



Toxicity and bio-effects of CuO nanoparticles on transgenic Ipt-cotton

Nhan Le Van, Yukui Rui, Weidong Cao, Jianying Shang, Shutong Liu, Trung Nguyen Quang & Liming Liu

To cite this article: Nhan Le Van, Yukui Rui, Weidong Cao, Jianying Shang, Shutong Liu, Trung Nguyen Quang & Liming Liu (2016) Toxicity and bio-effects of CuO nanoparticles on transgenic Ipt-cotton, Journal of Plant Interactions, 11:1, 108-116, DOI: [10.1080/17429145.2016.1217434](https://doi.org/10.1080/17429145.2016.1217434)

To link to this article: <https://doi.org/10.1080/17429145.2016.1217434>



© 2016 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group



Published online: 08 Aug 2016.



Submit your article to this journal [↗](#)



Article views: 1319



View related articles [↗](#)



View Crossmark data [↗](#)



Citing articles: 10 View citing articles [↗](#)

RESEARCH ARTICLE

 OPEN ACCESS

Toxicity and bio-effects of CuO nanoparticles on transgenic *lpt*-cotton

Nhan Le Van^{a,b}, Yukui Rui^{a,c}, Weidong Cao^d, Jianying Shang^a, Shutong Liu^a, Trung Nguyen Quang^b and Liming Liu^a

^aCollege of Resources and Environmental Sciences, China Agricultural University, Beijing, People's Republic of China; ^bCenter for Training, Consultancy and Technology Transfer, Viet Nam Academy of Science and Technology, Cau Giay, Ha Noi, Vietnam; ^cStockbridge School of Agriculture, University of Massachusetts, Amherst, MA, USA; ^dKey Laboratory of Plant Nutrition and Fertilizer, Ministry of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, People's Republic of China

ABSTRACT

This study investigated the effects of copper oxide nanoparticles (CuO NPs) on the growth and development of transgenic cotton harboring the *lpt* gene, which encodes isopentenyl transferase (*lpt*). Three concentrations of CuO NPs were evaluated: 10, 200, and 1000 mg·L⁻¹, each with three replicates. The height and the root length were 26.91% and 42.80% decreased after 10-day exposure with 1000 mg·L⁻¹ CuO NPs, respectively. In addition, less abundant root hairs and lower shoot biomass of *lpt*-cotton when compared with the control group. The growth of *lpt*-cotton was not affected by 10 mg·L⁻¹ CuO NPs, but a high concentration of CuO NPs promoted the absorption of Fe and Na into roots, and inhibited the production of phytohormones in *lpt*-cotton. The CuO NPs increased the concentration of iPA in shoots, which can delay senescence. The extent of the increase in iPA in response to CuO NPs should be relative to the amount of *lpt* immobilized onto the NPs in the plant tissue. To our knowledge, this is the first study to evaluate the phytotoxicity of CuO NPs to *lpt*-transgenic cotton. These results establish a baseline for further research on the effects of nanoparticles on transgenic crops harboring the *lpt* gene.

ARTICLE HISTORY

Received 14 April 2016
Accepted 23 July 2016

KEYWORDS

CuO nanoparticle; *lpt*-cotton; iPA; nutrient content; phytohormone; phytotoxicity

Abbreviations

NPs	nanoparticles
<i>lpt</i>	isopentenyl transferase
iPA	isopentenyl adenine
CKs	cytokinins
DI	deionized
ICP-MS	inductively coupled plasma mass spectrometry
ICP-AES	inductively coupled plasma-atomic emission spectroscopy
ABA	abscisic acid
IAA	indole-3-acetic acid
t-ZR	trans-zeatinriboside
GA	gibberellic acid
RPM	round per minute
TEM	transmission electron microscope
ANOVA	one-way analysis of variance
SD	standard deviation

Highlights

- High concentration CuO NPs significantly decreased the height, root length, root hairs, and shoot biomass of *lpt*-cotton.
- High concentration of CuO NPs promoted the absorption of Fe and Na into roots, and inhibited the production of some phytohormones of transgenic *lpt*-cotton.
- The effects of CuO NPs on exogenous gene expression of transgenic crops.

Introduction

Copper oxide nanoparticles (CuO NPs) have been one of the most widely used metal nanoparticles (NPs) since 2011, and

have attracted much interest because of their catalytic, electric, optical, photonic, textile, and nanofluid properties, and their antibacterial activity (Perelshtein et al. 2009; Devi & Singh 2014; Tamuly et al. 2014). The CuO NPs and other Cu complexes have been used for various purposes: as water purifiers, algacides, fungicides, antibacterial compounds, and antifouling agents (Raul et al. 2014). The CuO NPs have been shown to be effective in killing a range of bacterial pathogens (Ravishankar & Jamuna 2011; Raul et al. 2014), and a high concentration of CuO NPs was required to achieve a bactericidal effect (Aruoja et al. 2009). However, soluble Cu ions derived from CuO NPs showed some toxicity to algae *Pseudokirchneriella subcapitata* (Bouwmeester et al. 2009).

Nanomaterials have been used as insecticides and herbicides in the agriculture (Bergeson 2010; Nair et al. 2010; Sharon et al. 2010) and have been used to enhance plant germination and growth (Khodakovskaya et al. 2009). In another study, TiO₂ NPs promoted photosynthesis and nitrogen metabolism, and improved the growth of spinach, which relate to the change of nitrogen metabolism (Yang et al. 2006). However, NPs have also been shown to have negative effects on plants. Alumina NPs inhibited root elongation in corn, cucumber, soybean, cabbage, and carrot (Ma et al. 2010). Nanoparticles of Zn (35 nm) and ZnO (20 nm) at 2000 mg L⁻¹ decreased the germination rates of ryegrass and corn, respectively (Kasemets et al. 2013). The growth of *Saccharomyces cerevisiae* BY4741 was inhibited by CuO NPs (Gregersen et al. 2013). These studies have increased our knowledge about the phytotoxicity of NPs; however, less is known about the phytotoxic effects of lower concentrations of NPs and the environment impacts of nanoparticles remain uncertain (Syu et al. 2014).

Senescence is a normal developmental process in annual crop plants, but it can reduce crop yield if it is induced prematurely under adverse environmental conditions. Leaf senescence plays an important role in the recycling of nutrients from old, non-functional leaves to young productive leaves and developing seeds (Liu et al. 2012). The expression of the *Ipt* gene under the control of senescence-associated promoters in transgenic plants has been successful in delaying senescence (Rubio-Wilhelmi et al. 2011). Cytokinins (CKs) are phytohormones that control plant development (Roitsch & Ehneß 2000), and play a role in delaying senescence. These hormones play a role in carbohydrate transport and metabolism, and in sink–source relationships (Cowan et al. 2005). The *Ipt* gene has been studied using different promoters in various dicots (Lin et al. 2002; Khodakovskaya et al. 2005) and in some monocots such as rice (*Oryza sativa*), tall fescue (*Festuca arundinacea*), and Italian ryegrass (*Lolium multiflorum*) (Li et al. 2004; Hu et al. 2005). Introduction of the *Ipt* gene, which was located close to the right T-DNA border in the fusion construct, into transgenic tobacco plants resulted in transcriptional fusions between plant promoters and the *Ipt* gene (Guo et al. 2010). As a group of phytohormones, CKs show activities in many aspects of plant growth and development, such as cell division, shoot-growth initiation, the release of axillary buds, delayed leaf senescence, and induction of photomorphogenic development (Le et al. 2014).

Beside nanotechnology, transgenic plants are also widely applied in agriculture in the world and particularly in China. Therefore, NPs might be interacted with *Ipt*-transgenic plants together in agricultural production in the near future. In this study, we investigated the effects of CuO NPs on the growth and development of transgenic cotton harboring the *Ipt* gene (*Ipt*-cotton). We determined the effects of CuO NPs at different concentrations on plant height, root length, number of root hairs, biomass, nutrient contents, and concentrations of isopentenyl adenine (iPA) and other phytohormones. This is the first study to evaluate the phytotoxicity of CuO NPs to *Ipt*-cotton. Our results establish a baseline for further research on the effects of NPs on transgenic crops harboring the *Ipt* gene.

Materials and methods

Characterization of CuO NPs

The CuO NPs (30 ± 10 nm) were purchased from the Shanghai Hufeng Bioscience Technology Company (Shanghai, China), and other chemicals were purchased from the Beijing Chemical Plant (Beijing, China). Deionized (DI) water was used in all of these experiments. The CuO NPs were characterized as described by Amrut and co-workers (Wang et al. 2012b) by observations under a high-resolution scanning electron microscope (JEOL JSM 5600, Tokyo, Japan) and a transmission electron microscope (TEM) (JEM 200CX, Tokyo, Japan).

Experiment exposure

The *Ipt*-cotton was purchased from the Chinese Academy of Agricultural Sciences, China Agricultural University (CAU). The experiments were performed as described previously (Le et al. 2014; Li et al. 2014). Cotton seeds were randomly selected and sterilized in (30%) H_2O_2 for 15 min, rinsed

with DI water, and then immersed in DI water for 12–15 h before germination in sterilized, moist sand and two seedlings were then transplanted to 3.0-L pots containing 2.0 L of nutrient solution. After 4 days, the nutrient solution was added with different concentrations of CuO nanoparticles (0-control, 10, 200, and 1000 mg L^{-1}) for 10 days. All experiments were repeated three times and were conducted from September to October, 2014, in a greenhouse at the CAU.

Measurement of biomass and plant height, root length, and root hairs

After 10-day treatments with CuO NPs, plant height was measured from the growing point to the cotyledon node using a ruler. Root length was measured from the growing point to the root point (cm). The root hairs were counted. Biomass was measured by separating plants into roots and shoots, drying the materials at 80°C for 24–36 h to constant weight, and then weighing them.

Measurement of nutrient contents

Dried shoots and roots were separately ground to a powder to measure nutrient contents, as described previously (Li et al. 2014). Briefly, samples (20–30 mg) were soaked in 5 mL (98%) HNO_3 for 24 h, and then 3 mL H_2O_2 was added. The mixture was digested at 180°C for 4–5 h until 1 mL solution remained, and then the sample was diluted with deionized water. The Zn and Cu contents were determined by inductively coupled plasma mass spectrometry (ICP-MS) with a DRC-II instrument (PerkinElmer, Norwalk, CT, USA). The contents of Ca, Na, K, Mg, Fe, Mn, B, Mo, and P in the shoots and roots were determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) with an iCap 6000 instrument (Thermo Scientific, Waltham, MA, USA).

Determination of hormone and iPA concentrations

Abscisic acid (ABA), indole-3-acetic acid (IAA), trans-zeatin-riboside (t-ZR), isopentenyl adenosine (iPA), and gibberellic acid (GA) were extracted and purified as described elsewhere (Dong et al. 2008; Wang et al. 2012a). The hormone concentrations were determined by ELISA kits using monoclonal antibodies (Phytodetek, Agdia, Elkhart, IN, USA) (Ni et al. 2005). Approximately 0.2 g fresh leaf and root samples were separately homogenized in 2 mL of 80% methanol (containing 40 mg L^{-1} butylated hydroxytoluene) and stored at -20°C for 48 h. The solution was then centrifuged at 3500 rpm for 15 min. The precipitates were re-suspended in 1 mL 80% methanol at -20°C for 16 h. C_{18} Sep-Pak cartridges (Waters, Milford, USA) were applied for purification of the combined extracts. Then the samples were evaporated under vacuum to remove the organic solvent, and were dissolved in 2.0 mL of TBS buffer (TRIS-buffered saline; 50 mM TRIS, pH 7.8, 1 mM MgCl_2 , 10 mM NaCl, 0.1% Tween, 0.1% gelatin).

Transmission electron microscopy

Fresh leaves and roots were collected from *Ipt*-cotton treated with 1000 mg L^{-1} CuO NPs for 10 days for TEM observations. The leaves and roots were washed thoroughly with tap water and then deionized water, and then prefixed in 2.5% glutaraldehyde. The leaves and roots were then

dehydrated in a graded series of ethanol and embedded in Spurr's resin. The samples were sectioned using an UC6i ultramicrotome (Leica, Austria) with a diamond knife. The sections (around 90 nm) were collected on copper grids followed by staining with uranyl acetate and lead citrate and observed on a JEM-1230 TEM (TEOL, Tokyo, Japan) operating at 80 kV. TEM/EDS spectra were collected on a TEM (Oxford Instruments, Oxfordshire, UK) equipped with energy dispersive X-ray spectrometer with a beam diameter of 25 nm. More than 10 sections cut from different leaves and roots were examined (Zhang et al. 2012).

Data analysis

Data were subjected to one-way analysis of variance (ANOVA) using SPSS 22.0 software. The results are shown as mean \pm standard deviation (SD), and a confidence interval of 95% ($p < .05$) was considered significant.

Results and discussion

Characterization of CuO NPs

In the SEM analysis, the CuO NPs showed the typical particle morphology of CuO NPs with a spherical shape (Figure 1). The observed particle size was that reported by the manufacturers: 30 ± 10 nm. The zeta potential and zeta average diameter were 0.416 mV and 388.2 nm, respectively.

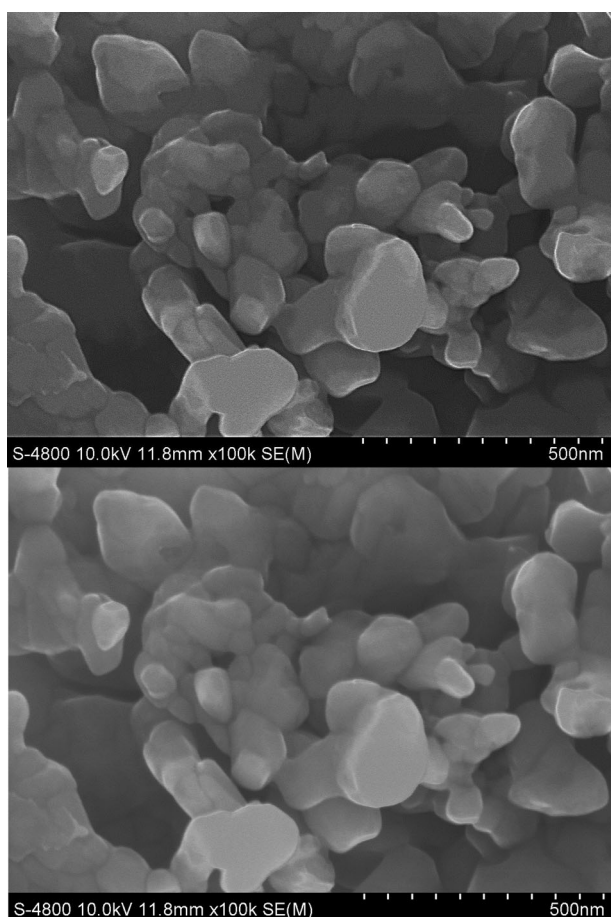


Figure 1. SEM images of CuO nanoparticles.

Effects of CuO NPs on growth and biomass of *Ipt-cotton*

The plant height and root length of *Ipt-cotton* were significantly lower in plants treated with 200 and 1000 mg L⁻¹ CuO NPs than in the control (Figure 2(a) and 2(b)). In plants treated with 10 mg L⁻¹ CuO NPs, plant height and root length were not significantly different from those of the control. In plants treated with 200 and 1000 mg L⁻¹ CuO NPs, plant height was 13.99% and 26.91% lower, respectively, than that of the control. Similarly, their root length was 45.79% and 42.80% lower, respectively, than that of the control. The root length of plants treated with 10 mg L⁻¹ CuO NPs was not significantly different from that of the control. Root hairs were less abundant on roots of plants treated with 1000 mg L⁻¹ CuO NPs than on roots of the control plants (Figure 2(c)). Together, these results showed that plant height, root length, and the number of root hairs were affected by CuO NPs at high concentrations, but not by CuO NPs at low concentrations. These results are similar to those obtained in our previous study, in which SiO₂ NPs affected cotton plant height (Raul et al. 2014), but differ from those of another study in which CeO₂ did not affect the height of Bt-transgenic cotton (Remya et al. 2014). These findings show that different NPs have different effects on transgenic cotton plants.

The low concentration of CuO NPs did not affect the shoot biomass or root biomass of *Ipt-cotton*, compared with those of the control. However, the shoot biomass was significantly lower in the 1000 mg L⁻¹ CuO-NP treatment than in the 10 mg L⁻¹ CuO-NP treatment and the control (Figure 2(d) and 2(e)). This result indicates that only the high concentration (1000 mg L⁻¹) of CuO NPs affected the shoot biomass of *Ipt-cotton*.

Effects of CuO NPs on nutrient contents in shoots and roots of *Ipt-cotton*

The low concentration of CuO NPs (10 mg L⁻¹) did not affect the contents of most of the nutrients analyzed in the shoots, when compared with the control group (Table 1). However, higher concentrations of CuO NPs affected the uptake of several nutrients. The Fe content in shoots was significantly lower in the 200 mg L⁻¹ CuO-NP treatment than in the control. The Zn content in shoots was significantly higher in the 10 mg L⁻¹ CuO treatment than in the control, but significantly lower in the 200 and 1000 mg L⁻¹ CuO-NP treatments than in the control (Table 1). These data indicate that, at a low concentration, CuO NPs enhanced Zn uptake in *Ipt-cotton*; therefore, CuO NPs may be useful to increase the uptake of Zn fertilizer.

There were similar trends in Mg, Ca, Mn, Mo, B, and P contents in the shoots of *Ipt-cotton* in response to CuO NPs (Table 1); that is, the low concentration of CuO NPs did not affect their contents in shoots, when compared with their respective contents in the control. The contents of Mg, Ca, Mn, Mo, B, and P in shoots were significantly lower in the 200 and 1000 mg L⁻¹ CuO treatments than in the 10 mg L⁻¹ CuO-NP treatment and the control. However, there was no significant difference in the contents of these nutrients in shoots between the 200 and 1000 mg L⁻¹ CuO treatments (Table 1). Similarly, the K and Na contents in shoots were not affected by the low concentration of CuO

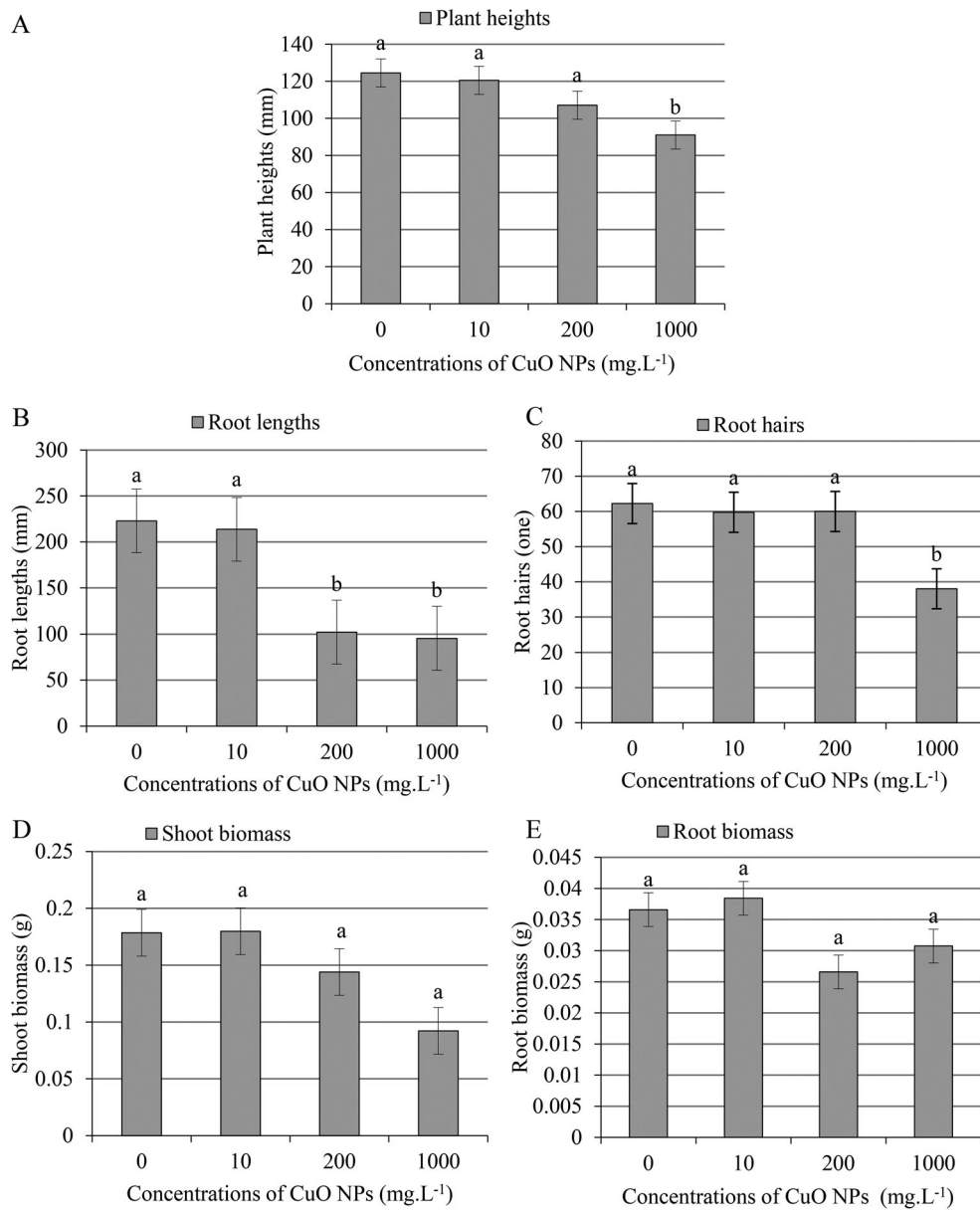


Figure 2. Effects of CuO NPs on the growth of Ipt-transgenic cotton after a 10-day exposure. The means are averaged from three replicates and error bars corresponded to standard derivations of three values.

NPs (10 mg L⁻¹), but higher concentrations of CuO NPs had different effects on K and Na contents in the shoots. The K contents in shoots were significantly lower in the 200 and 1000 mg L⁻¹ CuO treatments than in the control, whereas the Na contents were significantly higher in the 200 and 1000 mg L⁻¹ CuO treatments than in the control. The Cu

contents in shoots and roots increased with increasing CuO-NP concentrations (Figure 3(a) and 3(b)). These results suggested that nutrient contents in shoots of Ipt-cotton were impacted differently upon exposure to CuO nanoparticles. Most of nutrients were not affected by low concentration of CuO nanoparticles but were inhibited by high concentration

Table 1. Effects of CuO NPs on nutrient contents in shoots of Ipt-transgenic cotton after a 10-day exposure.

Nutrient contents (µg gFW ⁻¹)	CuO NPs concentration (mg L ⁻¹)			
	0	10	200	1000
Fe	143.29 ± 14.07 ^a	161.33 ± 6.79 ^a	114.58 ± 10.77 ^b	136.49 ± 11.84 ^a
Zn	43.71 ± 1.91 ^a	49.69 ± 4.42 ^b	37.27 ± 1.96 ^c	34.59 ± 4.82 ^c
Mg	7877.51 ± 229.27 ^a	8056.63 ± 82.98 ^a	5610.30 ± 537.74 ^b	4761.98 ± 78.87 ^b
Ca	33664.24 ± 2015.51 ^a	35369.13 ± 833.37 ^a	17763.96 ± 3410.35 ^b	12264.32 ± 258.17 ^b
K	38401.35 ± 1997.58 ^a	39893.51 ± 2549.73 ^a	29019.94 ± 571.97 ^b	25258.13 ± 556.36 ^c
Na	356.18 ± 14.04 ^a	356.20 ± 13.71 ^a	410.32 ± 39.72 ^b	480.25 ± 39.96 ^c
Mn	36.13 ± 2.73 ^a	39.09 ± 2.86 ^a	20.14 ± 3.08 ^b	17.33 ± 1.76 ^b
Mo	4.24 ± 0.26 ^a	4.29 ± 0.12 ^a	1.52 ± 0.29 ^b	0.79 ± 0.01 ^b
B	36.79 ± 1.53 ^a	38.79 ± 1.65 ^a	27.94 ± 1.89 ^b	25.92 ± 2.36 ^b
P	16082.44 ± 1473.89 ^a	16078.90 ± 545.29 ^a	7490.22 ± 642.26 ^b	6261.92 ± 256.73 ^b

Notes: The values presented as mean ± standard deviation (SD). Different small letters mean significant difference at *p* < .05 level between different CuO NPs concentrations.

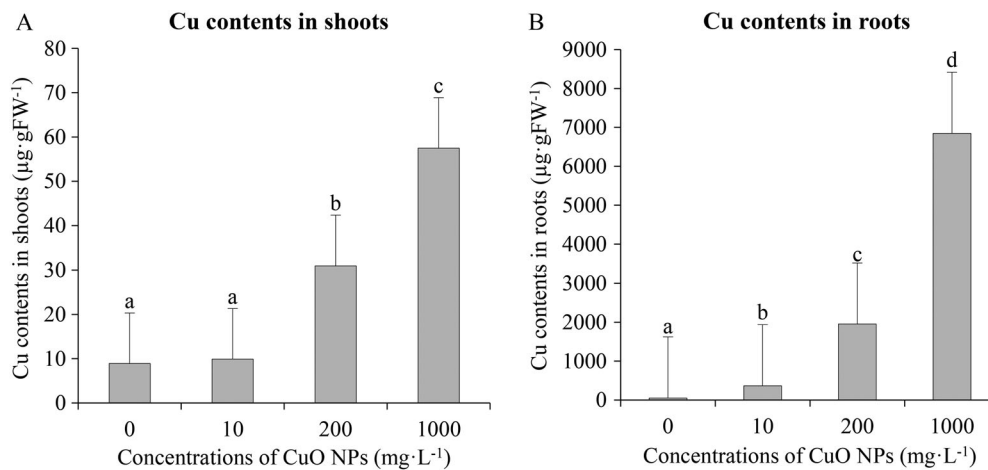


Figure 3. Cu concentrations in shoots and roots of *Ipt*-transgenic cotton after a 10-day exposure with CuO NPs. The means are averaged from three replicates and error bars corresponded to standard derivations of three values. Different small letters mean significant difference at $p < .05$ level between different CuO NPs treatments.

of CuO NPs. In particular, Zn content was enhanced by a low concentration of CuO nanoparticles, whereas a high concentration of CuO nanoparticles promoted the uptake and transport of Na contents from roots to shoots in *Ipt*-cotton.

The low concentration of CuO NPs (10 mg L⁻¹) had no effect on the contents of almost all of the nutrients analyzed in the roots of *Ipt*-cotton, when compared with their respective concentrations in the control (Table 2). The effects of CuO NPs depended on the concentration of the CuO NPs and the type of nutrient. The Fe and Na contents in roots were significantly higher in the 200 and 1000 mg L⁻¹ CuO-NP treatments than in the control (Table 2). This finding indicated that CuO NPs promoted the absorption of Fe and Na by the roots of *Ipt*-cotton. In contrast, the contents of Zn, Ca, B, and P in roots were significantly lower in the 200 and 1000 mg L⁻¹ CuO-NP treatments than in the control. There were significantly higher K and Mo contents in roots in the 10 mg L⁻¹ CuO-NP treatment than in the control, but lower K and Mo contents in roots in the 200 and 1000 mg L⁻¹ CuO-NP treatments than in the control. Only the roots of plants treated with 200 mg L⁻¹ CuO NPs showed a higher Mn content than that in the control (Table 2). In the previous studies, we found that CeO₂ NPs significantly reduced the absorption of Zn, Mg, Fe, and P in conventional cotton in comparison with control group. In the other studies, the contents of Cu, Mg in shoots, and Na in roots of transgenic cotton were affected under SiO₂ NPs treatments (Le et al. 2014; Nhan et al. 2015). Furthermore, Li et al. (2014) found that cerium oxide (CeO₂) NPs disrupt the uptake of

nutrient elements in Bt-transgenic cotton compared with its parental conventional cotton plant. Higher Cu concentrations in plants have been shown to result in toxicity, growth inhibition, disrupted photosynthesis, and increased oxidative stress (Shobha et al. 2014). Also, higher Cu concentrations have been shown to interfere with the uptake of other nutrients in tomato (Passam et al. 2007). These results suggest that after a 10-day treatment, the toxicity of CuO NPs affected the uptake of other nutrients into roots of *Ipt*-cotton. It is consistent with the studies of Peralta-Videa et al. (2014) that both the nCeO₂ and nZnO, even at a concentration as low as 100 and 50 mg.kg⁻¹, respectively, have the potential to disturb the accumulation of some nutritional elements in soybean plants growth in NP-amended farm soil (Peralta-Videa et al. 2014).

Copper concentration in *Ipt*-cotton and TEM observations

Microelements, including Cu, are important requirements for plant development and various metabolic processes in plant cells (Maksymiec 1997; Shobha et al. 2014). We measured the Cu contents in shoots and roots of *Ipt*-cotton after a 10-day exposure to CuO NPs (Figure 3). The Cu contents in plant tissues increased with increasing CuO concentrations. There was no significant difference in shoot Cu contents between the 10 mg L⁻¹ CuO treatment and the control. In contrast, plants treated with 200 and 1000 mg L⁻¹ CuO NPs showed shoot Cu contents 3.47 and 6.44 times higher, respectively, than

Table 2. Effects of CuO NPs on nutrient contents in roots of *Ipt*-transgenic cotton after a 10-day exposure.

Nutrient contents (µg gFW ⁻¹)	CuO NPs concentration (mg L ⁻¹)			
	0	10	200	1000
Fe	3569.68 ± 416.58 ^a	3858.45 ± 365.22 ^a	6858.99 ± 104.95 ^b	7475.60 ± 296.76 ^b
Zn	163.16 ± 12.27 ^a	180.33 ± 4.44 ^a	100.45 ± 1.36 ^b	64.61 ± 4.89 ^c
Mg	3777.96 ± 135.02 ^a	3821.13 ± 131.41 ^a	3685.25 ± 230.71 ^a	4205.03 ± 305.51 ^a
Ca	13035.43 ± 1136.04 ^a	12545.39 ± 863.81 ^a	10121.20 ± 874.35 ^b	9628.67 ± 483.02 ^b
K	29467.60 ± 2688.61 ^a	39154.37 ± 1694.34 ^b	28351.49 ± 934.81 ^a	23187.61 ± 2483.20 ^a
Na	974.93 ± 118.14 ^a	1101.90 ± 47.25 ^a	1398.71 ± 58.02 ^b	1632.38 ± 149.52 ^b
Mn	65.51 ± 7.54 ^a	61.54 ± 4.70 ^a	83.73 ± 5.65 ^b	72.24 ± 8.59 ^{ab}
Mo	10.66 ± 0.69 ^a	14.63 ± 0.46 ^b	13.14 ± 1.62 ^{ab}	9.50 ± 0.70 ^a
B	18.62 ± 0.56 ^a	18.33 ± 0.64 ^a	15.83 ± 0.98 ^a	11.45 ± 1.00 ^b
P	8621.19 ± 403.86 ^{ab}	9313.63 ± 213.06 ^a	7688.89 ± 223.50 ^b	4858.88 ± 602.44 ^c

Notes: The values presented as mean ± standard deviation (SD). Different small letters mean significant difference at $p < .05$ level between different CuO NPs concentrations.

that in the control. The Cu contents in roots of *Ipt*-cotton also differed among the CuO-NP treatments. In plants treated with 10 mg L^{-1} CuO NPs, the Cu content in roots was 7.09 times that in the control. At 200 mg L^{-1} CuO NPs exposure, root Cu content was 37.67 times higher than that in the control. The highest Cu content in roots ($6845.08 \text{ ng g}^{-1}$; 132.26 times that in the control) was in plants treated with 1000 mg L^{-1} CuO NPs. The Cu contents in shoots and roots increased with increasing concentrations of CuO NPs (Figure 3). These results indicated that CuO NPs were absorbed into roots and then transported to the shoots of *Ipt*-cotton. In another study on cotton, CeO_2 nanoparticles were absorbed through the roots and transported to shoots (Li et al. 2014). Similarly, CuO NPs were taken up through the root system in maize (Wang et al. 2012b). In our previous study, we observed that SiO_2 NPs were present in the xylem sap of *Bt*-transgenic and conventional cotton, illustrating that SiO_2 NPs could be transported to the shoots from the roots (Le et al. 2014).

The TEM analysis revealed that CuO NPs were present in the leaves and roots of *Ipt*-cotton after a 10-day exposure to CuO NPs (Figure 4). The CuO NPs were visible as dark dots in the endodermis and vascular cylinder of plants treated with 1000 mg L^{-1} CuO NPs (Figure 4(b1) and 4(b2)). In another study, one or several NPs could be visualized in

higher magnification TEM images (Maksymiec 1997). In the present study, most of the CuO NPs were found on the root outer epidermis of *Ipt*-cotton. The Cu contents in the shoots and roots significantly increased with increasing CuO-NP concentrations (Figure 3). The presence of CuO in leaves illustrated that the CuO NPs were absorbed into the roots, and then transported to shoots and leaves via the xylem sap (Figure 4(a1) and 4(a2)). In other studies, CeO_2 NPs were taken up by roots of both conventional and *Bt*-transgenic cotton (Li et al. 2014), and some NPs could be transferred from the roots to shoots of cucumber plants (Zhang et al. 2011). In rice plants, C_{70} NPs entered into the roots and were transported to the stem and leaves (Lin & Xing 2008). Nanoparticles of SiO_2 and CuO were transported to shoots from roots via the xylem sap in *Bt*-transgenic cotton, conventional cotton, and maize (Zhao 2010; Le et al. 2014). Based on these results, we concluded that CuO NPs can enter and be retained in *Ipt*-transgenic plants; this poses potential risks to human health.

Effects of CuO NPs on hormone concentrations in cotton leaves and roots

Table 3 shows the concentrations of phytohormones in leaves and roots of *Ipt*-cotton treated with various concentrations of

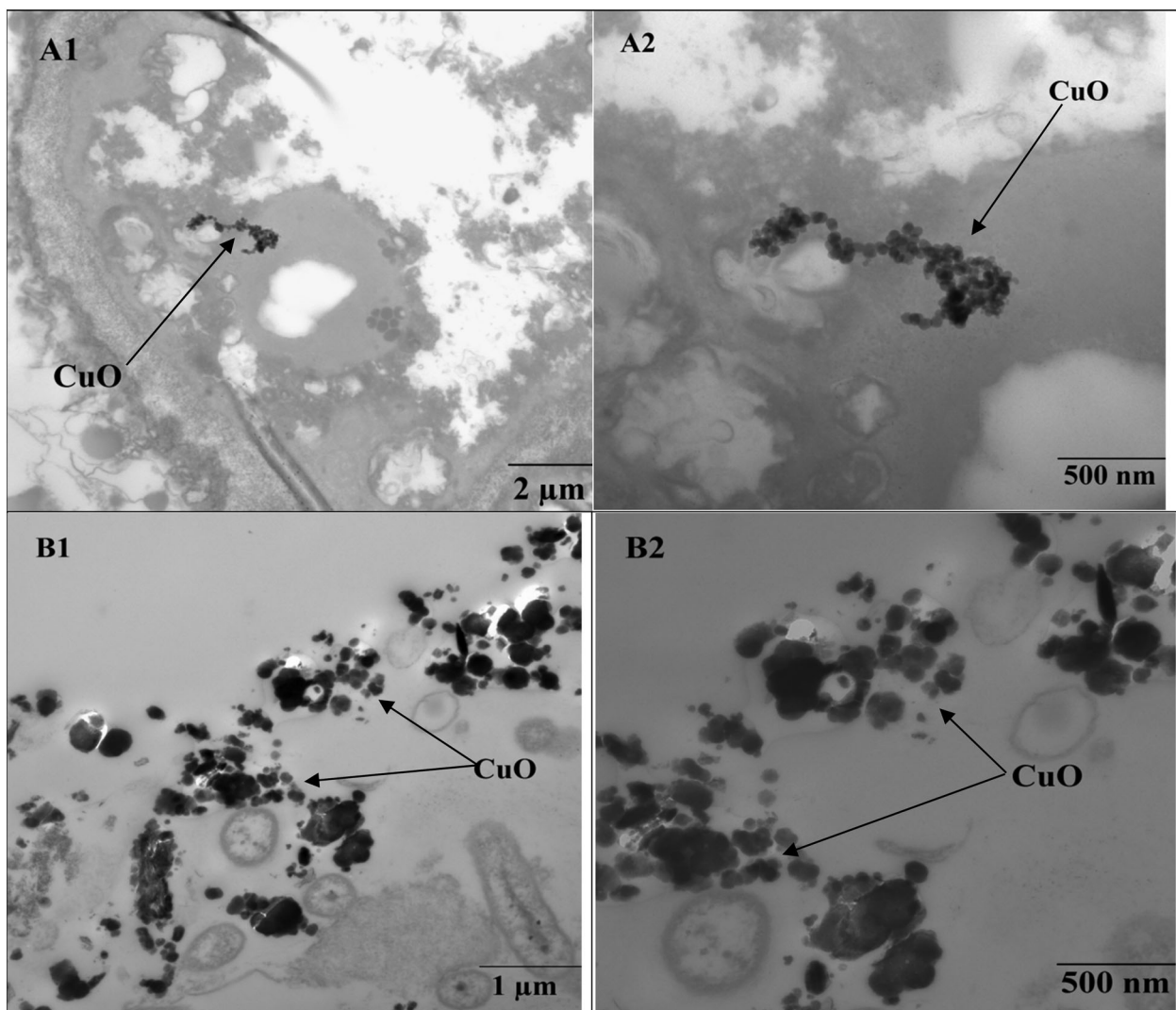


Figure 4. TEM images of *Ipt*-transgenic cotton leaves (a1), (a2) and roots (b1), (b2) after a 10-day treatment with 1000 mg L^{-1} CuO NPs.

Table 3. Effects of CuO nanoparticles on hormone concentrations of *Ipt*-transgenic cotton after a 10-day exposure.

Hormones (ng gFW ⁻¹)	Position	CuO NPs concentrations (mg L ⁻¹)			
		0	10	200	1000
IAA	Leaves	87.94 ± 1.23 ^a	77.86 ± 1.09 ^b	78.65 ± 0.30 ^b	70.71 ± 0.63 ^c
	Roots	59.01 ± 0.53 ^a	57.73 ± 0.37 ^a	56.24 ± 0.43 ^a	69.27 ± 0.97 ^b
ABA	Leaves	108.30 ± 0.47 ^a	93.12 ± 0.65 ^b	169.48 ± 2.36 ^c	183.13 ± 0.80 ^d
	Roots	56.33 ± 0.35 ^a	52.58 ± 0.41 ^b	62.18 ± 0.47 ^c	83.67 ± 0.29 ^d
GA	Leaves	7.52 ± 0.06 ^a	6.41 ± 0.05 ^b	5.83 ± 0.23 ^b	6.20 ± 0.04 ^b
	Roots	3.82 ± 0.06 ^a	4.66 ± 0.08 ^b	4.31 ± 0.06 ^{ba}	4.84 ± 0.27 ^b
t-ZR	Leaves	11.29 ± 0.11 ^a	10.70 ± 0.11 ^a	11.23 ± 0.18 ^a	9.04 ± 0.12 ^b
	Roots	8.68 ± 0.17 ^a	8.56 ± 0.13 ^a	7.96 ± 0.06 ^b	6.75 ± 0.04 ^c

Notes: The values presented as mean ± standard deviation (SD). Different small letters mean significant difference at $p < .05$ level between different CuO NPs concentrations.

CuO NPs. The main auxin in higher plants is IAA; this phytohormone profoundly affects plant growth and development (Santner et al. 2009). The IAA concentration in leaves was significantly lower in the 10 and 200 mg L⁻¹ CuO-NP treatments than in the control (88.5%, 89.4%, and 80% lower in the 10, 200, and 1000 mg L⁻¹ CuO-NP treatments, respectively, than that in the control). The IAA concentration in roots was similar among the control and the 10 and 200 mg L⁻¹ CuO-NP treatments, but significantly higher in the 1000 mg L⁻¹ CuO-NP treatment than in the control (Table 3). These results suggested that CuO NPs inhibited IAA production in the leaves, leading to reduced plant height and biomass.

In plants, ABA is a stress hormone with versatile functions in regulating many developmental processes and adaptive stress processes (Cutler et al. 2010). The plant response to ABA depends on its concentration in the tissue, as well as the sensitivity of the tissue to ABA (Amrut et al. 2010). We determined the ABA concentration in leaves and roots of *Ipt*-cotton exposed to CuO NPs for 10 days (Table 3). The ABA concentrations in leaves and roots were lower in the 10 mg L⁻¹ CuO-NP treatment than in the control, but higher in the 200 and 1000 mg L⁻¹ CuO-NP treatments than in the control. Stress conditions have been shown to increase ABA concentrations in plants (Amrut et al. 2010). However, our findings showed that the ABA concentration in *Ipt*-cotton depended on the concentration of CuO NPs. The ABA concentration increased in response to higher CuO-NP concentrations, and decreased in response to the lower concentration.

In leaves of *Ipt*-cotton, the GA concentration significantly decreased in response to CuO NPs, but there was no significant difference in GA concentrations among the different CuO-NP treatments. In contrast, the GA content in roots was significantly higher in plants treated with CuO NPs than in the control. This result suggested that in *Ipt*-cotton, CuO NPs may induce GA production in roots, but inhibit GA production in leaves. At low concentrations, CuO NPs did not affect the t-ZR concentration in leaves and roots of *Ipt*-cotton. However, the t-ZR concentrations in leaves and roots were significantly different between the 1000 mg L⁻¹ CuO-NP treatment and the control. These results indicated that CuO NPs affected the various phytohormones differently in leaves and roots of *Ipt*-cotton.

Effects of CuO NPs on iPA contents in *Ipt*-cotton

As the expression product of *Ipt* gene, iPA can inhibit leaf senescence and increase resistance to pathogens; therefore, the iPA concentration is the most important index to evaluate the expression of exogenous gene (*Ipt*) and the quality of *Ipt*-transgenic crops. After exposure to CuO NPs, the concentration of iPA in leaves of *Ipt*-cotton increased significantly with increasing CuO-NP concentrations, but the concentration of iPA in the roots decreased significantly. These data indicated that CuO NPs can inhibit leaf senescence, and the increase in iPA in leaves resulted from the transport of iPA produced in the roots (Figure 5). When the concentration of CuO NPs was higher than 200 mg L⁻¹, the growth and development of *Ipt*-cotton plants were inhibited, but the

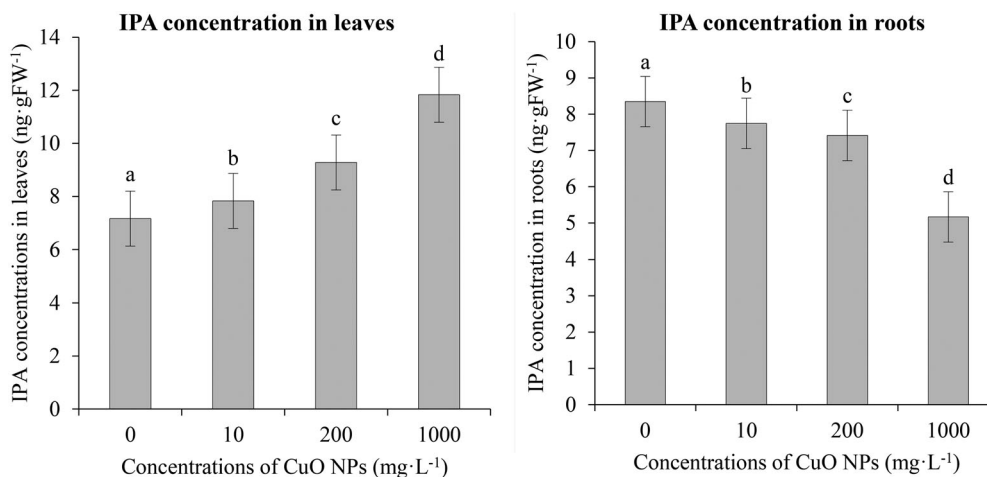


Figure 5. The content of iPA-toxin in leaves and roots of *Ipt*-transgenic cotton after a 10-day exposure with CuO NPs. The mean is averaged from three replicates and error bars correspond to standard deviation of mean. Different small letters mean significant difference at $p < .05$ level between different CuO NPs concentrations.

increased iPA concentration in plants treated with a high concentration of CuO NPs delayed their death.

At a low concentration (10 mg L^{-1}), CuO NPs did not affect the growth and development of cotton plants, but induced an increase in iPA contents in the leaves, which delayed senescence. Therefore, a low concentration of CuO NPs could be used to regulate plant growth or improve the performance of *Ipt*-transgenic plants. The CuO NPs were absorbed by *Ipt*-transgenic plants, and then isopentenyl transferase produced in the transgenic plants was adsorbed onto the surface of the CuO NPs, which increased the stability of isopentenyl transferase and inhibited its degradation, and the adsorption reduced the free isopentenyl transferase, which will also promote the expression of exogenous *Ipt* gene. According to Sanpui et al. (2015), the mutual interaction of CuO NPs and proteins at physiological conditions may result in the aggregation of protein, which can ultimately lead to the formation of CuO NPs – protein agglomerates. The increase in iPA in response to CuO NPs should be relative to the amount of *Ipt* immobilized onto the CuO NPs. Consequently, the increase in iPA in response to CuO NPs can be expected to delay the senescence of leaves of plants at a late stage of development. These results suggest that NPs should be used at a later stage to enhance the expression of the *Ipt* gene and delay senescence in *Ipt*-transgenic plants. In the previous studies of Kole et al. (2013), NPs enhanced contents of two anticancer phytochemicals as cucurbitacin-B and lycopene up to 74% and 82%, respectively, and contents of two antidiabetic phytochemicals (charantin and insulin) were augmented up to 20% and 91%, respectively in bitter melon (*Momordica charantia*). On the other hand, protein of spinach was obviously enhanced under nano-anatase TiO_2 conditions in comparison with control group (Yang et al. 2007). To our knowledge, this should be the first report on the effects of CuO NPs on exogenous gene expression of transgenic crops.

Conclusion

At a high concentration, CuO NPs negatively affected the plant height, root length, abundance of root hairs, and shoot biomass of *Ipt*-cotton. At low concentrations, CuO NPs had no effect on most of the nutrients analyzed in the shoots and roots of cotton. The CuO NPs promoted the absorption of Fe and Na into roots, and inhibited the production of some phytohormones (IAA, ABA, GA, and t-ZR). The iPA concentration in *Ipt*-cotton was significantly increased by CuO NPs, suggesting that they could be used to delay senescence. The Cu content in roots and shoots of *Ipt*-cotton increased with increasing concentrations of CuO NPs. The CuO NPs were detected in leaves and roots of *Ipt*-cotton in the TEM analysis, illustrating that CuO NPs were able to enter roots and be transported to the leaves. This is the first study to evaluate the phytotoxicity of CuO NPs to *Ipt*-transgenic cotton, and to our knowledge, this might be the first report on the effects CuO NPs on exogenous gene expression of transgenic crops. Our data establish a baseline for further studies on the effects of NPs on transgenic crops harboring the *Ipt* gene.

Acknowledgements

LVN and YR designed the experiments. LVN, YR, WC, PB, JS, SL and LL performed the experiments. LVN and YR analyzed the data and drafted

the manuscript. We also acknowledge assistance with IPA and other plant hormones' determination that provided by Professor Wang Baomin of college of Agriculture and Biotechnology, China Agricultural University.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

We appreciate the support of the National Natural Science Foundation of China [grant nos. 41130526 and 41371471]

References

- Amrut SL, Sharma SJ, Pode RB, Ningthoujam RS. 2010. Synthesis and optical characterization of copper oxide nanoparticles. *Adv Appl Sci Res.* 1:36–40.
- Aruoja V, Dubourguier HC, Kasemets K, Kahru A. 2009. Toxicity of nanoparticles of CuO, ZnO and TiO_2 to microalgae *Pseudokirchneriella subcapitata*. *Sci Total Environ.* 407:1461–1468.
- Bergeson LL. 2010. Nanosilver pesticide products: what does the future hold. *Environ Qual Manage.* 19:73–82.
- Bouwmeester H, Dekkers S, Noordam MY, Hagens WI, Bulder AS, de Heer C, ten Voorde SECGS, Wijnhoven WP, Marvin HJP, Sips AJAM. 2009. Review of health safety aspects of nanotechnologies in food production. *Regul Toxicol Pharm.* 53:52–62.
- Cowan AK, Freeman M, Bjorkman PO, Nicander B, Sitbon F, Tillberg E. 2005. Effects of senescence-induced alteration in cytokinin metabolism on source–sink relationships and ontogenic stress-induced transitions in tobacco. *Planta.* 221:801–814.
- Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR. 2010. Abscisic acid: emergence of a core signaling network. *Annu Rev Plant Biol.* 61:651–679.
- Devi HS, Singh TD. 2014. Synthesis of copper oxide nanoparticles by a novel method and its application in the degradation of methyl orange. *Adv Electron Electr Eng.* 4:83–88.
- Dong H, Niu Y, Li W, Zhang D. 2008. Effects of cotton rootstock on endogenous cytokinins and abscisic acid in xylem sap and leaves in relation to leaf senescence. *J Exp Bot.* 59:1295–1304.
- Gregersen, PL, Culetic, A, Boschian L, Krupinska K. 2013. Plant senescence and crop productivity. *Plant Mol Biol.* 82:603–622.
- Guo J-C, Hu X-W, Li K-M, Fu S-P. 2010. Isopentenyl transferase gene (*ipt*) downstream transcriptionally fused with gene expression improves the growth of transgenic plants. *Transgenic Res.* 19:197–209.
- Hu YL, Jia WL, Wang JD, Zhang YQ, Yang LL Lin ZP. 2005. Transgenic tall fescue containing the agrobacterium tumefaciens IPT gene shows enhanced cold tolerance. *Plant Cell Rep.* 23:705–709.
- Kasemets K, Suppi S, Kunnis-Beres K, Kahru A. 2013. Toxicity of CuO nanoparticles to yeast *Saccharomyces cerevisiae* BY4741 wild-type and its nine isogenic single-gene deletion mutants. *Chem Res Toxicol.* 26:356–367.
- Khodakovskaya M, Dervishi E, Mahmood M, Xu Y, Li Z, Watanabe F, Biris AS. 2009. Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. *ACS Nano.* 3:3221–3227.
- Khodakovskaya M, Li Y, Li JS, Vankova R, Malbeck J, McAvoy R. 2005. Effects of *cor15a*-IPT gene expression on leaf senescence in transgenic *Petunia hybrida* and *Dendranthema grandiflorum*. *J Exp Bot.* 56:1165–1175.
- Kole C, Kole P, Randunu KM, Choudhary P, Podila R, Ke PC, Rao AM, Marcus RK. 2013. Nanobiotechnology can boost crop production and quality: first evidence from increased plant biomass, fruit yield and phytochemical content in bitter melon (*Momordica charantia*). *BMC Biotechnol.* 13(37):1–10.
- Li Q, Robson PRH, Bettany AJE, Donnison IS, Thomas H Scott IM. 2004. Modification of senescence in ryegrass transformed with IPT under the control of a monocot senescence-enhanced promoter. *Plant Cell Rep.* 22:816–821.

- Li X, Gui X, Rui Y, Ji W, Le VN, Yu Z, Peng S. 2014. Bt-transgenic cotton is more sensitive to CeO₂ nanoparticles than its parental non-transgenic cotton. *J Hazard Mater.* 274:173–180.
- Le VN, Rui Y, Gui X, Li X, Liu S Han Y. 2014. Uptake, transport, distribution and Bio-effects of SiO₂ nanoparticles in Bt-transgenic cotton. *J Nanobiotechnol.* 12:50.
- Lin D, Xing B. 2008. Root uptake and phytotoxicity of ZnO nanoparticles. *Environ Sci Technol.* 42:5580–5585.
- Lin YJ, Cao ML, Xu CG, Chen H, Wei J, Zhang QF. 2002. Cultivating rice with delaying leaf-senescence by P-SAG12-IPT gene transformation. *Acta Bot Sin.* 44:1333–1338.
- Liu YD, Yin ZJ, Yu JW, Li J, Wei HL, Han XL, Shen FF. 2012. Improved salt tolerance and delayed leaf senescence in transgenic cotton expressing the *Agrobacterium* Ipt gene. *Biol Plant.* 56:237–246.
- Ma Y, Kuang L, He X, Bai W, Ding Y, Zhang Z, Zhao Y, Chai Z. 2010. Effects of rare earth oxide nanoparticles on root elongation of plants. *Chemosphere.* 78:273–279.
- Maksymiec V. 1997. Review: effect of copper on cellular processes in higher plants. *Photosynthetica.* 34:321–342.
- Nair R, Varghese SH, Nair BG, Maekawa T, Yoshida Y, Kumar DS. 2010. Nanoparticulate material delivery to plants. *Plant Sci.* 179:154–163.
- Nhan LV, Ma C, Rui Y, Liu S, Li X, Xing B, Liu L. 2015. Phytotoxic mechanism of nanoparticles: destruction of chloroplasts and vascular bundles and alteration of nutrient absorption. doi:10.1038/srep11618.
- Ni Y, Chen YX, Lin Q, Tian GM. 2005. Subcellular localization of copper in tolerant and non-tolerant plant. *J Environ Sci.* 17:452–456.
- Passam HC, Karapanos IC, Bebeli PJ, Savvas D. 2007. A review of recent research on tomato nutrition, breeding and post-harvest technology with reference to fruit quality. *Eur J Plant Sci Biotechnol.* 1:1–21.
- Peralta-Videa JR, Hernandez-Viezas JA, Zhao L, Diaz BC, Ge Y., Priester JH, Holden PA, Gardea-Torresdey JL. 2014. Cerium dioxide and zinc oxide nanoparticles alter the nutritional value of soil cultivated soybean plants. *Plant Physiol Biochem.* 80:128–135.
- Perelshtein I, Applerot G, Perkash N, Wehrschuetz-Sigl E, Hasmann A, Guebitz G, Gedankena A. 2009. CuO-cotton nanocomposite: formation, morphology, and antibacterial activity. *Surf Coat Tech.* 204:54–57.
- Raul, PK, Senapati S, Sahoo AK, Umlong IM, Devi RR, Thakur AJ, Veer V. 2014. CuO nanorods: a potential and efficient adsorbent in water purification. *RSC Adv.* 4:40580–40587.
- Ravishankar RV, Jamuna BA. 2011. Nanoparticles and their potential application as antimicrobials. *Sci Microb Pathog Commun Curr Res Technol Adv.* 197–209. Available from: <http://www.formatex.info/microbiology3/book/197-209.pdf>
- Remya NS, Syama S, Gayathri V, Varma HK, Mohanan PV. 2014. An in vitro study on the interaction of hydroxyapatite nanoparticles and bone marrow mesenchymal stem cell for assessing the toxicological behaviour. *Colloids Surf B: Biointerfaces.* 117:389–397.
- Roitsch T, Ehneß R. 2000. Regulation of source /sink relations by cytokinins. *Plant Growth Regul.* 32:359–367.
- Rubio-Wilhelmi M del M, Sanchez-Rodriguez E, Rosales MA, Begoña B, Rios JJ, Romero L, Blumwald E, Ruiz JM. 2011. Cytokinin-dependent improvement in transgenic PSARK: IPT Tobacco under Nitrogen deficiency. *J Agr Food Chem.* 59:10491–10495.
- Sanpui, P, Paul A, Chattopadhyay A. 2015. Theranostic potential of gold nanoparticle-protein agglomerates. *Nanoscale.* 7:18411–18423.
- Santner A, Calderon-Villalobos LI, Estelle M. 2009. Plant hormones are versatile chemical regulators of plant growth. *Nat Chem Biol.* 5:301–307.
- Sharon M, Choudhary A, Kumar R. 2010. Nanotechnology in agricultural diseases and food safety. *J Phytol.* 2:83–92.
- Shobha G, Vinutha M, Ananda S. 2014. Biological synthesis of copper nanoparticles and its impact – a review. *Int J Pharm Sci Invent.* 3:28–38.
- Syu Y-Y, Hung J-H, Chen J-C, Chuang H-W. 2014. Impacts of size and shape of silver nanoparticles on Arabidopsis plant growth and gene expression. *Plant Physiol Biochem.* 83:57–64.
- Tamuly C, Saikia I, Hazarika M, Das MR. 2014. Reduction of aromatic nitro compounds catalyzed by biogenic CuO nanoparticles. *RSC Adv.* 4:53229–53236.
- Wang Y, Li B, Du M, Eneji AE, Wang B, Duan L, Li Z, Tian X. 2012a. Mechanism of phytohormone involvement in feedback regulation of cotton leaf senescence induced by potassium deficiency. *J Exp Bot.* 63:5887–5901.
- Wang Z, Xie X, Zhao J, Liu X, Feng W, Jason C. 2012b. White and Baoshang Xing. Xylem-and Phloem-based transport of CuO nanoparticles in Maize (*Zea mays* L.). *Environ Sci Technol.* 46:4434–4441.
- Yang F, Hong FS, You WJ, Liu C, Gao FQ, Wu C, Yang P. 2006. Influences of nano-anatase TiO₂ on the nitrogen metabolism of growing spinach. *Biol Trace Elem Res.* 110:179–190.
- Yang F, Liu C, Gao F, Su M, Wu X, Zheng L, Hong F, Yang P. 2007. The improvement of spinach growth by nano-anatase TiO₂ treatment is related to nitrogen photoreduction. *Biol Trace Elem Res.* 119:77–88.
- Zhang P, Ma Y, Zhang Z, He X, Zhang J, Guo Z, Tai R, Zhao Y. 2012. Biotransformation of Ceria nanoparticles in cucumber plants. *ACS Nano.* 6:9943–9950.
- Zhang ZY, He X, Zhang HF, Ma YH, Zhang P, Ding YY, Zhao YL. 2011. Uptake and distribution of ceria nanoparticles in cucumber plants. *Metallomics.* 3:816–822.
- Zhao Y. 2010. Indole-3-acetic acid (IAA), the main auxin in higher plants, has profound effects on plant growth and development. *Annu Rev Plant Biol.* 61: 49–64.