attained a length of 1 mm and the plumule had just unfolded.

##### 2.2.2. Assessment of morphological and biochemical responses

The imbibed seeds (10 seeds/pot) were transferred to pots to study the morphological and biochemical response. The pots were irrigated with the nanoparticles suspensions of respective concentration and a control (distilled water) on alternate days. After 30 days of growth the plants were uprooted for morphometric analysis such as measurement of root length, shoot length, number of...
root hairs and number of leaves as a morphological response. In order to study the biochemical responses, the leaves of treated and untreated (control) plants were used for estimation of the chlorophyll, protein, proline and carbohydrate content. The chlorophyll content was estimated using the Arnon’s method [10], protein content using Bradford’s method [11], proline content using Bates method [12] and the carbohydrate content was estimated using Anthrone method [13].

2.2.3. Data analysis. All the experiments were conducted in triplicate; the values are expressed as mean ± statistical error (mean ± SE). Duncan’s post hoc test and one way analysis of variance were used to test for differences using SPSS ver.17.0 software at 5% probability level.

3. Results and discussion

3.1. Effect of zinc nanoparticles on seed germination

Results showed that the leachate conductivity is more in nanoparticles treatment compared to the control (distilled water) and also the amount of soluble carbohydrates leached out from the seeds is more in nanoparticles treatments compared to control. When seeds are imbibed in water, internal seed substances such as potassium, phosphate, sugar, amino acid etc are leached out due to membrane deterioration [14] and it was proved that as the membrane deterioration increases, the leachate conductivity also increases [15].

The germination experiment conducted revealed the positive effect of Zn NPs treatment on germination of Abelmoschus esculentus (L.) Moench seeds. The percentage of seed germination increased in all treatments. The highest seed germination percentage of 92.5% was observed in 100 ppm and the lowest of 45% was observed in control (table 1). This enhanced germination may be attributed to the photo-sterilization and photo-generation of active oxygen such as superoxide and hydroxide anions that enhance seed stress resistance and encouraged capsule penetration for intake of water and oxygen needed for quick germination [16]. Similar results were obtained for TiO$_2$ nanoparticles in the case of wheat seeds [17]. The germination percentage increased in treatments (1, 2, 10 and 100 ppm) of TiO$_2$ nanoparticles. However, the higher concentration 500 ppm did not show any effect on germination percentage and was similar to control. The multiwalled carbon nanotube (MWCNT) treatment also increased the germination percentage of mustard seeds indicating their beneficial role [18].

3.2. Effect of zinc nanoparticles on plant growth

The effect of Zn NPs treatment on the root length (table 1) was not significant but they had a significant impact on shoot length. The root length increased in treatments 100 ppm and 500 ppm but decreased in 50 and 200 ppm compared to control. The highest root length of 7.8 cm was observed in 100 ppm followed by 7.6 cm in 500 ppm and the lowest of 4.25 cm in 50 ppm followed by 4.97 in 200 ppm. Similarly, the treatment of TiO$_2$ in concentrations (5, 20, 40, 60 and 80 ppm) increased the root length in wheat grass [19]. But no significant effect of Zn NPs treatment was observed on root growth of Cucumis sativus [20].

The shoot length decreased in all concentrations of Zn NPs indicating the negative impact of Zn NPs on the shoot growth of Abelmoschus esculentus (L.) Moench (table 1). However, the Zn NPs treatment increased the shoot length in Cucumis sativus [20]. Similarly the shoot length of Phaseolus vulgaris and Zea mays increased on exposure to the silver nanoparticles (Ag NPs) of the concentration 20, 40 and 60 ppm but decreased in higher concentration of 80 and 100 ppm [21]. Lower and higher concentrations 50 and 100 ppm of alumina nanoparticles increased the shoot length in Triticum aestivum but, the decrease was observed in optimal concentrations of 200 and 500 ppm [22].
The Zn NPs treatment had a significant impact on the number of root hairs but they did not show any significant impact on the number of leaves (table 1). The number of root hairs increased in all treatments except for the higher concentration of 500 ppm, indicating the beneficial impact of Zn NPs on absorption machinery of the plant system.

3.3. *Effect of zinc nanoparticles on biochemical constituents*

Zinc is used in chlorophyll synthesis, protein synthesis, membrane function, cell elongation and tolerance to environmental stresses [23]. The chlorophyll content of the leaves increased in all the treatments except 50 ppm, which was similar to the control (table 2). Similarly, the Ag NPs treatment at the concentration of 20, 40 and 60 ppm increased the chlorophyll content in *Phaseolus vulgaris* and *Zea mays*, but the decrease in chlorophyll content was observed in 80 and 100 ppm [21]. The protein content of the leaves increased in all treatments except 200 ppm (table 2). The highest protein content of 48.88 μmol g⁻¹ was observed in 100 ppm and the least of 21.33 μmol g⁻¹ in 200 ppm. Similarly, Ag NPs treatment to *Phaseolus vulgaris* and *Zea mays* at 20, 40 and 60 ppm increased the protein content but it decreased in 80 and 100 ppm treatment [21]. The proline content of the leaves increased in all treatments indicating the stress of Zn NPs on plants. The highest proline content of 17.57 μmol g⁻¹ was observed in 100 ppm and the lowest of 2.05 μmol g⁻¹ was observed in control. The carbohydrate content decreased in all treatments, the lowest carbohydrate content of 0.57 mg g⁻¹ was observed in 500 ppm followed by 1.78, 5.74, 8.34 and 10.62 mg g⁻¹ in 50, 200, 100 ppm and control respectively (figure 4). Opposite results were reported for silver nanoparticles treatment [21]. The carbohydrate content was enhanced in *Phaseolus vulgaris* and *Zea mays* on exposure to Ag NPs with concentrations of 20, 40 and 60 ppm. However, it decreased in the concentrations of 80 and 100 ppm. The generation of reactive oxygen species (ROS) is one important toxicity mechanism, as ROS are known to damage cell membranes, cellular organelles and nucleic acids contained in DNA and RNA [24]. Radicals are formed as necessary intermediates in a variety of normal biochemical reactions, but when generated in excess, or not appropriately controlled, radicals can damage a broad range of macromolecules. ROS can clearly be toxic to cells. By definition, radicals possess an unpaired electron, which makes them highly reactive and thereby able to damage all macromolecules, including lipids and proteins.

4. Conclusion

The present investigation was carried out to evaluate the morphological and biochemical responses of *Abelmoschus esculentus* (L.) Moench to Zn NPs treatment. The present investigation revealed the positive impact of Zn NPs on the seed germination, root length, number of root hairs, number of leaves per plant, chlorophyll and protein content. The percentage of seed germination increased in Zn NPs treatment compared to control. The root length increased in 100 and 500 ppm but decreased in 50 and 100 ppm. The number of root hairs per plants increased on exposure to the Zn NPs enhancing the absorption machinery of the plant system. The chlorophyll content increased in all treatment showing the positive impact of nanoparticles treatment on synthesis of chloroplast pigments. The enhanced proline content was observed in all treatments indicating the stress of Zn NPs on plants. The carbohydrate content decreased in all treatments showing the negative impact of Zn NPs on carbohydrate production. It is suggested that further studies are needed to

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**Table 1.** Effect of nZn on seed germination the seedling growth of *Abelmoschus esculentus* (L.) Moench.

| Concentration (ppm) | Germination (%) | Root length (cm) | Shoot length (cm) | No. of root hairs (mean ± SE) | No. of leaves (mean ± SE) |
|---------------------|-----------------|------------------|------------------|-------------------------------|--------------------------|
| Control             | 45 ± 20887a     | 6.15 ± 0.1c      | 25.45 ± 0.342d   | 16.5 ± 0.645a                 | 6 ± 0d                   |
| 50                  | 52 ± 2.5ab      | 4.25 ± 0.967a    | 20.97 ± 1.874e   | 16.75 ± 0.750a                | 6.25 ± 0.25a             |
| 100                 | 92.5 ± 4.787c   | 7.8 ± 0.46d      | 17.85 ± 0.309ab  | 23.75 ± 1.548b                | 6.5 ± 0.5a               |
| 200                 | 57.5 ± 6.0292ab | 4.97 ± 0.853b    | 20.50 ± 0.404bc  | 26.75 ± 2.594d                | 5.75 ± 0.25a             |
| 500                 | 62.5 ± 4.787b   | 7.6 ± 0.2d       | 17.57 ± 1.125a   | 13.5 ± 0.645a                 | 6.25 ± 0.479a            |

Means in each column followed by similar letters are not significantly different at the 5% probability level using Duncan’s test.

**Table 2.** Biochemical effect of Zn nanoparticle on *Abelmoschus esculentus* (L.) Moench.

| Concentration (ppm) | Chl a (μmol g⁻¹) | Chl b (μmol g⁻¹) | total Chl (μmol g⁻¹) | Protein (mg g⁻¹) | Proline (μmol g⁻¹) | Carbohydrates (mg g⁻¹) |
|---------------------|-----------------|-----------------|---------------------|-----------------|-----------------|----------------------|
| Control             | 1.571 ± 0.0017a | 1.257 ± 0.009a  | 0.540 ± 0.0173a     | 24.88 ± 0.256b  | 2.046 ± 0.211a  | 10.62 ± 0.0057c      |
| 50                  | 1.586 ± 0.0057b | 1.520 ± 0.0002b | 0.776 ± 0.0057d     | 33.47 ± 0.146c  | 3.57 ± 0.590b    | 1.776 ± 0.0033b      |
| 100                 | 1.622 ± 0.0057a | 1.243 ± 0.012a  | 0.540 ± 0.0000      | 48.88 ± 0.009c  | 3.57 ± 0.590b    | 8.366 ± 0.0088d      |
| 200                 | 1.590 ± 0.004b  | 1.430 ± 0.0000  | 0.710 ± 0.0173c     | 21.33 ± 0.010a  | 15.966 ± 0.066c  | 6.25 ± 0.0057a       |
| 500                 | 1.590 ± 0.011b  | 1.353 ± 0.0057b | 0.630 ± 0.0050      | 31.25 ± 0.146e  | 3.430 ± 0.047b   | 5.736 ± 0.0088c      |

Means in each column followed by similar letters are not significantly different at the 5% probability level using Duncan’s test.
examine the interaction of nanoparticles with micro and macro molecules resulting in phytotoxicity and positive impacts on growth.

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