

REVIEW

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# Impact of metal oxide nanoparticles on cotton (*Gossypium hirsutum* L.): a physiological perspective

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## Abstract

Cotton production substantiated a crucial part in the escalating economic development of many countries. To realize the increasing global demand for cotton, the emphasis should be laid on to improve cotton fiber growth and production. The bioengineered transgenic cotton proved expedient in resolving inadequacies of conventional cotton, but still required improvements to encounter heightened demand of textile industries. One possible solution pertaining to this has been provided by nanoscience in the form of metal or metal oxide nanoparticles. These metal oxide nanoparticles have easy access to the various parts of cotton plants through its transportation system, and thus significantly influence several parameters relative to the growth and production of cotton fiber. This review summarizes the distribution and accumulation of metal oxide nanoparticles in cotton plant and its impact on different plant growth-promoting factors, which resulted in the improved cotton yields.

**Keywords:** Cotton, Bt-transgenic, Nanoparticles, Metal oxide nanoparticles, Phytohormones, Superoxide dismutase, Nutrient element

## Background

Cotton, *Gossypium hirsutum* L. (Malvaceae), is a natural plant fiber of great economic significance, grown worldwide and now dominates the natural textile industry (Wegier et al. 2016). Additionally, cottonseed is a well-known livestock and poultry feed rich in fiber (24%), fat (20%) and protein (23%). Refined and deodorized cottonseed oil is one of the nutritious edible oils due to the presence of high levels of antioxidants such as tocopherol (Nix et al. 2017; Yang et al. 2019; Yang et al. 2017). The supply, however, has not grown proportionately to its diverse uses. Further, the gap between demand and supply has widened with the exponential growth of the population. To fill this gap, a genetically-modified Bt-cotton comprising the parasporal crystal protein genes of bacteria *Bacillus thuringiensis* (Bt) with insecticidal proteins

( $\delta$ -endotoxin) was developed with many advantages over conventional cotton to improve yield, quality, and pest resistance, etc., which has encouraged the commercialization of the transgenic cotton in recent years (Abdelmoteleb et al. 2018; Roh et al. 2007). The high yield Bt-cotton resulted in a 30% reduction of the land area used for cotton cultivation globally over the last 30 years, as well as the global cotton production has increased around 400% (Mehboob-ur-Rahman et al. 2012; Witjaksono et al. 2014). Many factors such as climate (temperature, light, rainfall, dew, wind, etc.), duration of growing season, availability of nutrients, relative humidity, soil moisture, pests, heavy metal contamination and cultivation practices may have unexpected responses to the growth of cotton plants (Sawan 2017; Mei et al. 2018; Xu et al. 2019). The target for the improved cotton yield can be realized by using advanced agricultural technologies and genetically-improved cotton breeds, understanding of climatic conditions, and soil fertilizer management, etc. (Thorp et al. 2014). Other than these technological developments for

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improving crop production, the utilization of metal or metal oxide based nanoparticles is a relatively new topic of study.

Nanoparticles (NPs) have been widely utilized for the different applications like biosensing (Salata 2004; Nehra et al. 2019), biofuel production (Sekoai et al. 2019), and organic and photochemical reactions (Song 2015), etc. The major advantage of nanofertilizer is that these are not only the best micro-nutrients but also aids in reclamation of soil. There are several reports where the presence of a certain amount of various nanoparticles has shown substantial beneficial effects on different plant species. (Nair 2016; Zhu et al. 2019; Kumar et al. 2019; Rastogi et al. 2017). However, the influence of a particular nanoparticle is dependent upon the dose, type, shape, structure, solubility and duration of the treatment (Aslani et al. 2014). NPs are prepared either with organic polymers (organic NPs) and/or inorganic elements (inorganic NPs). Inorganic NPs includes metals like Aluminium (Al), Cobalt (Co), Bismuth (Bi), Iron (Fe), Copper (Cu), Gold (Au), Molybdenum (Mo), Nickel (Ni), Tin (Sn), Silver (Ag), Titanium (Ti), Tungsten (W), Zinc (Zn), metal oxides ( $\text{SnO}_2$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{In}_2\text{O}_3$ ,  $\text{CuO}$ ,  $\text{ZrO}_2$ ,  $\text{Cu}_2\text{O}$ ,  $\text{MgO}$ ,  $\text{La}_2\text{O}_3$ ,  $\text{NiO}$ ,  $\text{ZnO}$ ,  $\text{TiO}_2$ ,  $\text{CeO}_2$ ) and quantum dots, while liposomes, dendrimers, carbon nanomaterials, and polymeric micelles are examples of bio-organic NPs (Rajput et al. 2017; Nie et al. 2010; Kango et al. 2013). NPs get absorbed 15–20 times more by the plants than the bulk nutrients (Lv et al. 2019; Srivastav et al. 2016). NPs have been deployed in agriculture to escalate the rate of seed germination and plant growth (Vera-Reyes et al. 2018) and also to protect plants from various abiotic stresses such as high and/or low temperature, salinity, drought, and flooding, and biotic stress such as fungi, bacteria, and insects (Jalil and Ansari, 2019, Elhawati et al. 2018; Hao et al. 2018; Hao et al. 2017).

### Recent beneficial applications of nanoparticles in plants

Silicon is found beneficial for plants under stress and help to rescue from drought stress as well as from micronutrient and other metal toxicities, i.e., copper, aluminum, iron, zinc, etc. (Siddiqui et al. 2015; Emamverdian et al. 2018). Nano- $\text{SiO}_2$  also affects maize seed germination positively by making available nutrients in better amounts, adjusting the pH and conductivity to the growing medium (Suriyaprabha et al. 2012). Quantum dot (QD) and silica-coated quantum dot have also been used for the study of root growth in rice plants (Wang et al. 2014). ZnONPs has been used to investigate the seed germination process in soybean, wheat, and onion and exhibits a beneficial effect under the lower concentration (Ali et al. 2021; Boonyanitipong et al. 2011; Sedghi et al. 2013; Raskar and Laware 2014). Additionally, some experiments on in vitro cultures and organogenic renaissance of bananas

supplemented with ZnONPs and found promoting effect on somatic embryogenesis and reconstruction of plantlets. A noticeable elevated level of antioxidant enzymes (SOD, POD, and CAT) and biochemicals (such as proline) were found responsible for the observed tolerance to various biotic stresses (Helaly et al. 2014). A study to assess the effect of ZnONPs in tomato plants showed significant improvement in growth, photosynthetic efficacy, carbonic anhydrase, and free radical scavenging activity (Faizan et al. 2018). Carbon based nanoparticles in the form of carbon nanotubes (CNTs) have been widely employed for the promotion of plant growth. The CNTs delivered into chloroplast, worked as artificial antennae, were capable of capturing light of different wavelengths (ultraviolet, green, and near-infrared), and hence, enhanced the seed germination, growth, and overall development in plants (Patel et al. 2017; Mukesh and Jha 2017; Siddiqui et al. 2015; Lahiani et al. 2013). The ability of the single and multi-walled CNTs to penetrate the plant cell has also been explored to develop the delivery system for DNA and other biochemicals (Lara-Romero et al. 2017; Oloumi et al. 2018; Srinivasan and Saraswathi 2010). Multi-walled CNTs were found a possible influencing factor for improved seed germination and plant growth in important crop plants (barley, corn, soybean) by inducing water and essential nutrients (Fe and Ca) uptake efficacy. Multi-walled CNTs also have a gene-regulating effect on various kinds of water channel proteins in soybean, barley, and corn (Lahiani et al. 2013). Noble metal nanoparticles such as Au and Ag have also been used for several crops and non-crop plants (Dykman and Shchyogolev 2018). Improved seed germination has been observed in lettuce (*Lactuca sativa*) (Barrena et al. 2009), mustard (*Brassica juncea*) (Arora et al. 2012; Sharma et al. 2012), common bean (*Phaseolus vulgaris*), and corn (*Zea mays*) (Salama 2012). Further, the improvement in the number of leaves, leaf area, plant height, chlorophyll content, and sugar content has also been reported, which resulted in better crop yield (Arora et al. 2012; Gopinath et al. 2014). In a study, *Arabidopsis thaliana* was treated with AuNPs and its remarkable effects on seed germination and free radical scavenging activity were noticed (Kumar et al. 2013). In this study, the expression levels of various miRNAs was also found correlated with seed germination, growth, and antioxidant potential. Interestingly, in a study where the NPs morphology-based effects were analyzed, the decahedral shaped AgNPs were found to have maximum root growth promoting effect; while, the spherical-shaped showed the maximum anthocyanin accumulation in *Arabidopsis* seedlings, but had no effect on root growth (Syu et al. 2014). The effects of conventionally synthesized and plant extract-based green AgNPs were compared in terms of their effects on *Phaseolus vulgaris* growth, as well as soil physicochemical properties. Under the low dose treatment of green AgNPs, the leaf number, leaf area index, pod yield, and nitrate reductase activity, etc., were found remarkably improved in comparison with conventional AgNPs. These

nanoparticles had also successfully modified the soil pH from originally acidic to neutral range, and thus, remarkably improved the cation exchange capacity, water holding capacity, and N/P content (Das et al. 2018).

Similar to these above discussed NPs, some other NPs such as Manganese (Mn), Titanium (Ti), etc., were also analyzed for their impacts on different plants (Pradhan et al. 2013; Jacob et al. 2013; Jaberzadeh et al. 2013). TiO<sub>2</sub>NPs elevated the growth of *Brassica napus* and *Triticum aestivum* (Mahmoodzadeh et al. 2013; Jaberzadeh et al. 2013). A study on *Triticum aestivum* to analyze the effect of nano Mn<sub>2</sub>O<sub>3</sub> (spherical-shaped, 30 nm size) along with bulk Mn<sub>2</sub>O<sub>3</sub>, and manganese chloride (MnCl<sub>2</sub>·4H<sub>2</sub>O) resulted in declined nitrogen content in the plant shoot by 9–18%. However, MnNPs in soil reduced the Mn, P, and K contents in the shoot by 25, 33 and 7%, respectively, while soil residual nitrate-N content were increased by 30%. The translocation efficiency of Mn was increased in the grain by nano Mn<sub>2</sub>O<sub>3</sub>, in comparison to other forms, i.e., salt and bulk-Mn. However, foliar exposure of MnNPs showed improved Mn contents in shoot and grain, P content in shoot, along with lesser soil nitrate (Dimkpa et al. 2018). TiO<sub>2</sub> nanoparticle induced elevated growth of *Brassica napus* was observed via concentration dependent improved radicle and plumule growth resulting in enhanced seed germination and seedling vigor (Mahmoodzadeh et al. 2013). The effect of foliar application of nano and bulk TiO<sub>2</sub> was studied to evaluate the agronomic traits such as height, ear weight and number, seed number and weight, seed gluten and starch contents under water deficit stress conditions in *Triticum aestivum*. The nano TiO<sub>2</sub> foliar treatment resulted in increased agronomic traits in comparison to bulk TiO<sub>2</sub> NPs (Jaberzadeh et al. 2013).

### Disadvantages of nanoparticles on plants or ecological environments

In recent years, various metal NPs have been used as nanopesticides to restrict the attack of different pests and microbes (Worrall et al. 2018; Deshpande 2019), nanoherbicides to reduce the negative effects of herbicides (Abigail and Chidambaram 2017) and nanofertilizers to improve productivity and natural fertility in plants by conquering the deficiencies of micronutrients (Bala et al. 2019). Along with their beneficial impacts, metal-based NPs are also applied and monitored for their harmful effects on flora and fauna (Jeevanandam et al. 2018; Ebrahimi et al. 2016; McGee et al. 2017; Priester et al. 2017; Rajput et al. 2018; Singh and Kumar 2016). Nanotoxicology helps to recognize the interaction mechanisms of a nanomaterial with plants or animals (Hobson 2016). The toxicity or poisonous level of NPs is not directly related to its dose or exposure concentration, but to the parameters like size, surface activity,

number, aggregate formation etc. (Singh 2016). The concentration dependent toxicity effect of rare earth metal oxides and their respective metals were observed on the aquatic microorganisms *Vibrio fischeri* and *Tetrahymena thermophila* (Kurvev et al. 2017).

These days, various reports show that NPs could be a health hazard and toxicity at primary level or secondary level as they could get entered in the food chain through plants. Presence of various nanoparticles (TiO<sub>2</sub>, ZnO, Ag NPs, etc.) in cosmetics are also very common and could penetrate the human skin (Fytianos et al. 2020). The NPs damage mitochondria (Meyer et al. 2011; Assadian et al. 2018), leakage in lysosomal membrane of blood lymphocytes (Assadian et al. 2018), reduce cell viability etc. (Umar et al. 2019) which ultimately harms the cells. NPs can also show toxicity by inducing oxidative stress that causes cell damage, increased inflammation, and altered immune responses (Shabbir et al. 2021).

NPs that are being used in agriculture are also found toxic for crops too. NPs were found toxic for the crop plants like to *Triticum aestivum* (Gorczyca et al. 2021), Tobacco (Peharec et al. 2021; Biba et al. 2021), *Oryza sativa* (Thuesombat et al. 2014), *Arabidopsis thaliana* (Sosan et al. 2016), *Hordeum vulgare* (El-Temsah and Joner 2012), *Lettuce sativa* (Ruttkay-Nedecky et al. 2017), *Vicia faba* (Falco et al. 2020) *Pisum sativum* (Mukherjee et al. 2016), etc.

The impact of various metal/metal oxide nanoparticles on the growth and production of major cultivation crops have always remained a topic of great interest (Table 1). Therefore, in this comprehensive review, we are going to represent those literature reports, where the impacts of various metal or metal oxide-based nanoparticles have been explored on the physiological parameters of cotton plants (Fig. 1).

### Distribution/accumulation of nanoparticles in cotton plant

Before going into the detail of various aspects of NPs impact on the transgenic or non-transgenic cotton plant, it is necessary to have insight into the distribution of nanomaterials in various parts of the cotton plant upon treatment with different concentrations of nanomaterials. In this section, the uptake, distribution, and the accumulation of various nanomaterials (viz. CeO<sub>2</sub>, CuO, Fe<sub>2</sub>O<sub>3</sub>, and SiO<sub>2</sub>) in cotton plants will be discussed.

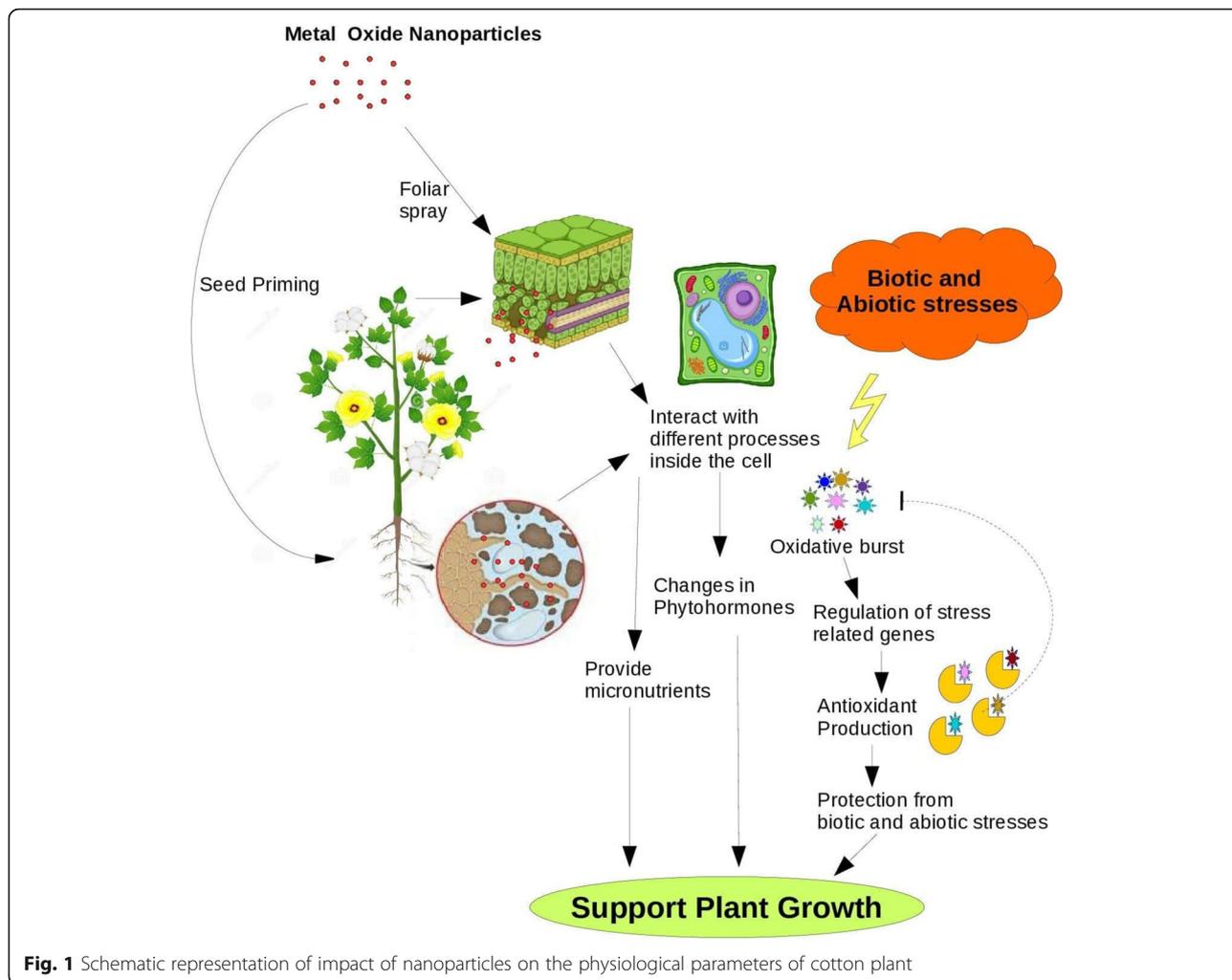
In both transgenic (Bt 29317) and non-transgenic (Jihe 321) cotton, most of the CeO<sub>2</sub> NPs aggregates were found to accumulate in the outer epidermis of the root, with fewer in the intercellular spaces as confirmed by ICP-MS analysis, which reflects the poor penetration tendencies of CeO<sub>2</sub> NPs into roots of plants (Li et al. 2014). The transgenic cotton exhibits greater accumulation of CeO<sub>2</sub> NPs in the intercellular spaces than

**Table 1** Impact of various metal/metal oxide nanoparticles on major cultivation crops

Metal/Metal oxide nanoparticles	Concentrations	Plant/Crops	Exposure Methodology	Physiological Impacts on plants	Reference
Au NPs	0–10 mg L <sup>-1</sup>	Mustard greens ( <i>Brassica juncea</i> )	Field	Improved seedling growth with increased productivity in terms of seed yield	Arora et al. 2012
	0–100 mg L <sup>-1</sup>	<i>Arabidopsis</i> ( <i>Arabidopsis thaliana</i> L.)	Growth chamber	Decrease in root length with increased dose of NPs	Taylor et al. 2014
Ag NPs	0–5 000 mg L <sup>-1</sup>	Barley ( <i>Hordeum vulgare</i> L.), Ryegrass ( <i>Lolium perenne</i> L.)	Growth chamber	Decrease in seed germination and shoot length	EI-Temseh and Joner 2012
	1–10 mg L <sup>-1</sup>	Lettuce ( <i>Lactuca sativa</i> ), Barley ( <i>Hordeum vulgare</i> L.)	Growth chamber	Significant increase in root length for barely and reduction in case of lettuce,	Gruyer et al. 2013
	20–100 mg L <sup>-1</sup>	Common bean ( <i>Phaseolus vulgaris</i> L.) Corn ( <i>Zea mays</i> L.)	Field	Protein content increased up to 60 g·kg <sup>-1</sup> concentration of Ag NPs. Further increase show toxic effects	Salama 2012
	0–100 mg L <sup>-1</sup>	Mungbean ( <i>Phaseolus radiatus</i> )	Growth chamber	Reduction in seedling growth, less toxicity in soil medium	Lee et al. 2012
	0–40 mg L <sup>-1</sup>	Sorghum ( <i>Sorghum bicolor</i> )			
	0–1 mg L <sup>-1</sup>	Rice ( <i>Oryza sativa</i> L.)	Growth chamber	Significant decrease in root growth, plant biomass, total chlorophyll and carotenoids content and photosynthetic pigments in rice seedlings	Nair and Chung 2014
Al <sub>2</sub> O <sub>3</sub>	2000 mg L <sup>-1</sup>	Corn ( <i>Zea mays</i> )	Growth chamber	Inhibition in root elongation	Lin and Xing 2007
	0.02–20 g·L <sup>-1</sup>	Cucumber ( <i>Cucumis sativus</i> ) Soybean ( <i>Glycine max</i> ) Cabbage ( <i>Brassica oleracea</i> ) Carrot ( <i>Daucus carota</i> )	Growth chamber	Inhibition in root elongation	Yang and Watts 2005
	400–4 000 g·L <sup>-1</sup>	<i>Arabidopsis</i> ( <i>Arabidopsis thaliana</i> L.)	Growth chamber	Significant Increase in root elongation	Lee et al. 2010
	50 mg/ml	Wheat ( <i>Triticum aestivum</i> )	Growth chamber	Reduction in root elongation, lignin deposition, cellular deformation, increase in peroxidase activity and decrease in total protein content	Yanik and Vardar 2015
CeO <sub>2</sub>	0.1–10 mg L <sup>-1</sup>	Tomato ( <i>Solanum lycopersicum</i> L.)	Green house	Increased plant growth and production with accumulation of Ce in tomato fruit	Wang et al. 2012a
	500–2000 mg L <sup>-1</sup>	<i>Arabidopsis</i> ( <i>Arabidopsis thaliana</i> L.)	Glasshouse	Reduction in plant growth and chlorophyll content at higher concentration	Ma et al. 2013
	0–500 mg·kg <sup>-1</sup> 0–400 mg·kg <sup>-1</sup>	Wheat ( <i>Triticum aestivum</i> )	Field Greenhouse	Significant increase plant height, biomass, and grain yield Toxic to wheat seedlings and increase in grain protein content	Du et al. 2015 Rico et al. 2014

**Table 1** Impact of various metal/metal oxide nanoparticles on major cultivation crops (*Continued*)

Metal/Metal oxide nanoparticles	Concentrations	Plant/Crops	Exposure Methodology	Physiological Impacts on plants	Reference
CeO <sub>2</sub> & ZnO	0–800 mg·kg <sup>-1</sup>	Cucumber ( <i>Cucumis sativus</i> )	Greenhouse	Bioaccumulation of Ce and Zn	Zhao et al. 2013
Cr <sub>2</sub> O <sub>3</sub>	0–100 mg·L <sup>-1</sup>	Wheat ( <i>Triticum aestivum</i> )	Growth chamber	Inhibition of seed germination, biomass, shoot and root length	Vajpayee et al. 2011
CuO	10–100 mg·L <sup>-1</sup>	Maize ( <i>Zea mays</i> L.)	Growth chamber	No effect on seed germination	Wang et al. 2012b
	0–1 000 mg·L <sup>-1</sup>	Rice ( <i>Oriza sativa</i> var. <i>Jyoti</i> )	Growth chamber	Increased level of oxidative and osmotic stress, decrease in germination rate, root and shoot length, and biomass	Da Costa and Sharma 2016
Fe <sub>3</sub> O <sub>4</sub> & ZnO	0 – 20 mg·L <sup>-1</sup>	Wheat ( <i>Triticum aestivum</i> )	Field	Increase in nutrients, biomass and decreased Cd toxicity	Rizwan et al. 2019
Fe <sub>3</sub> O <sub>4</sub>	0–100 µL·L <sup>-1</sup>	Sunflower ( <i>Helianthus annuus</i> L.)	Growth chamber	Reduction in chlorophyll content	Ursache-Oprisan et al. 2011
	2000 mg·L <sup>-1</sup>	Wheat ( <i>Triticum aestivum</i> )	Growth chamber	Growth inhibition & reduce oxidative stress induced by heavy metals (Zn, Pb, Cu and Cd)	Konate et al. 2017
SiO <sub>2</sub>	0–100 mg·L <sup>-1</sup>	Rice ( <i>Oriza sativa</i> L.)	Growth chamber	Positive effect on seed germination and seedlings growth	Adhikari et al. 2013
TiO <sub>2</sub>	0–400 mg·L <sup>-1</sup>	Tomato ( <i>Lycopersicum esculentum</i> L.) Onion ( <i>Allium cepa</i> L.) Radish ( <i>Raphanus sativus</i> L.)	Green House	Improved seed germination at 100 and 200 mg·L <sup>-1</sup> concentration	Haghighi and da Silva, 2014
	100 mg·L <sup>-1</sup>	Wheat ( <i>Triticum aestivum</i> )	Growth chamber	No effect on seed germination and total biomass	Larue et al. 2012
TiO <sub>2</sub> & ZnO	100–500 mg·kg <sup>-1</sup>	Wheat ( <i>Triticum aestivum</i> )	Field	Reduced plant Growth	Du et al. 2011
	0–1 000 mg·L <sup>-1</sup>	Rice ( <i>Oriza sativa</i> L.)	Growth chamber	Root elongation inhibition with decreased number of roots	Boonyanitipong et al. 2011
ZnO	0–500 mg·kg <sup>-1</sup>	Soybean ( <i>Glycine max</i> L.)	Green House	Reduced growth of plant	Yoon et al. 2014
	400–2000 mg·L <sup>-1</sup>	Peanut ( <i>Arachis hypogaea</i> )	Growth chamber	1g·L <sup>-1</sup> NPs concentration improved seedling germination but showed negative effect at 2 g·L <sup>-1</sup>	Prasad et al. 2012
	0–1 600 mg·L <sup>-1</sup>	Tomato ( <i>Solanum lycopersicum</i> L.) Alfalfa ( <i>Medicago sativa</i> ) Cucumber ( <i>Cucumis sativus</i> )	Growth chamber	Germination rate reduced in Tomato and Alfalfa but increased in Cucumber	de la Rosa et al. 2013
	0–500 mg·kg <sup>-1</sup>	Green peas ( <i>Pisum sativum</i> L.)	Field	Increased root elongation	Mukherjee et al. 2014
	0–16 mg·L <sup>-1</sup>	Tomato ( <i>Lycopersicum esculentum</i> L.)	Net house	Increased growth, enhanced photosynthetic efficiency at 8 mg·L <sup>-1</sup> treatment	Faizan et al. 2018



**Fig. 1** Schematic representation of impact of nanoparticles on the physiological parameters of cotton plant

the conventional cotton as observed in transmission electron microscope (TEM) images of roots of conventional (A) and Bt-transgenic cotton (B) under  $500 \text{ mg}\cdot\text{L}^{-1}$   $\text{CeO}_2$  NPs treatments (Fig. 2). The enhanced accumulation of  $\text{CeO}_2$  NPs aggregates was observed in the leaves and stems of both Bt-transgenic cotton and conventional cotton, but to different extents, when these cotton plants were treated with increased concentrations of  $\text{CeO}_2$  NPs. The Ce content was nearly 1.8 times higher in leaves and stem of Bt-cotton compared with the conventional cotton, when treated with lower concentrations ( $100 \text{ mg}\cdot\text{L}^{-1}$ ) of  $\text{CeO}_2$  NPs solutions. But the Ce content was significantly increased in the leaves and stems of Bt-transgenic cotton, when the dose of  $\text{CeO}_2$  NPs solution was increased to  $500 \text{ mg}\cdot\text{L}^{-1}$  and was approximately three-fold as compared with that observed in the conventional cotton (Li et al. 2014; Nhan et al. 2015).

These results revealed that after uptake by the root system, the  $\text{CeO}_2$  NPs were transported to the leaves

and stem in both transgenic and nontransgenic cotton. The greater accumulation of  $\text{CeO}_2$  NPs was observed in the transgenic Bt 29317 cotton compared with the conventional Jihe 321. TEM images showed aggregation of  $\text{CeO}_2$  NPs on the outer surface of chloroplasts, which resulted in the rupturing of later and release of an essential component of chlorophyll viz. Zn, Mg, Fe, and P from xylem sap (Fig. 3).

The CuO uptake studies carried out on both transgenic and nontransgenic cotton showed enhanced nanoparticle concentrations in the roots of cotton plants irrespective of its type (Nhan et al. 2016b). A noticeable increase in Cu content was observed in the shoots and roots of the conventional cotton, when CuO NPs solution concentrations were increased from  $200 \text{ mg}\cdot\text{L}^{-1}$  to  $1\ 000 \text{ mg}\cdot\text{L}^{-1}$ , and this was significantly high as compared with that observed in the transgenic cotton. The TEM analysis of Ipt-cotton plant pre-exposed for 10 days to copper oxide nanoparticle concentrations ( $1\ 000 \text{ mg}\cdot\text{L}^{-1}$ ) showed dark dots in leaves and roots, which

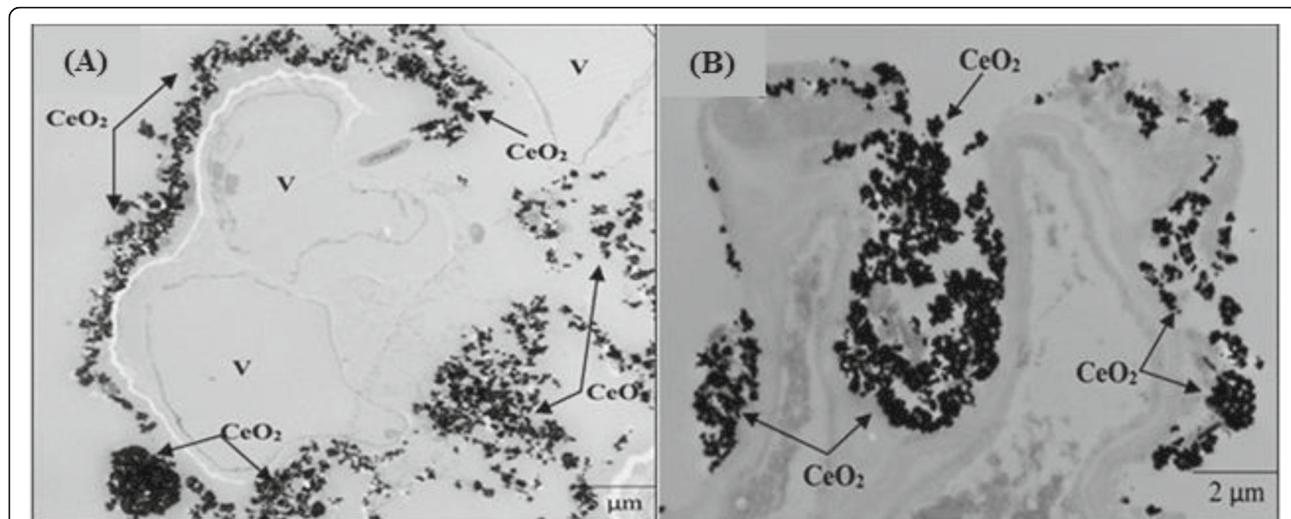
**Table 2** Impact of various metal/metal oxide nanoparticles on cotton plants

Metal Oxide Nanoparticles	Source/ Synthetic Method	Cotton Variety Studied	Exposure Methodology	Impact on Cotton Plant	Reference
Cerium Oxide (CeO <sub>2</sub> NPs) Size (nm): 10 ± 3.2 Conc. (mg L <sup>-1</sup> ): 0, 100, 500, 2 000	Merchant	Bt 29317 & Jihe 321	Growth chamber	Decreased plant height, shoot and root biomass with various dose treatments; Decreased nutrient element content (Na, Ca, Zn, Mg, and Fe) in roots.	Li et al. 2014; Nhan et al. 2015
Silicon Dioxide (SiO <sub>2</sub> NPs) Size (nm): < 30 Conc. (mg L <sup>-1</sup> ): 0, 10, 100, 500, 2 000	Merchant	Bt-29312 & Jihe 321	Growth chamber	Decreased plant height, shoot and root biomass; Altered Na content in roots and Mg, Cu contents in shoots; Increased SOD activity, IAA concentration.	Nhan et al. 2014
Copper Oxide (CuO NPs) Size (nm): < 30 Conc. (mg L <sup>-1</sup> ): 0, 10, 200, 1 000	Merchant	lpt-cotton	Growth chamber	Negatively effect on plant height, number of root hairs, root length, and shoot biomass with higher dose; Inhibition of the synthesis of phytohormones (IAA, ABA, GA, and t- ZR); Increased iPA concentration, that might have resulted in delayed senescence.	Nhan et al. 2016c
		Bt 29317 & Jihe 321	Growth chamber	Inhibited the growth in both transgenic and conventional cottons; Reduced uptake of nutrients (Mo, B, Mg, Zn, Fe, Mn); Lower dose treatment improved the Bt toxin expression in Bt-transgenic cotton.	Nhan et al. 2016b
Ferric Oxide (Fe <sub>2</sub> O <sub>3</sub> NPs) Size (nm): < 50 Conc.(mg L <sup>-1</sup> ): 0, 100, 1 000	Merchant	Bt 29317 & Jihe 321	Growth chamber	Inhibited plant height of Bt-transgenic cotton; Promoted abundance of root hairs, and biomass of conventional cotton; Increased Fe, Na and K nutrients content and phytohormones in the roots of Bt-transgenic cotton at low Fe <sub>2</sub> O <sub>3</sub> NP exposure; Decreased Zn contents in roots of Bt-transgenic cotton; Increased Bt-toxin level in roots and leaves of Bt-transgenic cotton.	Nhan et al. 2016a
Zinc Oxide (ZnO NPs) Size (nm): 2 to 54 Conc. (mg L <sup>-1</sup> ): 25, 50, 75, 100, 200	Using algal extract (from <i>Halimeda tuna</i> ) as reducing agent	Non-transgenic	Growth chamber	Promoted the growth, photosynthetic pigment levels, biomass, and protein contents; Declined MDA production; Improved the activity of antioxidant defense enzymes; Enhanced SOD and POX isoenzymes expression levels.	Venkatachalam et al. 2017
Silver nanoparticles (AgNPs) Size (nm): 59.2 Conc. (mg L <sup>-1</sup> ): 100	Using plant extract (from <i>Pluchea sericea</i> & <i>Prosopis glandulosa</i> ) as reducing agent	Transgenic	Growth chamber	AgNPs from <i>P. glandulosa</i> exhibited greater efficiency than AgNPs from <i>P. sericea</i> in scaling down the infection in the plants contaminated with <i>Fusarium solani</i> .	Abdelmoteleb et al. 2018

confirmed the presence of CuO NPs (Fig. 4). The presence of dark dot in the endodermis and vascular cylinders further proved that the CuO NPs were first absorbed by roots (as higher concentrations of CuO NPs were found present in the epidermis of roots), and then were transported through xylem sap to shoots and leaves.

At a lower concentration of Fe<sub>2</sub>O<sub>3</sub> NPs (100 mg·L<sup>-1</sup>), there was an insignificant difference observed in the accumulation

of nanoparticles in the shoots of both Bt-cotton and conventional cotton. However, with the increased concentration (1 000 mg·L<sup>-1</sup>) Bt-cotton shoots exhibited higher accumulation of iron oxide nanoparticles compared with the non-transgenic cotton (Fig. 5). At 1 000 mg·L<sup>-1</sup> dose of Fe<sub>2</sub>O<sub>3</sub> NPs, the Fe content was also found higher in the roots of both Bt-transgenic cotton (5.3-fold) and non-transgenic cotton (2.8-fold) compared with the control groups. Further, Bt-transgenic cotton displayed higher capabilities towards Fe<sub>2</sub>O<sub>3</sub>

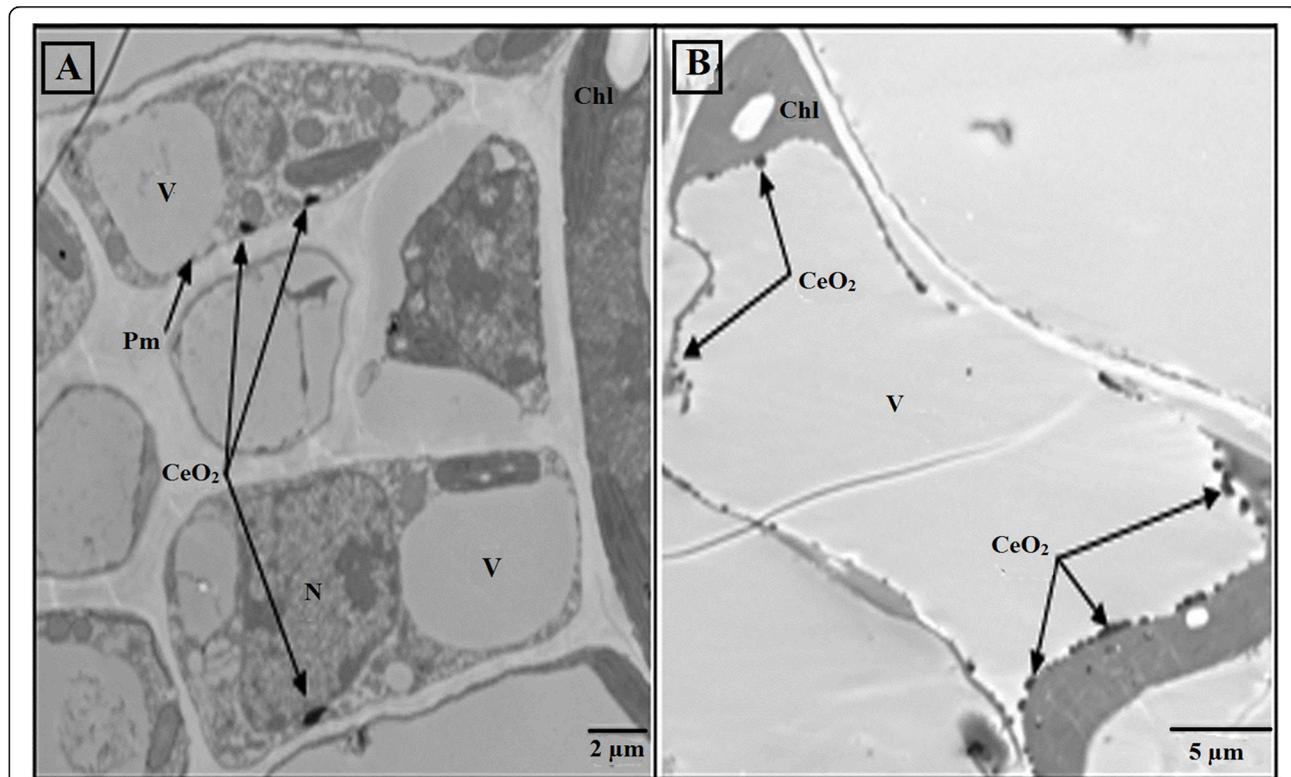


**Fig. 2** Transmission electron microscopic (TEM) images of cross section of roots of conventional (a); and Bt-transgenic cottons (b) under 500 mg·L<sup>-1</sup> CeO<sub>2</sub> NPs treatments. Image reproduced from Nhan et al. (2015) under a Creative Commons Attribution 4.0 International License, visit <https://creativecommons.org/licenses/by/4.0/>

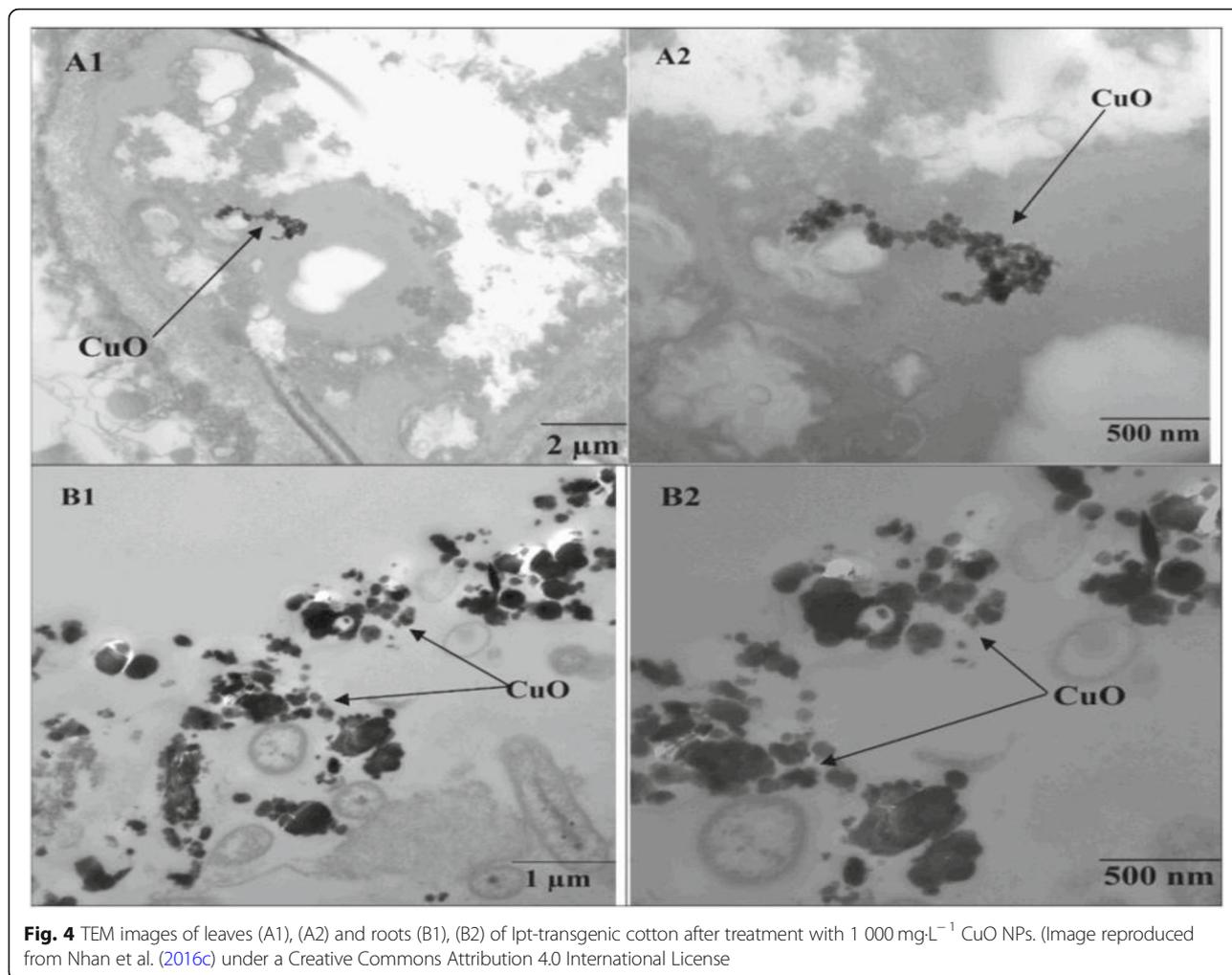
NPs uptake (1.27-fold) in comparison to the conventional cotton (Nhan et al. 2016a).

The TEM images of cross-sections of roots of both non-transgenic and Bt-transgenic cotton showed the presence of SiO<sub>2</sub> NPs in the form of dark dots with

abundance in the epidermis and fewer in the intercellular spaces. At 2000 mg·L<sup>-1</sup> SiO<sub>2</sub> NPs concentration the Si content observed in the Bt-transgenic roots was higher than that in the non-transgenic one, which suggests higher penetration of SiO<sub>2</sub> NPs into Bt-transgenic



**Fig. 3** TEM images of leaf section of conventional cotton (a); and Bt-transgenic cotton (b) under 500 mg·L<sup>-1</sup> CeO<sub>2</sub> NPs treatments. Chloroplast (Chl), plasma membrane (pm), Vacuole (V). Image reproduced from Nhan et al. (2015) under a Creative Commons Attribution 4.0 International License



**Fig. 4** TEM images of leaves (A1), (A2) and roots (B1), (B2) of lpt-transgenic cotton after treatment with 1 000 mg·L<sup>-1</sup> CuO NPs. (Image reproduced from Nhan *et al.* (2016c) under a Creative Commons Attribution 4.0 International License)

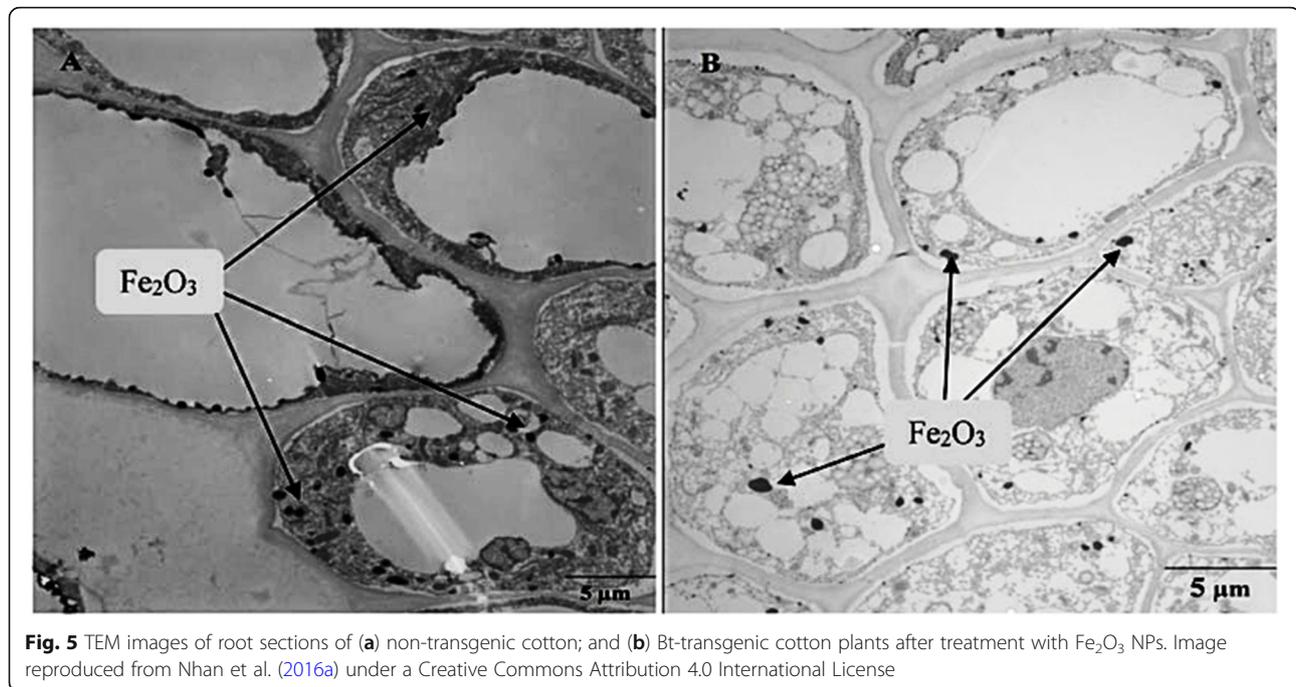
cotton and have a more adverse effect on it (Nhan *et al.* 2014).

#### Impact of metal oxide nanoparticles on nutrient element contents

After water, nutrients are the most significant factors in determining crop production. Out of 16 essential elements, the fertilizers provide three (N, P, K), while most of the rest of nutrients are obtained directly through either soil or atmosphere. Nanoparticles accumulation in various parts of the cotton plant affects the nutrient content levels to a significant extent. The following discussion will help us in understanding the impact of nanoparticles on the availability of nutrient elements in various parts of cotton. It has been reported that the presence of CeO<sub>2</sub> NPs significantly decreased the nutrient element content in roots and shoots of transgenic cotton (Bt 29317) as compared with the non-transgenic (Jihe 321) (Li *et al.* 2014; Nhan *et al.* 2015). A remarkable decrease in the nutrient elements levels of Zn, Mg,

Fe, and P in the xylem sap was observed in CeO<sub>2</sub> NPs treated plants; however, the Mn content was significantly increased. The decrease in nutrient element concentrations in the xylem sap was more prominent in the CeO<sub>2</sub> NPs-treated conventional cotton than that in Bt-transgenic cotton plants. The Ca and Mn content in the xylem sap of both CeO<sub>2</sub> NP-treated conventional and Bt-transgenic cotton were nearly the same and also comparable to control samples. The Cu content in the xylem sap of CeO<sub>2</sub> NPs-treated transgenic cotton was on the higher side compared with the non-transgenic cotton. A significant increase in Ce content was observed in the xylem sap of both Bt 29317 and Jihe 321 with an increased concentration of CeO<sub>2</sub> NPs.

Moreover, the treatment of Fe<sub>2</sub>O<sub>3</sub> NPs (100 mg·L<sup>-1</sup>) increased the K and Ca content of both Bt-transgenic and the non-transgenic cotton plants, but the more prominent effect was observed in the shoots and roots of the transgenic cotton plant (Nhan *et al.* 2016a). Increased uptake of Na was observed with increased Fe<sub>2</sub>O<sub>3</sub>



**Fig. 5** TEM images of root sections of (a) non-transgenic cotton; and (b) Bt-transgenic cotton plants after treatment with Fe<sub>2</sub>O<sub>3</sub> NPs. Image reproduced from Nhan et al. (2016a) under a Creative Commons Attribution 4.0 International License

NPs treatment (100 to 1 000 mg·L<sup>-1</sup>) in the Bt-transgenic cotton, but Na uptake was altogether inhibited in the shoots of the conventional cotton. Interestingly, an entirely opposite effect was observed with the Mg uptake in these cotton types. The treatment of Fe<sub>2</sub>O<sub>3</sub> NPs resulted in decreased concentration of Mg in the Bt-transgenic cotton and increased uptake of Mg in the conventional cotton. Mn, Zn, P, and Cu contents in the shoots were the same in the cotton plants upon Fe<sub>2</sub>O<sub>3</sub> NPs treatments irrespective of the plant type. Zn content was decreased while Cu content was increased in the roots of the Bt-transgenic cotton upon exposure to Fe<sub>2</sub>O<sub>3</sub> NPs (100 mg·L<sup>-1</sup>). The Bt-transgenic cotton was comprehensively found more sensitive to Fe<sub>2</sub>O<sub>3</sub> NPs compared with the non-transgenic cotton (Nhan et al. 2016a).

The uptakes of various nutrient content in the conventional and the transgenic cotton were differently affected with equal dose treatment of CuO NPs. Lower exposure of CuO NPs (10 mg·L<sup>-1</sup>) did not affect the Ca, Mg, Mn, Mo, K, B or P content in the shoots of the conventional or the transgenic cotton, but increased level (1 000 mg·L<sup>-1</sup>) resulted into enhanced nutrient uptake. CuO NPs (1 000 mg·L<sup>-1</sup>) treated Bt-transgenic cotton exhibited a decrease in Fe and Zn contents, whereas an increase in both Fe and Zn content was observed at lower CuO NPs (10 mg·L<sup>-1</sup>) treatment, and was relatively higher in the Bt-transgenic cotton ( $P < 0.05$ ) than that in the conventional cotton. Negligible differences were observed in Na content in the shoots of CuO NPs treated conventional and transgenic cotton in comparison to the control plants, however, the Na content in the roots was significantly

increased with increased CuO NPs concentrations. The Na content in CuO NPs treated (1 000 mg·L<sup>-1</sup>) Bt-transgenic cotton was lower than that in the conventional cotton, but higher compared with the control group. CuO NPs exposure (1 000 mg·L<sup>-1</sup>) did not affect the Ca, Mn, and P content, however, Fe, Na and Mo contents alter significantly in the roots of the conventional and transgenic cotton (Nhan et al. 2016b). In Ipt-cotton, most of the nutrient contents (Mg, Ca, Mn, Mo, B, and P) except Zn were undisturbed at a lower concentration treatment of CuO NPs (10 mg·L<sup>-1</sup>) in roots and shoots and was reduced with a higher concentration (200, 1 000 mg·L<sup>-1</sup>) compared with the control (Nhan et al. 2016c). The Fe content in roots and Na and Cu content in both roots and shoots were increased, whereas K content in shoots and Zn, Ca, B, and P contents in roots were significantly decreased, with enhanced exposure of CuO NPs (200, 1 000 mg·L<sup>-1</sup>).

SiO<sub>2</sub> NPs treated Bt-transgenic and non-transgenic cotton exhibited similar in Fe, Mn, K and Zn contents in the shoots and Mn, Zn, Mg and Cu contents in roots and was hardly shown any difference from control plants. However, the Fe content was observed high in roots of Bt-transgenic cotton than non-transgenic cotton on lower SiO<sub>2</sub> NPs treatments (10 and 100 mg·L<sup>-1</sup>), and exactly opposite was observed at higher SiO<sub>2</sub> NPs treatments (500 and 2 000 mg·L<sup>-1</sup>). The contents of Cu, K, and Na were higher in the roots of Bt-transgenic cotton at various concentrations of treatment, but significantly decreased in shoots. This reflects the greater tendencies of nutrient element absorptions by treated Bt-transgenic cotton, but poor transportation of the same to the

shoots. Further, SiO<sub>2</sub> NPs treatment had insignificant effects on the Mn, K, Na, Cu, Zn, and Ca contents in the xylem sap of both non-transgenic and Bt-transgenic cotton, but concentrations differ remarkably in the xylem sap of two cotton plants. Fe and Mg transportations were geared-up by the presence of SiO<sub>2</sub> NPs in the xylem sap of both non-transgenic and Bt-transgenic cotton, but concentrations of both nutrient elements were nearly same in the xylem sap of the genetically-modified and conventional cotton (Nhan et al. 2014).

### Impact of metal oxide nanoparticles on phytohormone concentrations

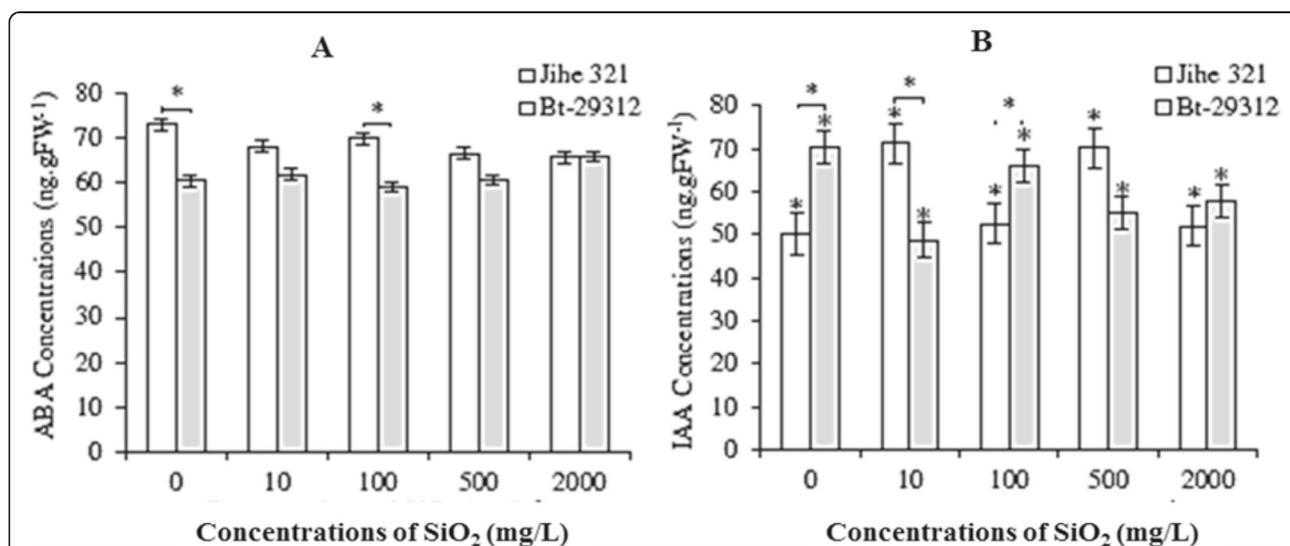
Plant growth hormones are chemical substances that regulate the various physiological activities in a plant viz. cell elongation and cell division, metabolism, stress relief, seed germination, and senescence, etc. There are mainly four plant growth hormones viz. Auxins (indole-3-acetic acid, IAA), cytokinins (trans-zeatinriboside, t-ZR), gibberellins (Gibberellic acid, GA), and abscisic acid (ABA) that regulate the growth and harmony in plants. The distribution and accumulation of various metal oxide-based nanoparticles (MNPs) in the different plant parts may pose beneficial and adverse impacts on the synthesis and regulation of these phytohormones.

CeO<sub>2</sub> NPs exposures had different effects on hormones in different parts of Bt-transgenic and conventional cotton plants. At 500 mg·L<sup>-1</sup> CeO<sub>2</sub> NP exposure did not alter the auxin hormone (indole-3-acetic acid, IAA) concentrations in the leaves and roots of Bt-transgenic cotton and the control groups, but it was

found significantly higher (1.29 times) in the leaves of the conventional cotton compared with the control plants. In contrast, the roots of CeO<sub>2</sub> NPs treated Bt-transgenic cotton exhibited higher IAA levels as compared with the conventional cotton, but comparatively lower than the control plants. The t-ZR content in the leaves and roots of CeO<sub>2</sub> NPs exposed Bt-transgenic cotton was not perturbed, however, a significant decrease in t-ZR levels were observed in the leaves (76.6%) and roots (91.3%) of conventional cotton as compared with the control groups. The GA content in the leaves of CeO<sub>2</sub> NP treated conventional cotton was higher than that in Bt-transgenic cotton ( $P < 0.05$ ). These results showed greater sensitivity of the conventional cotton towards phytohormones under CeO<sub>2</sub> NPs treatment.

The abscisic acid (ABA) contents in the roots of non-transgenic cotton were maximum (72.98 ng·g<sup>-1</sup> (FW) ) at 500 mg·L<sup>-1</sup>, and minimum (65.57 ng·g<sup>-1</sup> (FW) ) at 2000 mg·L<sup>-1</sup> SiO<sub>2</sub> NPs treatments, and were evidently higher than Bt-transgenic cotton at the control treatment, but exactly opposite effect was observed at 100 and 2 000 mg·L<sup>-1</sup> SiO<sub>2</sub> NP treatments in the former cotton plant (Fig. 6).

Moreover, the exogenous application of Fe<sub>2</sub>O<sub>3</sub> NPs to different concentrations had a negative effect on the root hormone contents in both Bt-transgenic and conventional cotton, and hence retarded the plant growth and development (Nhan et al. 2016a). The ABA and GA contents in the leaves were decreased in the Fe<sub>2</sub>O<sub>3</sub> NP-treated conventional cotton as compared with the transgenic one which displayed an increase in GA content in the leaves of Bt-transgenic cotton with no influence on ABA levels. Additionally, the exposure of Fe<sub>2</sub>O<sub>3</sub> NPs (100 mg·L<sup>-1</sup>) to



**Fig. 6** Effect of SiO<sub>2</sub> NPs concentrations on ABA concentration level (a); and IAA concentration levels (b); in cotton plants. Image reproduced from Nhan et al. (2014) under a Creative Commons Attribution 4.0 International License

the roots of Bt-transgenic cotton resulted in enhancement of all phytohormone concentrations, but with the opposite effect in the non-transgenic cotton.

Furthermore, various CuO NPs treatments resulted in elevated hormonal levels (IAA, ABA, and GA), in both conventional and transgenic cotton plants relative to control groups, with the more prominent effects observed in the conventional cotton plants. Although, the t-ZR content was slightly decreased with CuO NP treatment in the conventional cotton plant. However, the t-ZR and IAA hormone contents were comparatively higher in the transgenic roots than those in the control groups, when treated with varying concentrations of CuO NPs (Nhan et al. 2016b). In Ipt-cotton, CuO NPs treatments with lower doses, i.e., 10 and 200 mg·L<sup>-1</sup>, caused a decrease in the level of IAA content in leaves, whereas an increase was observed in the roots of treated cotton plants compared with the control groups (Nhan et al. 2016c). The CuO NPs influenced inhibited production of IAA in leaves was in concordance with diminished plant height and biomass. The ABA content in the leaves and roots showed its direct correlation with the dose of CuO NPs treatment. The GA synthesis was inhibited in leaves, and was promoted in roots on treatment with CuO NPs. The t-ZR hormone in the leaves and roots was unaltered at lower dose treatments of CuO NPs, but differs significantly with higher concentration of CuO NPs from that in controls.

### Impact of metal oxide nanoparticles on the enzyme activities

Recent studies showed that various metal oxide nanoparticles at different levels play a significant role in detoxifying the reactive oxygen species by stimulating antioxidative machinery in both conventional and transgenic cotton.

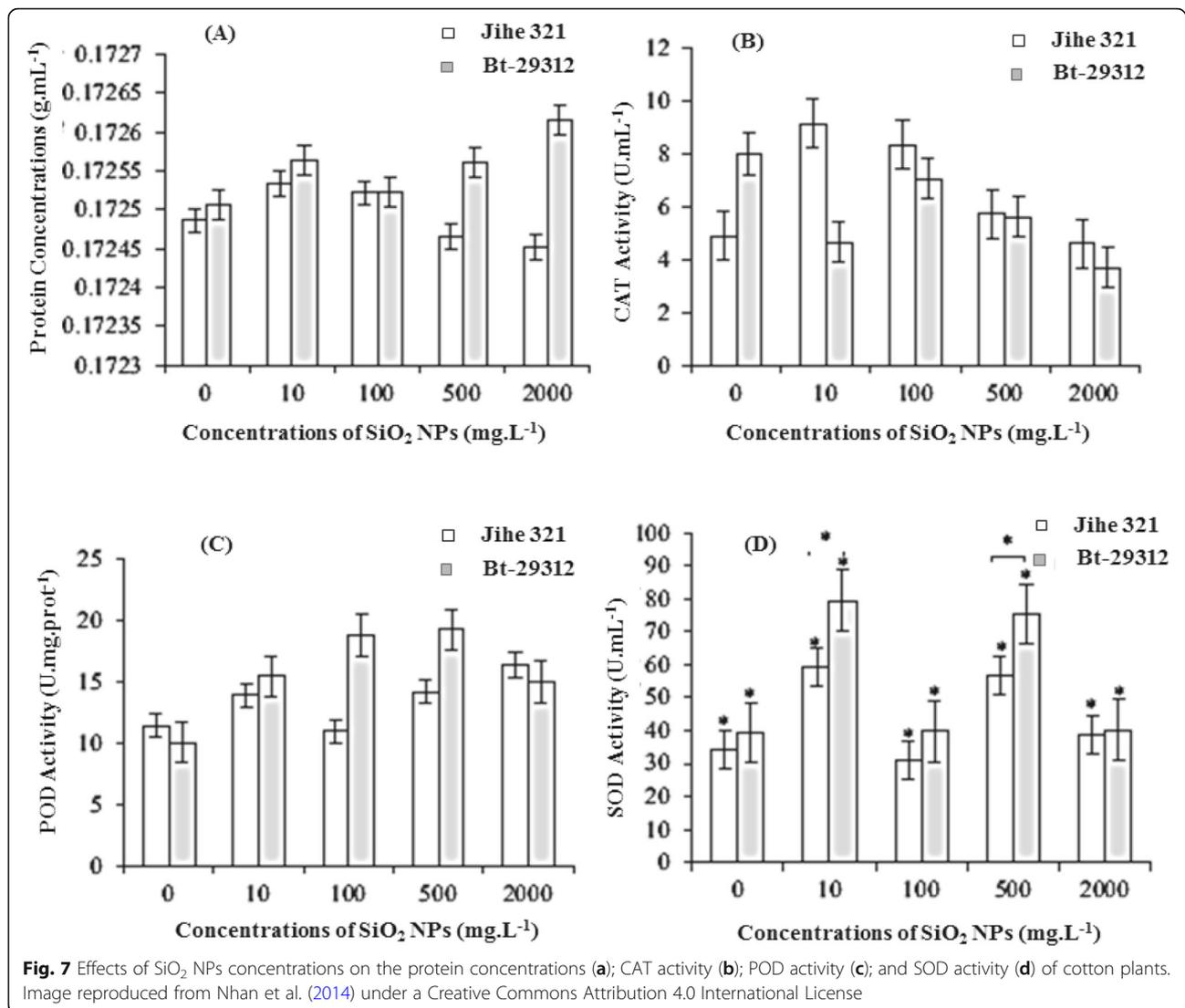
When cotton was treated with ZnO NPs at different concentrations, the SOD activity in plants treated with 75 mg·L<sup>-1</sup> was increased up to 267.8% compared with the untreated plant, while it decreased at higher doses of ZnO NPs (Venkatachalam et al. 2017). Subsequently, slight improvement was noticed in CAT activity (106.9%) at 25 mg·L<sup>-1</sup>, and which declined significantly with an increasing concentration of ZnO NPs. Along with SOD and CAT, the POX activity was also improved by 174.5% under 100 mg·L<sup>-1</sup> ZnO NPs application, however, it decreased at higher doses of the treatment. Such concomitant decreased activity of SOD, CAT, and POX enzyme with a higher dose of ZnO NPs in cotton leaves with a decreased level of malondialdehyde (MDA) content suggested that the cotton augments antioxidant enzymes level to alleviate the accumulated H<sub>2</sub>O<sub>2</sub>. Additionally, ZnO NPs exposure (200 mg·L<sup>-1</sup>) caused a significant decline in the MDA content (73.8%) in the leaves of treated

cotton compared with the control. Although, boosting of SOD and POX activities in ZnO NPs treated plants were found successful to neutralize free radical-mediated oxidative damage in *G. hirsutum*.

Native PAGE was performed to analyze the expression pattern for SOD and POX isozymes in the cotton leaves treated with ZnO NPs (Venkatachalam et al. 2017). For SOD, 2 isoforms were noticed in the isozyme banding pattern, where SOD isoform 1 was visible, while the SOD isoform 2 was absent in the leaves exposed with higher ZnO NPs doses (100 and 200 mg·L<sup>-1</sup>). Like SOD, two isoforms were also observed for POX isoenzyme, among them, POX isoform 2 bands were clearly observed in ZnO NP treated plants, although it was absent in the control. Interestingly, expression of POX isoform 1 was found 2–3 folds higher in leaf tissue treated with higher doses of NPs over the control. Here, the interesting fact to be noted was that, the level of SOD and POX isoenzymes expression was on a par with the quantitative analysis results of the respective enzymes.

Additionally, no significant differences were observed in POD and CAT activities between Bt and conventional cotton cultivars, and even between the treated and the control plants exposed to SiO<sub>2</sub>NP. At higher SiO<sub>2</sub>NP treatment (2 000 mg·L<sup>-1</sup>), the CAT activities were decreased from 9.15 µg·mL<sup>-1</sup> to 4.63 µg·mL<sup>-1</sup> in the roots of non-transgenic cotton, however, in the Bt-cotton, these were declined from 7.99 µg·mL<sup>-1</sup> to 3.70 µg·mL<sup>-1</sup>. This study clearly indicated that the CAT activities were negatively affected by SiO<sub>2</sub> NPs in the roots of both Bt and conventional cotton. Unlike CAT, Bt and conventional cotton showed higher POD activities with increased SiO<sub>2</sub> NPs concentration up to 500 mg·L<sup>-1</sup> and followed by declined at 2 000 mg·L<sup>-1</sup>, in comparison to the controls, suggesting that the POD activities were triggered by SiO<sub>2</sub> NPs with lower concentrations (> 500 mg·L<sup>-1</sup>) in both kinds of cotton. Additionally, non-transgenic and Bt-cotton showed significantly different SOD activities between the control and NPs treatments. The maximum SOD activity was observed at 10 and 500 mg·L<sup>-1</sup> SiO<sub>2</sub> NPs treatment for both non-transgenic (58.98 µg·mL<sup>-1</sup>) and Bt-cotton (79.51 µg·mL<sup>-1</sup>), while lesser activity was observed at 0.0 mg·L<sup>-1</sup> (the control treatment), 100 and 2 000 mg·L<sup>-1</sup> SiO<sub>2</sub> NPs treatments (Fig. 7) (Nhan et al. 2014).

There were insignificant differences between POD and SOD activities, observed in roots and leaves, respectively, in Bt or conventional cotton and the control samples, when treated with CeO<sub>2</sub> NPs. Bt-transgenic cotton showed significantly higher POD activity in the leaves than that in conventional cotton (*P* < 0.05), when exposed to 500 mg·L<sup>-1</sup> CeO<sub>2</sub> NPs (Li et al. 2014). However, the insignificant difference was shown by SOD in the leaves as well as roots of Bt-cotton, and the leaves of CeO<sub>2</sub> NP-treated conventional cotton and the control plants, while SOD activity in roots of Bt-cotton was



significantly lesser ( $P < 0.05$ ) than untreated conventional cotton. Although, SOD activity under CeO<sub>2</sub> treatment in root of the conventional and Bt-cotton plants showed no significant difference.

### Impact of metal oxide nanoparticles on Bt-toxin expression

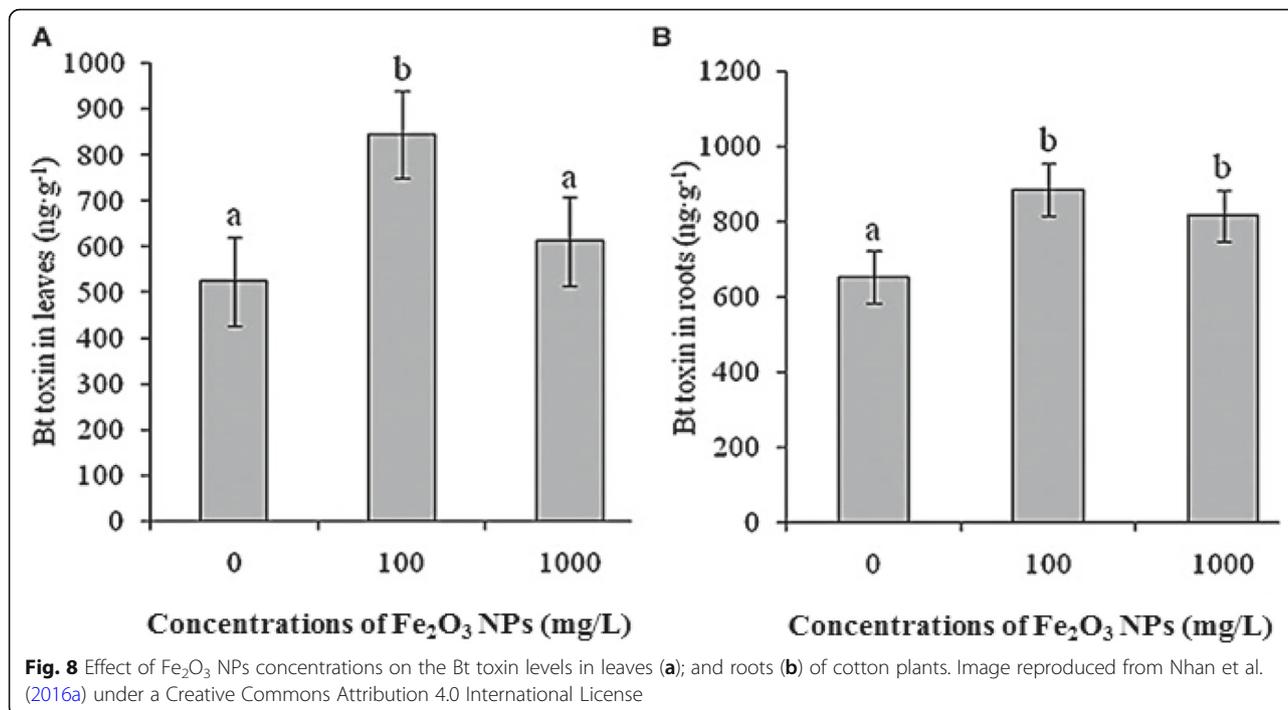
The Bt toxins defend transgenic cotton against biotic stress resulting from vandalization by other living organisms like viruses, fungi, parasites, bacteria, insects, weeds, etc. The presence of metal oxide nanoparticles considerably influences the levels of Bt toxins in the different parts of the cotton plant and thus is directly linked to growth and biomass production of cotton.

Bt toxin levels were improved with lower dose treatment of CuO NPs (10 mg.L<sup>-1</sup>) in the leaves and roots of Bt-transgenic cotton and were significantly higher than

those observed in the control plants (Nhan et al. 2016b). However, the remarkably decreased Bt toxin expression was observed with increased exposure of CuO NPs (1 000 mg.L<sup>-1</sup>) and was assigned to the absorption of Bt toxin protein by CuO NPs present in large amounts.

Fe<sub>2</sub>O<sub>3</sub> NPs exposure to lower concentration dose (100 mg.L<sup>-1</sup>) explicitly enhanced Bt toxin levels in leaves (845.89 ng.g<sup>-1</sup>) and roots (886.94 ng.g<sup>-1</sup>) of Bt cotton, which was higher by manifolds than their respective control groups (Fig. 8). The treatment of a higher dose of Fe<sub>2</sub>O<sub>3</sub> NPs (1 000 mg.L<sup>-1</sup>) resulted in decreased expression level of Bt toxin in leaves and roots, but was still higher than in control groups (Nhan et al. 2016a).

Bioengineered phycocyanin coated ZnO NPs (200 mg.L<sup>-1</sup>) induced enhancement in the rate of formation of photosynthetic pigments, i.e., chlorophyll-a, 134.7%; chlorophyll-b, 132.6%; and carotenoids, 160.1% in cotton



(Venkatachalam et al. 2017). RAPD-DNA fingerprinting analysis is used as a powerful tool to detect the genomic changes/alterations that occurred in the plants. ZnO NPs treatment did not change any banding pattern excluding DNA amplicon intensity. Ten out of 80 primers showed distinct DNA patterns, but only 4 primers showed DNA fingerprinting bands with changed intensity under the effect of ZnO NPs. The ineffectiveness ZnO NPs on the cotton genome could be either because the formation of ZnO nano complexes capped with different growth-promoting factors, or the Zn ions becomes available by phycocompound-coated ZnO NPs to dividing cells without harming DNA of the plants.

Fusarium wilt caused by *Fusarium solani*, is an important disease that occurs in cotton plants in various countries (Gonzalez-Soto et al. 2015). The AgNPs were synthesized using leaf extract of *Prosopis glandulosa* and *P. sericea* and were diluted at 100 mg·L<sup>-1</sup> with demineralized water (Abdelmoteleb et al. 2018). The cotton plants were grown up to seedlings, developed few roots and were transferred to infested soil with *F. solani* T-ICA04. The AgNPs were applied weekly and caused a curtailment of fungal growth after 30 days of treatment. In terms of limiting infection (antifungal activity) in the roots of *F. solani* infected plants AgNPs from *P. glandulosa* were found more potent. Additionally, the application of these AgNPs from *P. sericea* and *P. glandulosa* showed a significant increase of stomata conductance

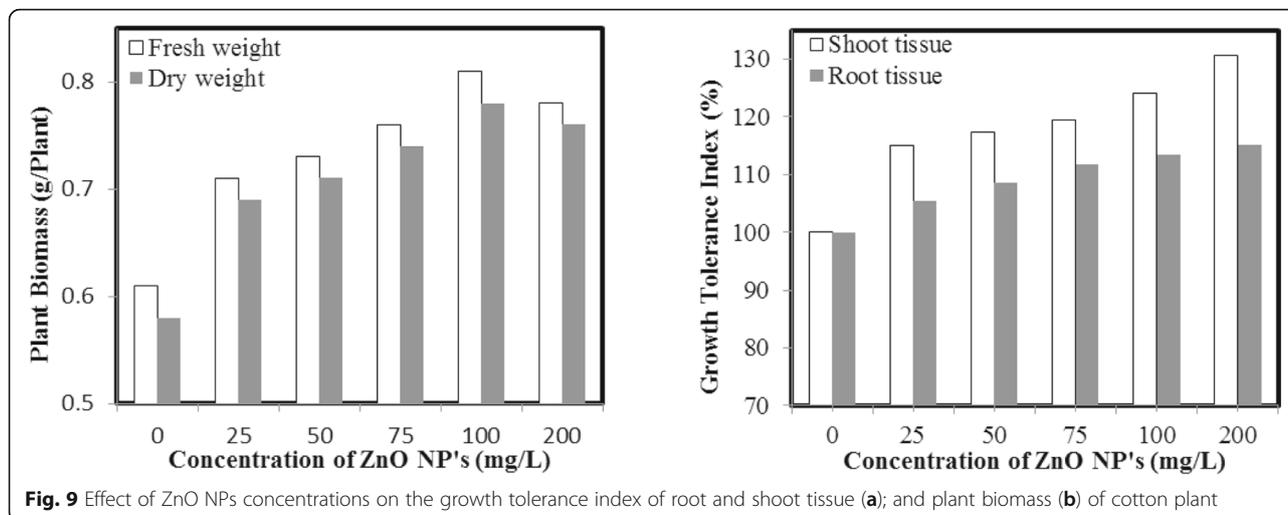
(gs), optimum quantum efficiency ( $F_v/F_m$ ), and the number of lateral roots in transgenic cotton, when compared with the control after 30 days of NPs exposure.

### Impact of metal oxide nanoparticles on plant height and biomass

Different concentrations of various nanoparticles and their time of exposure have both positive and negative impacts on various growth parameters, viz., plant height, root length, root and shoot biomass. The growth of cotton plants was increased with the increasing dose of ZnO NPs. The growth tolerance index was increased to 115.2% & 130.6% for root and shoot, respectively, at 200 mg·L<sup>-1</sup> ZnO concentration (Fig. 9). No toxicity was found even with a high dose of treatment. For different concentrations of ZnO (25 to 200 mg·L<sup>-1</sup>), fresh and dry weight was improved from 113.7 to 125.4% & 115.7 to 131%, respectively (Venkatachalam et al. 2017).

On the contrary, the decrease in biomass and plant height of conventional and transgenic cotton was observed with increased proportions of SiO<sub>2</sub> NPs (500, 1 000, 2 000 mg·L<sup>-1</sup>). No significant decrease was detected up to 500 mg·L<sup>-1</sup>, but the dose treatment of 2 000 mg·L<sup>-1</sup> resulted in less plant height for both non-transgenic and transgenic cotton (Nhan et al. 2014).

The treatment of various concentrations of Fe<sub>2</sub>O<sub>3</sub> NPs showed no significant difference in plant height between the conventional and Bt-cotton plants of the control



groups. However, in conventional cotton, the enlargement in the root (length, hair) with the enhancement in root biomass was observed (30.8 to 41.2%) in comparison to the control. But in transgenic cotton, there was no significant change observed in plant height, root (length, hair, & biomass) or shoot biomass (Nhan et al. 2016a).

In both conventional and transgenic cotton, the growth parameters were negatively affected by the increasing concentration of CuO NPs accounted for the toxic effect of NPs. The harmful effect on the root growth was more prominent than the shoot growth and was attributed to greater accumulation of CuO NPs in the root. More than 50% reduction in root biomass (hair, length) was observed at higher dose treatment (1 000 mg·L<sup>-1</sup>). Plant height, root length, root and shoot biomass were not much affected at low concentrations of CuO NPs (10 mg·L<sup>-1</sup>) than those at high concentration (200 & 1 000 mg·L<sup>-1</sup>). CuO NPs caused a significant reduction in root lengths of 45.79 and 42.80% at higher concentrations of 200 and 1 000 mg·L<sup>-1</sup>, respectively (Nhan et al. 2016b).

The treatments of the transgenic and non-transgenic cotton plants with different concentrations of CeO<sub>2</sub> NPs (100, 500, 2 000 mg·L<sup>-1</sup>) had no effect on plant height and shoot biomass as compared with the control. But a significant difference in plant biomass was obtained between both types at higher concentrations (500 and 2 000 mg·L<sup>-1</sup>). Only the root biomass of transgenic cotton was reduced at 100 and 500 mg·L<sup>-1</sup> concentrations of CeO<sub>2</sub> NPs (Li et al. 2014). The exposure methodology and impact of various metal/metal oxide nanoparticles on different cotton varieties have been summarized in Table 2.

## Discussion and future directions

Advances in the field of biotechnology helped in the development of new cotton species (e.g., Bt 29317, Ipt-cotton) with better characteristics than their conventional counterpart (e.g., Jihe 321). To some extent, this facilitated to achieve goals like better production, disease, and stress resistance, etc. Further, the assistance was provided by nanotechnology in the form of metal or metal-oxide nanoparticles that helped in the improvement of physiological parameters in various plant parts like leaves, shoots, and roots. These nanoparticles were having easy access to the various parts of the cotton plant through its transportation system. However, it has been found that fewer NPs are being transported from roots to shoots in Bt cotton as compared with the conventional cotton, indicating the difficulty in translocation through vascular tissues which is an energy intensive process. The possible mechanism behind this could be the more energy consumption in the formation of Bt-toxin as compared with the transportation of NPs (Zhao et al. 2012). This was evident from the TEM images of the area of cross-sections of leaves, shoots, and roots, etc., and displayed an accumulation of nanoparticles in the form of black dots. The presence of these nanoparticles affected the availability of nutrient elements in different plant parts, and thus altered the plant growth parameters like height and biomass. A correlation was found between the nutrient content/growth parameters and the altered dose concentration of nanoparticles. Some nanoparticles were more effective with lower dose concentrations; however, others displayed their potency with higher values of dose treatments. Comparative studies were also performed to explore the concentration-dependent effect of nanoparticles on the growth parameters of both transgenic and conventional cotton. Some growth parameters were significantly affected by the increased/decreased concentrations

of the nanoparticles in transgenic cotton; while the same were remain unaltered in the conventional plants or *vice versa*. This suggested that various nanomaterials have different effects on different cultivars. However, the mechanism underlying this difference is unknown so far and thus, provide a wider scope for further studies in this extensive area. Growth parameters like phytohormone concentrations, cellular enzyme activities and Bt-toxin levels were effectively maintained via careful applications of different concentrations of nanoparticles. This comprehensive review will help the researchers in selecting the correct dose concentrations of the selected nanoparticle to achieve the desired targets in the selected cotton species. The limitations of the studied nanoparticles, which are discussed in this literature review can be easily overcome by experimenting with varied concentrations of other metal or metal oxide nanoparticles, and their effects on the physiological parameters of cotton plants can be explored. The ultimate solution to the problem of improvement of cotton fiber yield can only be realized via exploring the impacts of various nanoparticles on cotton plants.

## Conclusion

Bt-transgenic and conventional cotton have a great affinity to metal and metal oxide nanoparticles (MNPs) as evident from their uptake and distribution into various plant parts. Plant growth factors, viz., nutrient plant content, phytohormone levels, cellular enzyme activities, Bt toxin expression, etc., exhibited altered sensitivities towards varied dose treatments of different NPs. Some NPs significantly affected the plant growth parameters in roots, while others showed their sensitivities in leaves and shoots, but to different extents. The beneficial effects of these nanoparticles can be explored to accomplish the necessities of agricultural fields; however, the first emphasis should be laid on understanding the mechanism of interaction between various growth parameters and NPs. Meticulous usage of these NPs may ascertain fruitful improvements in controlling morphological variations, and thus the overall growth and production of cotton fibers.

## Abbreviations

Bt: *Bacillus thuringiensis*; NPs: Nanoparticles; QD: Quantum dot; SOD: Superoxide dismutase; POD: Peroxidase; CAT: Catalase; CNT: Carbon nanotube; DNA: Deoxyribose nucleic acid; miRNA: MicroRNA; ICP-MS: Inductively coupled plasma mass spectrometry; TEM: Transmission electron microscopy; IAA: Indole-3-acetic acid; t-ZR: Trans-zeatinriboside; GA: Gibberellic acid; ABA: Abscisic acid; MNP: Metal oxide nanoparticle; GR: Glutathione reductase; DHAR: Dehydroascorbate reductase; MDA: Malondialdehyde; POX: Proline oxidase; PAGE: Polyacrylamide gel electrophoresis; RAPD: Random amplified polymorphic DNA

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## Authors' contributions

All authors contributed equally in collecting and conceptualizing the literature material related to the manuscript topic, helping shape the manuscript and providing critical feedback. The author(s) read and approved the final manuscript.

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## Declarations

## Ethics approval and consent to participate

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## Consent for publication

All data used for writing the manuscript has been cited with proper references. Images used for the review manuscript are cited with reference under a Creative Commons Attribution 4.0 International License.

## Competing interests

The authors have no competing interests.

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