Chapter 9
Nanotechnology for Crop Improvement

Pragati Misra, Pradeep Kumar Shukla, Krishnendu Pramanik, Sanghdeep Gautam and Chittaranjan Kole

Abstract Nanotechnology has the potential to reinforce the mission toward evergreen revolution by enhancing agricultural productivity with limited inputs. It is emerging as a paradigm shift and evolving as a promising tool to begin a new era of precise farming techniques and therefore may provide a possible solution for crop improvement, even in challenging environments. Employment of engineered nanoparticles (ENPs), whether carbon- or metal-based, may be the future solution to increase crop production for feeding the fast-growing world population. This chapter provides an overview of the current knowledge on the effects of nanoparticles for

P. Misra (✉) · S. Gautam
Department of Molecular and Cellular Engineering,
Jacob School of Biotechnology and Bioengineering,
Sam Higginbottom Institute of Agriculture, Technology and Sciences,
Allahabad 211007, India
e-mail: pragatimisra3@rediffmail.com; pragati.misra@shiats.edu.in

S. Gautam
e-mail: sanghdeep007@gmail.com

P.K. Shukla
Department of Biological Sciences, School of Basic Sciences,
Sam Higginbottom Institute of Agriculture, Technology and Sciences,
Allahabad 211007, India
e-mail: pradeepshuklak@yahoo.co.in

K. Pramanik
Department of Agricultural Biotechnology, BC Agricultural University,
Mohanpur 741252, India
e-mail: kpramanik7@gmail.com

C. Kole
Bidhan Chandra Agricultural University, Mohanpur 741252, West Bengal, India
e-mail: ckole2012@gmail.com

Present Address:
C. Kole
Jacob School of Biotechnology and Bioengineering,
Sam Higginbottom Institute of Agriculture, Technology and Sciences,
Allahabad 211007, India

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crop improvement. Overriding influences of different carbon, metal-based and metal oxide nanoparticles on different growth parameters (number of seminal root initiation, root elongation, shoot length, number of seeds, flowers and its quality), ultimately leading to increased plant biomass and yield have been presented. Throughout this chapter, the beneficial role of nanoparticles through enhanced seed germination, increased root and shoot length, fruit and crop yield, and substantial increase in vegetative biomass of seedlings and plants in many crops including maize, wheat, alfalfa, soybean, mustard, mung bean, tomato, potato, lettuce, spinach, onion, peanut, borage, Arabidopsis, cluster bean, and bitter melon is highlighted. The experimental evidences for enhancement of secondary metabolites through nanoparticle treatment under in vivo and in vitro conditions are presented. Although implementation of nanotechnology for agriculture sustainability via enhanced yield, biomass, and secondary metabolite is at juvenile stage, world will witness exceptional and unparalleled prospective of nanoparticles for invigorating agriculture in many ways. It is evident that more investigations are urgently required to know the type of nanoparticle, size, concentration, and mode of application to enable its application on large scale for crop improvement.

Keywords Nanoparticles · Agriculture · Yield · Biomass · Secondary metabolite

9.1 Introduction

Agriculture in the twenty-first century is facing manifold challenges for producing more food by addressing the problems of rapidly growing global population, unpredictable climate change, decreasing agricultural productivity, variable labor force, and increased urbanization. These problems seem to intensify ferociously by 2050 when we have to feed the population of over 9 billion. Agriculture as a source of food, feed, and fiber has always been increasingly important in a world of diminishing resources and with an ever-increasing global population (Brennan 2012).

To counteract this scenario, the agriculture-dependent countries have to adopt more advanced technologies, labor-saving practices, and methods. Nanotechnology is a promising tool and has the potential to foster a new era of precise farming techniques and therefore may emerge as a possible solution for these problems. Nanotechnology may increase agricultural potential to harvest higher yields in an ecofriendly way even in the challenging environments (Sugunan and Dutta 2008). This is expected to be a potential complement to plant molecular breeding and genetic engineering besides traditional plant breeding in the near future.

The potential of nanotechnologies in agricultural practices is still unrevealed and needs to be explored to a large extent. Nanotechnologies can benefit agriculture in multiple dimensions. Introduction of nanomaterial in agriculture aims particularly to increase the yield through optimized nutrient management, minimal loss of nutrient in fertilization, and reduced application of plant protection chemicals (Chen et al. 2013). Engineered nanomaterials (ENMs) can alter agronomic traits including plant growth, biomass production, physiological parameters that directly influence yield, and quality of produce of plants grown to full maturity (Gardea-Torresdey et al. 2014) (Fig. 9.1). The use of nanoparticles in plant science is attracting attention of the researchers due to its beneficial effects (Zheng et al. 2005). Nanoagrotechnology currently focuses target farming involving the use of nanoparticles (NPs) with unique properties to boost crop and livestock productivity (Scott and Chen 2002; Batsmanova et al. 2013).

Nanotechnology has the potential to improve global food production and food quality through increased plant protection, detection of diseases, monitoring plant growth, and reduced waste for strengthening agriculture sustainability (Frewer et al. 2011; Gruère et al. 2011; Biswal et al. 2012; Ditta 2012; Prasad et al. 2012; Sonkaria et al. 2012; Pérez-de-Luque and Hermosín 2013).

The application of nanotechnology in agriculture also involves precise delivery of fertilizers to increase plant growth and yield (Liu et al. 2006; Naderi and Danesh-Shahraki 2013), sensors for monitoring soil quality, and pesticides for pest and disease management (Liu et al. 2008).

In recent years, scientists have been trying to reveal the potential of the nanobiotechnology as a promising tool in the field of crop improvement through an array of experiments in different directions. The role of NPs, either metal-based (MBNPs) or carbon-based (CBNPs), has been documented in many research articles in relation to their uptake, internalization, translocation, persistence, and effect on growth and overall development in many plant species of different commercial importance. Some of these studies have shown beneficial role on plant growth and

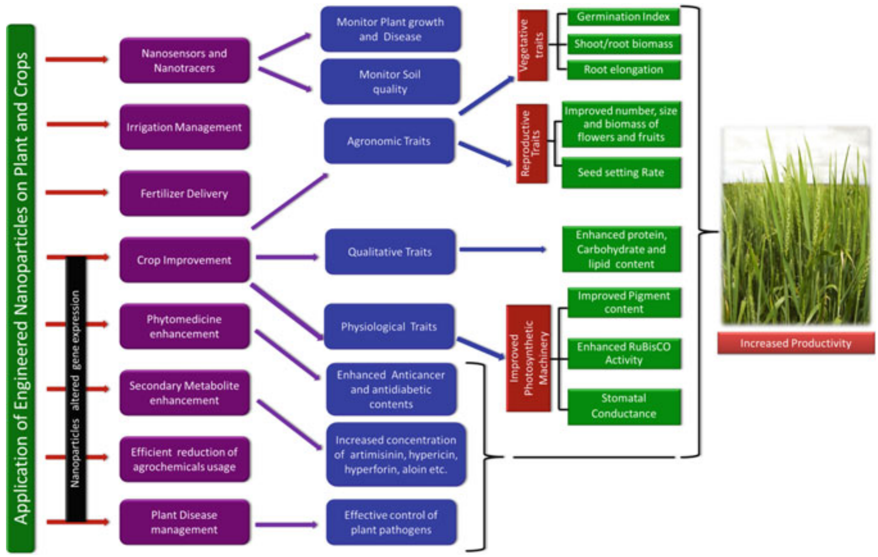

Fig. 9.1 Positive effects of engineered nanoparticles on plants and crops finally leading to increased productivity

development upon exposure to NPs (Lu et al. 2002; Shah and Belozerova 2009; Sharon et al. 2010; Sheykhbaglou et al. 2010; Kole et al. 2013; Razzaq et al. 2016), while others show negative effects (Lee et al. 2008, 2010, 2012, 2013; Zhu et al. 2008; Barrena et al. 2009; Kumari et al. 2009; Stampoulis et al. 2009; Yin et al. 2011).

The beneficial role of NPs has been evidenced through the successful demonstration of enhanced percentage in seed germination (Lu et al. 2002; Khodakovskaya et al. 2009; Nair et al. 2010; Gopinath et al. 2014), increased root and shoot length (Fig. 9.2) (Liu et al. 2005; Hafeez et al. 2015), increased fruit yield (Kole et al. 2013), enhanced phytomedicine content (Kole et al. 2013), and a substantial increase in vegetative biomass of seedlings and plants in many crops including wheat (*Triticum aestivum*), maize (*Zea mays*), ryegrass (*Lolium perenne*), alfalfa (*Medicago sativa*), soybean (*Glycine max*), rapeseed (*Brassica napus*), tomato (*Solanum lycopersicum*), radish (*Raphanus sativus*), lettuce (*Lactuca sativa*), spinach (*Spinacia oleracea*), onion (*Allium cepa*), pumpkin (*Cucurbita maxima*), cucumber (*Cucumis sativus*), and bitter melon (*Momordica charantia*). Augmentation in many biochemical parameters related to plant growth and development has also been reported that facilitates enhanced photosynthetic activity and nitrogen-use efficiency in many crops including soybean (Ngo et al. 2014), spinach (Hong et al. 2005; Zheng et al. 2005; Yang et al. 2006; Gao et al. 2008; Klaine et al. 2008; Linglan et al. 2008), and peanut (*Arachis hypogea*) (Liu et al. 2005; Prasad et al. 2012). However, the molecular mechanism underlying overall

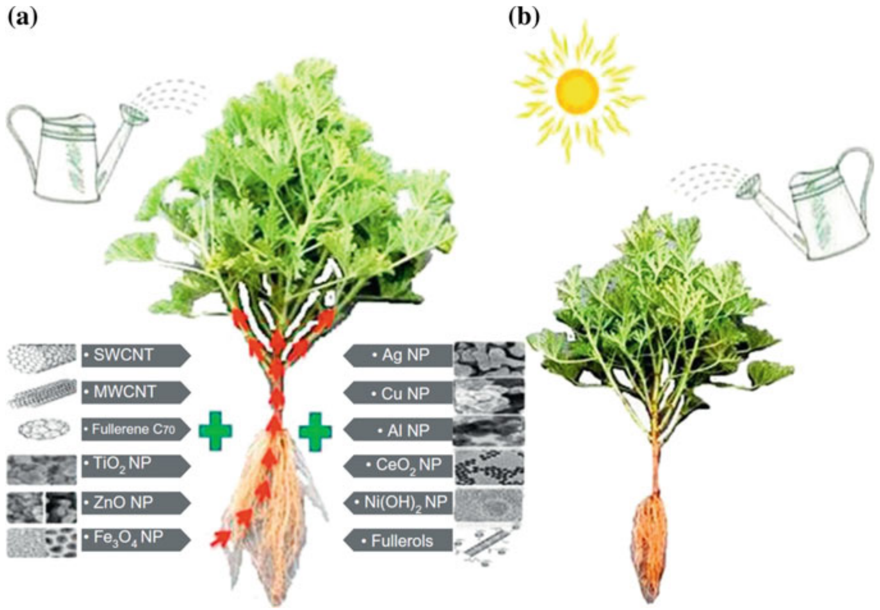

Fig. 9.2 Changes in plant growth on exposure to different NPs **a** plant treated with NPs in addition to other basic requirements, showing enhanced growth in comparison with **b** showing plant growth in the absence of NPs (adapted from Mishra et al. 2014)

development documented in various plant species on the application of different NP formulations is yet to be deciphered through rigorous experiments. Although the use of NPs in crop improvement is still under investigation, we can expect to see its use on a regular basis in farmers' fields in the near future.

Particular types of NPs in low concentrations have not displayed any harmful effect to plants but instead are capable of activating specific physiological and molecular responses. For example, TiO_2 nanoparticles (0.25–4 %) are able to promote photosynthesis and nitrogen metabolism in spinach and, therefore, improve the growth of the plants (Zheng et al. 2005; Klaine et al. 2008). Khodakovskaya et al. (2009) demonstrated that relatively low doses (10–40 μg/mL) of multiwalled carbon nanotubes (MWCNTs) were able to penetrate thick seed coats, increase germination, and stimulate growth in tomato plants (Khodakovskaya et al. 2009, 2012). However, the effects of NPs are influenced by the media and the mode of application. Zhu et al. (2008) studied the uptake of 20-nm-sized iron oxide NPs (Fe_3O_4 NPs) in pumpkin and lima beans (*Phaseolus lunatus*). Under hydroponic conditions, indications of magnetic NPs were found in roots, stems, and leaves, while the plants growing in soil or in sand did not show any signs of magnetic NPs confirming no particle uptake.

During the past few years, there has been extensive interest in applying NPs to plants for agricultural management (Nanotechnology in Agriculture and Food 2006; Torney et al. 2007; Khodakovskaya et al. 2009, 2012; Ashrafi et al. 2010; Serag et al. 2011b, 2012a; Husen and Siddiqi 2014; Razzaq et al. 2016). The genetic implications of such NP-induced positive changes have been validated through investigations on enhanced mRNA expression and protein level in spinach (Gao et al. 2008) by nano-TiO_2, generational transmission of fullerol through seeds in rice (Lin et al. 2009), and changes in gene expression at plant and cellular levels in tomato and tobacco (Khodakovskaya et al. 2009, 2012; Villagarcia et al. 2012) by MWCNTs.

9.2 Demonstration of Nanoparticle-Mediated Enhancement of Plant Biomass and Yield

Despite the high potential of NPs in enhancing plant growth and development, only few reports are available which document the improvement in agronomic traits in terms of an increased leaf and pod dry weight and improved grain yield in soybean when exposed to nanoiron oxide (Sheykhbaglou et al. 2010), borage (*Borago officinalis*) by silver nanoparticles (SNPs) (Seif et al. 2011), bitter melon by fullerols (Kole et al. 2013), mung bean by silver and $PbNO_3$ (Najafi and Jamei 2014), and wheat by SNPs (Razzaq et al. 2016). The role of ENPs of varying size and concentration on plants is given in Table 9.1. ENPs may be classified into the metal (or nonmetal) and metal oxide nanoparticles. Most widely used ENPs examined in the field of crop improvement include nanoferrous/ferric oxides (Liu et al. 2005; Sheykhbaglou et al. 2010; Alidoust and Isoda 2013; Bakhtiari et al. 2015), nanosilver (Vakhrouchev and Golubchikov 2007; Sharma et al. 2012; Razzaq et al.

Table 9.1 Enhanced biomass, productivity, and yield of different plants through nanoparticle treatment

NPs	Optimum concentration	Plant	Effects	Reference
CNT_S, $MWCNT_S$, fullerols	50 and 200 μg mL^{-1}	Tomato	Plant height and number of flowers	Khodakovskaya et al. (2013)
	47.2 nM	Bitter melon	Fruit yield	Kole et al. (2013)
Ag NPs	50 ppm	Potato	Weight and yield of potato mini-tubers	Tahmasbi et al. (2011)
	60 ppm	Common bean, maize	Dry weight of root and shoot	Salama (2012)
	60 ppm	Borage	Seed yield	Seif et al. (2011)
	–	Basil	Seed yield	Nejatzadeh-Barandozi et al. (2014)
	25–50 ppm	Wheat	Growth and yield	Razzaq et al. (2016)
Au NPs	10 ppm	Indian mustard	Growth and seed yield	Arora et al. (2012)
	10 μg mL^{-1}	Arabidopsis	Root and shoot length, early flowering	Kumar et al. (2013)
	1000 μM	Flame lily	Vegetative growth	Gopinath et al. (2014)
Ti NPs	0.25 % w/v	Spinach	Fresh and dry weights	Yang et al. (2007)
	20 g L^{-1}	Wheat	Biomass and yield	Jaberzadeh et al. (2013)
Si, Pd, Au, and Cu NPs	0.013 and 0.066 % w/w	Lettuce	Shoot–root ratio	Shah and Belozerova (2009)
Nanocrystalline powders (Fe, Co, and Cu)		Soybean	Growth and crop yield	Ngo et al. (2014)
Iron oxide NPs	0.5–75 g L^{-1}	Soybean	Yield and quality	Sheykhbaglou et al. (2010)
	50 ppm	Mung bean	Biomass yield	Dhoke et al. (2013)
	0.04 % w/v	Wheat	Grain yield, spike weight, protein content	Bakhtiari et al. (2015)

(continued)

Table 9.1 (continued)

NPs	Optimum concentration	Plant	Effects	Reference
	300 ppm and He, Xe irradiation 10 min	Pea	Growth and yield	Al Sherbini et al. (2015)
Nanoanatase-TiO_2 NPs Nano-TiO_2 Rutile (TiO_2)	0.25–4 %	Spinach (naturally aged)	Plant dry weight	Zheng et al. (2005)
	0.01 and 0.03 %	Maize	Content of carotenoids and anthocyanin	Morteza et al. (2013)
ZnO NPs	20 ppm 1 ppm	Mung bean Gram	Root and shoot biomass	Mahajan et al. (2011)
	20 mg L^{-1}	Tomato	Growth and biomass production	Panwar et al. (2012)
	1000 ppm	Peanut	Stem and root growth, high yield	Prasad et al. (2012)
	500, 1000, 2000, 4000 ppm	Mung bean	Dry weight	Patra et al. (2013)
	1.5 ppm	Chick pea	Shoot and dry weights	Burman et al. (2013)
	10–40 µg ml^{-1}	Onion	Seed yield	Laware and Raskar (2014)
	50 mg	Mung bean	Biomass weight	Jayarambabu et al. (2015)
	10 mg L^{-1}	Cluster bean	Shoot length, root area, and plant biomass	Raliya and Tarafdar (2013)
Silicon dioxide NPs	15 kg ha^{-1}	Maize	Growth and growth parameters	Yuvakumar et al. (2011)
	–	Tomato	Antioxidant system	Haghighi et al. (2012)
	–	Squash	Antioxidant system under salt stress condition	Siddiqui et al. (2014)
CuO NPs	500 mg Kg^{-1}	Wheat	Biomass	Dimkpa et al. (2012)
	30 ppm	Wheat	Growth and yield	Hafeez et al. (2015)

(continued)

Table 9.1 (continued)

NPs	Optimum concentration	Plant	Effects	Reference
CeO_2 NPs	2000 mg L^{-1} 4000 mg L^{-1}	Maize, alfalfa, soybean	Shoot growth and biomass	López-Moreno et al. (2010)
	125, 250, 500 mg Kg^{-1} soil	Wheat	Yield and nutritional parameter	Rico et al. (2014)
$CaCO_3$ NPs	–	Mung bean	Seedling growth and biomass	Yugandhar and Savithramma (2013)

2016), nanogold (Arora et al. 2012; Kumar et al. 2013), nanocopper (Ngo et al. 2014), nano zinc oxide (Prasad et al. 2012; Burman et al. 2013; Raliya and Tarafdar 2013), nanotitanium oxide (Zheng et al. 2005; Morteza et al. 2013; Feizi et al. 2013), nanocerium oxide (Rico et al. 2014, 2015), carbon nanotubes, and fullerols (Khodakovskaya et al. 2009, 2013; Villagarcia et al. 2012; Kole et al. 2013).

9.2.1 Carbon Nanomaterials

Among the NPs, carbon nanomaterials (CNMs) have acquired a significant place due to their unique mechanical, electrical, chemical, and thermal properties. Moreover, the information regarding the effect of nanomaterial such as carbon nanotubes (CNTs) on plant physiology and development is very limited and needs to be explored.

To achieve the goals of "nanoagriculture," exhaustive research on the effects of nanotubes on seed germination and development of seedlings of valuable agricultural plant species is required. Various studies have been reported showing contradictory results depending on the size and concentration of NPs and the species of plants. Canas et al. (2008) reported that CNTs enhanced root elongation in onion and cucumber, whereas it significantly reduced the root length in tomato. The tomato seeds, exposed to multiwalled CNTs (MWCNTs), showed significant enhancement in seed germination and increase in vegetative biomass (Khodakovskaya et al. 2009, 2011). Studies have proposed that the enhanced water uptake efficiency (Khodakovskaya et al. 2009) due to the surface chemistry of carbon nanotubes (Villagarcia et al. 2012) and activation of water channel proteins (aquaporins) (Khodakovskaya et al. 2009) resulted in increase in seed germination and plant growth.

Serag et al. (2013) reported that the diameter and length of single-walled CNTs (SWCNTs) are the major restraining features for their effective penetration into the plant cell wall. Many researchers have shown the penetration of chemically shortened SWCNTs into both the cell wall and the cell membrane of tobacco

(*Nicotiana tabacum*) and periwinkle (*Catharanthus roseus*) (Liu et al. 2009; Serag et al. 2011a, b, 2012a, b).

Some studies conducted on CNMs evidently indicated their potential to enhance plant growth, nutrient uptake, seed germination, and fruit yield/quality. Khodakovskaya et al. (2009), for the first time, demonstrated that CNTs can penetrate thick seed coat and support water uptake inside tomato seeds. Molecular mechanisms of CNT-induced water uptake inside plant seeds are not clear and necessitate further investigation. Researchers also found that the nanotubes migrate through the vascular tissues in the plant. However, such positive effects of CNTs on seed germination and biomass could have noteworthy economic importance for agriculture, horticulture, and the energy sector, such as for production of biofuels (Mondal et al. 2011). In another report, Srinivasan and Saraswathi (2010) showed enhanced seed germination and growth rate in tomato seeds when exposed to CNTs. Gonzalez-melendi et al. (2008) reported the use of carbon nanoparticles as smart treatment delivery system in plants.

Khodakovskaya et al. (2012) investigated the potential of CNTs as regulators of seed germination and growth of tobacco cell culture. Varying concentrations (5–500 μg/ml) of MWCNTs were used for enhanced growth of tobacco cell culture, and results confirmed 55–64 % increase over control. Improved cell growth (16 % increase) was observed under the effect of activated carbon (AC) at low concentrations (5 μg/mL), whereas intense inhibition in the cellular growth was recorded at higher concentrations (100–500 μg/mL). Correlation between the stimulation of growth of cells exposed to MWCNTs, the upregulatory genes participating in cell division/cell wall formation, and water transport was established. Unique molecular mechanism is involved in the regulation of cell division and plant growth by CNTs and is associated with the activation of water channels (aquaporins) and specific genes indulged in the regulation of cell division and extension (Khodakovskaya et al. 2012).

Recently, Tiwari et al. (2014) observed the beneficial role of pristine MWCNTs in enhanced growth and biomass of maize seedlings at low concentrations by enhancing water absorption and concentrations of the essential nutrients Ca and Fe, but their effectiveness could be diminished by high concentrations of ions/polar species in the medium. They proposed a plausible utilization of CNTs for optimizing water transport in arid zone agriculture and for improving crop biomass yields.

Kole et al. (2013) reported the effects of a carbon-based nanoparticle, fullerol, on agroeconomic traits in bitter melon. The uptake, translocation, and accumulation of fullerol were confirmed through bright-field imaging and Fourier transform infrared spectroscopy. Varied effects (positive and non-consequential) were recorded on yield, plant biomass, fruit yield, and component characters, when seeds were treated with five varying concentrations (0.943, 4.72, 9.43, 9.88, and 47.2 nM) of fullerols (Fig. 9.3). Increase in biomass yield by 54 % and water content in plants by 24 % over control was observed after treatment with fullerol, whereas fruit length, fruit number, and fruit weight increased up to 20, 59, and 70 %, respectively, that resulted in the improvement of up to 128 % in fruit yield. The accumulation of fullerol in tissues of root, stem, petiole, leaf, flower, and fruit at particular concentrations was stated as the causal factor for increase in biomass and fruit yield.

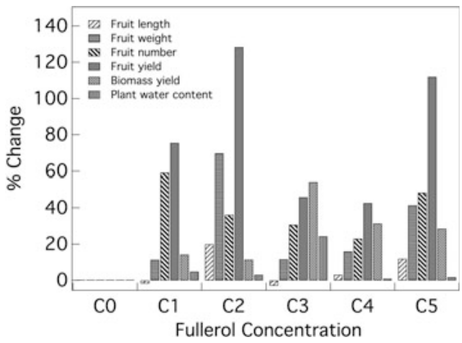

Fig. 9.3 Effect of fullerol at five concentrations (C_1–C_5) in comparison with control on changes (in %) in six plant characters. C_0 denotes control (without fullerol), and C_1–C_5 denote five fullerol concentrations (0.943, 4.72, 9.43, 9.88, and 47.2 nM), respectively (adapted from Kole et al. 2013)

Husen and Siddiqi (2014) proposed the potential of fullerene, C_{60}, and CNTs to improve the water retention capacity, biomass, and fruit yield in plants up to ~118 %. These findings can be taken into account as a remarkable achievement of agri-nanotechnology in the field of crop improvement.

Research-based evidences can confirm that CNMs may be considered as a promising nanoscale amendment for significantly suppressing microbial pathogens, improving plant growth, and promoting crop quality/yield. However, it is a challenge for research community to unravel the exact mechanism and dose-dependent pattern of CNMs on plant growth and development in physiological, metabolic, and molecular perspectives.

9.2.2 Metal-Based Nanoparticles

As already mentioned, nanoparticles can have both growth-promoting and harmful effects on crops. Toward this effort, different studies have been conducted to analyze the effect of metal-based nanoparticles (MBNP) such as silver (Ag), gold (Au), aluminum (Al), and copper (Cu) on plants. Application of MBNPs has been found to improve germination (Barrena et al. 2009; Yuvakumar et al. 2011), enhance growth and physiological activities (Shah and Belozerova 2009; López-Moreno et al. 2010; Salama 2012; Razzaq et al. 2016), increase water and fertilizer-use efficiency (Yuvakumar et al. 2011), inhibit abscission of reproductive organs of plant (Seif et al. 2011), and stimulate nodule formation (Taran et al. 2014).

Effects of super-dispersive iron, cobalt, and copper nanocrystalline powders on germination rate, plant growth, crop yield, and quality of soybean (Vietnamese species DT-51) were examined (Ngo et al. 2014). The soybean seeds treated with an extra low nanocrystalline dose (not more than 300 mg of each metal per hectare) were sowed on experimental landfill plot farming area of 180 m^2. Cobalt nanopowder exhibited a better germination effect than nanoscaled iron and copper wherein all the growth parameters exceeded the control ones with crop yield surpassing the control by 16 %.

SNPs have remarkable uses in crop production. Variable responses of SNPs have been reported by different researchers in various plants. SNPs affect plant growth by significantly inducing changes at physiological and molecular levels. Soaking of cotton seeds in SNPs produced favorable effects and reduced the amount of fertilizers applied through roots by half (Vakhrouchev and Golubchikov 2007). SNPs have catalytic effects (Ma et al. 2010), decreasing the abscission of reproductive organs of plants (Seif et al. 2011), and are known to increase chlorophyll contents (Sharma et al. 2012). Razzaq et al. (2016) reported positive effect of SNPs on wheat growth and yield when applied to soil. Exposure to 25–50 ppm SNPs significantly increased plant height and fresh and dry weights over the control. Application of SNPs at low concentrations (25 and 50 ppm) positively affected the number of seminal roots (Fig. 9.4). Favorable effects of soil-applied SNPs on growth might be due to more bioavailability and accumulation in plants, thereby stimulating growth. The highest grain number per spike, 100-grain weight, and yield per pot were recorded at 25 ppm SNPs (Fig. 9.5) (Razzaq et al. 2016). Sensible use of SNPs to soil can, therefore, improve the yield of wheat. However, further investigations are needed to explore precise concentration, suitable mode, and time of application to realize growth- and yield-enhancing potential of SNPs for wheat and other crops in an ecofriendly manner. Similarly, SNPs at 50 ppm was observed to increase remarkably total chlorophyll, chl-a, chl-b, root fresh weight in mung bean plants (Najafi and Jamei 2014). Similar effects of SNPs including increased fresh weight, root and shoot length, vigor index, and chlorophyll contents of seedlings of Indian mustard (*Brassica juncea*) were also reported (Sharma et al. 2012). Increased weight and yield of potato mini-tubers were reported by the

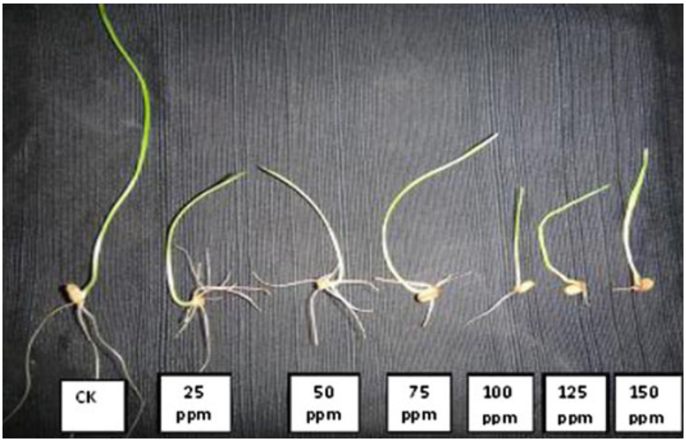

Fig. 9.4 Effect of varying concentrations of Ag NPs on the number of seminal roots in wheat (adapted from Razzaq et al. 2016)

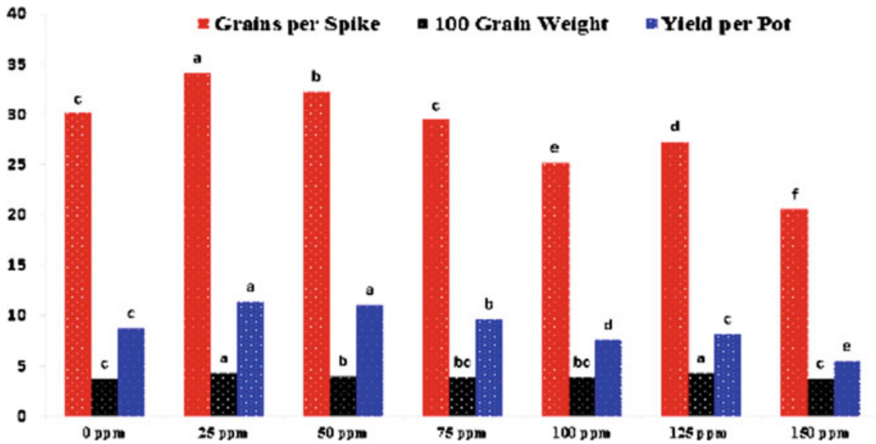

Fig. 9.5 Impact of different concentrations of soil-applied Ag NPs nanoparticles on crop yield in wheat (adapted from Razzaq et al. 2016)

application of 50 ppm nanosilver in combination with nitrogen and nitroxin (Tahmasbi et al. 2011).

Another study by Seif et al. (2011) studied the effect of nanosilver and silver nitrate on abscission and seed yield in borage plants. Varying concentrations (20, 40, and 60 ppm) of SNPs were used. Improvement in the seed yield was observed on raising the concentration of nanosilver from 20 to 60 ppm. In contrast, increasing the concentration of silver nitrate from 100 to 300 ppm led to the reduction in seed yield. Similar results were reported by Salama (2012) in common bean (*Phaseolus vulgaris* L.) and maize when exposed to five levels of SNPs (20, 40, 60, 80, and 100 ppm). Effects of SNPs on plant growth parameters such as shoot and root lengths, leaf surface area, chlorophyll, carbohydrate, and protein contents were investigated. The results revealed that lower concentrations (20, 40, and 60 ppm) of SNPs had positive impact on the growth of the plantlets, and in contrast, higher concentrations (80 and 100 ppm) of SNPs showed inhibitory effect. Enhancement in shoot and root lengths, leaf area, chlorophyll, carbohydrate, and protein contents was reported in both common bean and maize on exposure to increasing concentration of SNPs from 20 to 60 ppm.

SNPs also have strong antimicrobial effects and therefore effectively control and avoid plant diseases. SNPs at concentrations of 0.5–1000 ppm cause faster growth of plants and control pathogens (Ashrafi et al. 2010). Increased germination and enhanced seedling growth were reported by SNPs (Lu et al. 2002) that act as growth simulators (Sharon et al. 2010). SNPs can facilitate the plant to delay the senescence provoked by reactive oxygen species (ROS) formed due to oxidative stress. Oxidative stress induced senescence followed by 2,4-D triggered ROS generation in mung bean was suppressed by the application of 100 μL of SNPs (Karuppanapandian et al. 2011). The studies suggest that MBNPs enhance plant growth and development. Ag NPs increased root length in maize and cabbage (*Brassica oleracea* var. *capitata*) plants in comparison with $AgNO_3$ (Pokhrel and Dubey 2013).

The potential of Au nanoparticle as a promising tool to enhance seed yield was reported by Kumar et al. (2013). Total seed yield increased by threefold over the control when Arabidopsis (*Arabidopsis thaliana*) seeds were exposed to 10 μg/mL of Au NPs (24 nm size). Au NP treatment at both 10 and 80 μg/mL concentrations significantly improved seed germination rate, vegetative growth, and free radical scavenging activity. A significant correlation was found between expression of key plant regulatory molecules, microRNAs (miRNAs), seed germination, growth, and antioxidant potential of Arabidopsis upon Au NP exposure (Kumar et al. 2013). In another report, the role of gold NP on yield of Indian mustard was investigated. The positive effect of Au NPs was visible on various growth- and yield-related parameters including plant height, number of branches, stem diameter, number of pods, and seed yield. The average leaf area was not affected; however, there was an increase in the number of leaves per plant. At 10 ppm concentration of Au NP, the seed yield increased optimally, whereas the reducing sugar and total sugar content increased up to 25 ppm concentration of Au NP treatment (Arora et al. 2012).

Au NPs showed positive impact on seed germination and vegetative growth in flame lily (*Gloriosa superba*), an endangered medicinal plant (Gopinath et al. 2014). Two concentrations of Au NPs (500 and 1000 μM) were used for seed treatment. Seed germination rate (enhanced by 39.67 % than control) and vegetative growth of flame lily were significantly affected at 1000 μM concentration. Seed coat of Au NP-treated seeds showed increased permeability facilitating the entry of H_2O and O_2 into the cells and uptake of gold ions, which interacts with embryo cells. It stimulates the GA_3 activity resulting in the expression of α-amylase enzyme in the aleurone cell layer. α-amylase breaks down starch into simple sugar and accelerates the germination process (Fig. 9.6). More prominent effect on number of leaves, root initiation, and node elongation was observed in seeds exposed to

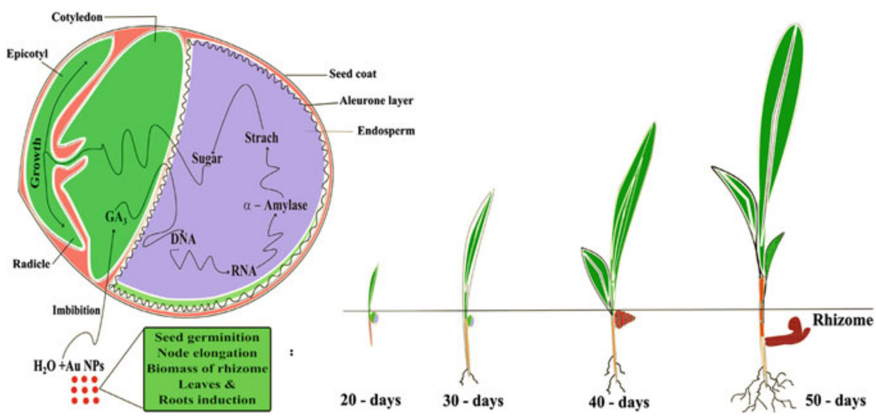

Fig. 9.6 Schematic representation of positive impact of Au NPs on *Gloriosa superba* resulting in enhanced germination, leaf and root initiation, node elongation, and biomass of rhizome (adapted from Gopinath et al. 2014)

1000 μM Au NPs (Fig. 9.7). Total biomass and fresh weight of flame lily rhizome were also increased by 2.40- and 5.18-fold after treatment with 500 and 1000 μM Au NPs respectively as compared to control.

Shah and Belozerova (2009) reported the influence of Si, Cu, Au, and Pd NPs on growth of lettuce plants after 15 days of incubation, which resulted in an increased shoot/root ratio compared to control.

Jaberzadeh et al. (2013) reported increased stem elongation, biomass, ear mass, seed number, yield, gluten and starch content, and early flowering in wheat when 20 gL^{-1} Ti NPs were applied through foliar spray as compared to their bulk material. Similarly, Yang et al. (2007) reported augmented fresh and dry weights as well as contents of total *N*, chlorophyll, and protein in leaves when spinach seeds were soaked in a solution of Ti NPs.

Iron is one of the essential elements for plant growth and plays an important role in the photosynthetic reactions. Iron is known to activate several enzymes contributing to RNA synthesis and enhance photosystem performance (Malakouti and Tehrani 2005). Soybean is sensitive to iron deficiency though different genotypes differ in iron consumption efficiency. Grain yield in soybean was increased by the application of iron in low-iron soils (Sheykhbaglou et al. 2010). Almeelbi and Bezbaruah (2012) reported the significant enhancement in plant growth and biomass of spinach by Fe NPs in hydroponic solution. Remarkably, Fe content in spinach leaves, stem, and root was increased by 11 to 21-fold. Amuamuha et al. (2012) also investigated the effect of different concentrations of Fe NPs (1, 2, and 3 gL^{-1}) on pot marigold (*Calendula officinalis*) at three growth stages, viz. stem elongation, flowering, and after harvest. Results revealed that the highest flower yield and essential oil percentage were achieved when 1 gL^{-1} Fe NPs were applied at the stem elongation stage.

Hafeez et al. (2015) examined the potential of copper NPs to increase growth and yield of wheat. The growth and yield were significantly increased in comparison with control when Cu NPs (at 10, 20, 30, 40, and 50 ppm) were applied to soil

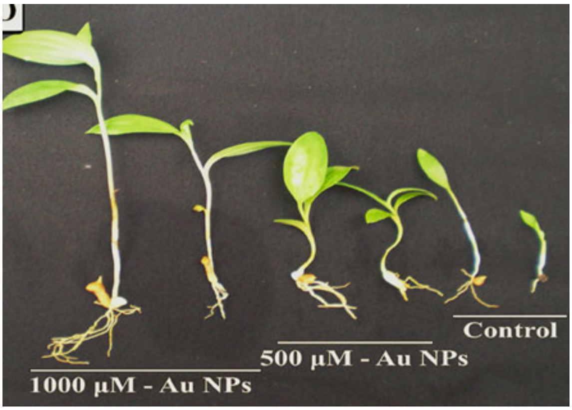

Fig. 9.7 Effect of Au NPs at two concentrations (1000 and 500 μM) as compared to control on *G. superba* leaf and root initiation, node elongation, and biomass of rhizome for duration of 40 days (adapted from Gopinath et al. 2014)

in pots. However, the significant increase in the chlorophyll content, leaf area, number of spikes/pot, number of grains/spike, 100-grain weight, and grain yield was observed at 30 ppm Cu NPs. Results revealed that the enhanced growth and yield in wheat due to Cu NPs are concentration-dependent and further experimentation is required for the dose optimization and mode of application to maximize the yield of wheat.

In a recent study, Khan et al. (2016) investigated the effect of nine types of metal nanoparticles including monometallic and bimetallic alloy nanoparticles [Ag, Au, Cu, AgCu (1:3), AgCu (3:1), AuCu (1:3), AuCu (3:1), AgAu (1:3), AgAu (3:1)] on seed germination and biochemical profile of milk thistle (*Silybum marianum*) plant. Significant increase in seed germination was reported upon treatment with all the NPs suspensions as compared to control and was recorded the highest for Ag NPs suspension. Significant effect was also observed on the biochemical profile of milk thistle on exposure to metal NPs. Enhancement in total protein content, DPPH, peroxidase, and superoxide dismutase activity was recorded for the first week and then declined as the time progressed. Among all the NPs being used, the maximum enhancement was observed with Ag NPs. Significant potential of different monometallic and bimetallic NPs on medicinal plant species was proposed.

Taran et al. (2014) studied the effect of colloidal solution of molybdenum nanoparticles (Mo NPs) on the microbial composition in the rhizosphere of chick pea (*Cicer arietinum*). It was reported that exposure of chick pea seeds with the combination of colloidal solution of Mo NPs (8 mg/L) and microbial preparation stimulated the development of "agronomically valuable" microflora. Combined treatment resulted in increase in number of nodules per plant by four times, while single treatment with colloidal solution of Mo NPs increased the number of nodules twofold as compared to control.

9.2.3 Metal Oxide Nanoparticles

Enormous studies on the effect of metal oxide NPs on varying parameters such as germination, growth, and yield of plants have been documented (Hong et al. 2005; Liu et al. 2005; Zheng et al. 2005; Sheykhbaglou et al. 2010; Mahajan et al. 2011; Alidoust and Isoda 2013; Laware and Raskar 2014; Bakhtiari et al. 2015; Razzaq et al. 2016). In spinach, enhanced chlorophyll formation, photosynthesis, and plant dry weight was observed when exposed to TiO_2 NPs (Hong et al. 2005; Zheng et al. 2005). In another study, increase of 58.2 and 69.8 % in fresh and dry weights, respectively, and substantial rise in chlorophyll content, Rubisco activity, and photosynthetic rate were recorded in spinach when treated with anatase TiO_2 NPs (Linglan et al. 2008). Low dosage of nanosized TiO_2 enhanced seed germination indices of fennel (Feizi et al. 2013). Germination percent was highly improved following exposure to 60 ppm nanosized TiO_2. Nano-TiO_2 was suggested to be used for improvement of seed germination of fennel. Morteza et al. (2013) reported that nano-TiO_2 plays a significant role in increasing pigments in maize—higher amounts of pigments were obtained when sprayed with nano-TiO_2 at reproductive stages of plant, which finally led to increase in yield. Thus, an application of nano-TiO_2 can foster an increase in crop yield, especially in maize.

Chutipaijit (2015) investigated, for the first time, on the effect of TiO_2 NPs on regeneration frequency in aromatic rice (*Oryza sativa*) (cultivar KDML105). Application of TiO_2 NPs at an optimum concentration, i.e., 25 mg L^{-1} showed elevated green spots, plant regeneration, and the ratio of seedling number to the number of regenerated calli. Therefore, application of TiO_2 NPs showed positive response on regeneration efficiency in rice, probably due to improved plant metabolism (Fig. 9.8) (Chutipaijit 2015). Improved nitrate reductase activity and stimulated antioxidant system by mixture of TiO_2 and SiO_2 NPs were reported in soybean (Lu et al. 2002).

Many researchers have reported the positive effect of iron NPs on photosynthetic potential, growth, biomass, and yield in crop plants (Liu et al. 2005; Sheykhbaglou et al. 2010; Amuamuha et al. 2012; Alidoust and Isoda 2013; Bakhtiari et al. 2015). Researchers have shown that the application of nanoferric oxide (nano-Fe_2O_3) significantly affected nutrient absorption in peanut and resulted in increased growth and photosynthesis. The photosynthate and iron transfer rate to the leaves of peanut was promoted by nano-Fe_2O_3 as compared to other organic materials and iron citrate treatments (Liu et al. 2005). In another study, the effect of Fe_2O_3 NPs when applied to soybean via foliar and soil route was investigated. The enhancement in root elongation and photosynthetic potential were significantly higher when Fe_2O_3 NPs were administered to plants by foliar spray as compared to soil route, which may be due to precipitation of Fe ions (Alidoust and Isoda 2013).

A study was conducted to investigate the effect of nanoiron oxide particles on soybean yield and agronomic traits (Sheykhbaglou et al. 2010). Nanoiron oxide was applied at 5 levels (0, 0.25, 0.5, 0.75, and 1 gL^{-1}). It was observed that exposure to

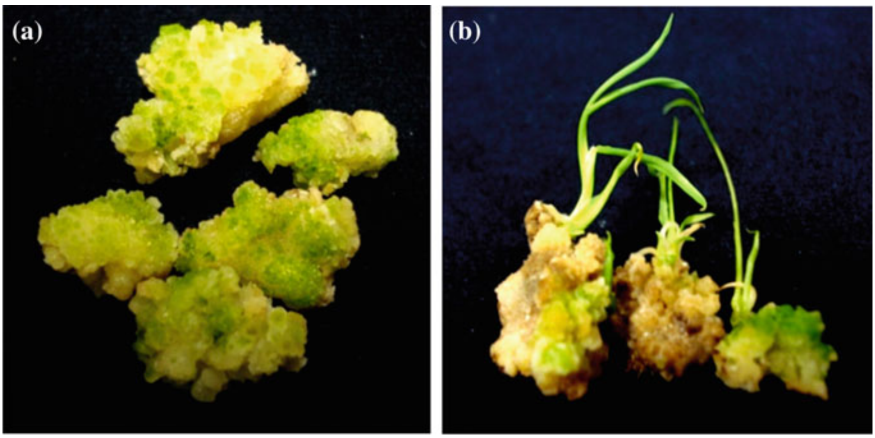

Fig. 9.8 Regeneration frequency of rice callus on medium **a** without treatment **b** after treatment with 25 mg L^{-1} TiO_2 nanoparticles (adapted from Chutipaijit 2015)

nanoiron oxide at 0.75 gL^{-1} concentration caused improvement in leaf and pod dry weight. Grain yield was augmented to 48 % as compared to control, by the treatment of 0.5 gL^{-1} nanoiron oxide. Thus, it can be suggested that 0.5 gL^{-1} nanoiron oxide treatment resulted in increased total yield by an increase in leaf and pod dry weight (Table 9.2). Bakhtiari et al. (2015) reported that spraying of iron oxide NPs solution in varying concentrations (0, 0.01, 0.02, 0.03, and 0.04 %) significantly affected the wheat growth and yield. The measured trait included: spike weight, 1000-grain weight, biological yield, grain yield, and protein content of wheat. The highest values of spike weight (666.96 g), 1000-grain weight (37.96 g), biological yield (8895.0 kg/ha), grain yield (3776.5 kg/ha), and protein content (16.44 %) were achieved at 0.04 % iron oxide concentration. It was evident that spraying time and concentration are the major factors affecting all the measured traits. Racuciu and Creanga (2007) observed similar results on plant growth in maize at early ontogenetic stages after application of magnetic NPs coated with tetramethyl ammonium hydroxide (TMA-OH).

In most of the studies, the effect of ZnO NPs on plant growth depends on concentration. Root elongation in soybean was reported at 500 mg L^{-1}, whereas higher concentrations resulted in the reduction of root length. No effect on seed germination in soybean was observed at even higher concentration (4000 mg L^{-1}) (López-Moreno et al. 2010). Mahajan et al. (2011) demonstrated the effect of nano-ZnO particles on the growth of plant seedlings of mung bean and chick pea (*C. arietinum*). ZnO NPs showed concentration-dependent growth pattern in mung bean and chick pea seedlings. The maximum growth was found at 20 ppm for mung bean and 1 ppm for chick pea seedlings, and beyond this concentration, the growth was inhibited (Mahajan et al. 2011).

The effect of ZnO NPs on growth, flowering, and seed productivity of onion was studied (Laware and Raskar 2014). Six-month-aged onion bulbs (cut in half portions) were subjected to pot plantation and sprayed three times with varying concentrations (0, 10, 20, 30, and 40 μg ml^{-1}) of ZnO NPs at the interval of 15 days. The growth parameters including plant height and number of leaves per plant were assessed at the time of flowering, and the seed yield parameters such as number of seeded fruits per umbel, seed yield per umbel, and 1000-seed weight were determined at the time of harvest. Seed samples obtained from treated plants along with control were tested for germination and early seedling growth. Results revealed that

Table 9.2 Effect of different concentrations of nanoiron oxide on some agronomic traits in soybean (adapted from Sheykhbaglou et al. 2010)

Nanoiron oxide (g/l)	Pod dry weight (g)	Leaf + pod dry weight (g)	Yield (g m^{-2})
0	0.41^{b}	32.35^{b}	60.94^{b}
0.25	0.42^{ab}	42.35^{ab}	76.78^{ab}
0.5	0.44^{ab}	42.45^{ab}	90.22^{a}
0.75	0.48^{a}	45.84^{a}	88.33^{a}
1	0.45^{ab}	42.32^{ab}	80.39^{ab}

Means with different letters at each column have statistically difference at 5 % level the plants treated with ZnO NPs at the concentration of 20 and 30 $\mu g\ ml^{-1}$ showed better growth and flowered 12–14 days earlier in comparison with control. Treated plants showed significantly higher values for seeded fruits per umbel, seed weight per umbel, and 1000-seed weight over control plants. It was confirmed that high-quality seed along with all other inputs (size, number, etc.) was responsible for enhancement in final yield. These results indicated that ZnO NPs can reduce flowering period in onion by 12–14 days and produce high-quality healthy seeds (Laware and Raskar 2014). The increase in vegetative growth in onion might be related to the fundamental role of ZnO in maintenance and protection of structural stability of cell membranes (Welch et al. 1982) and involvement in protein synthesis, functioning of membrane, cell elongation, as well as tolerance to various environmental stresses (Cakmak 2000). Prasad et al. (2012) suggested variable response of peanut seeds toward the treatment at various concentrations of both bulk $ZnSO_4$ and nanoscale ZnO particles. Absorption of ZnO NPs by plants was more as compared to $ZnSO_4$ bulk. Results also revealed the beneficial effects of NPs in enhancing plant growth, development, and yield in peanut at lower doses (1000 ppm), but at higher concentrations (2000 ppm), ZnO NPs were detrimental just as the bulk nutrients. Pod yield per plant was 34 % higher in plants treated with ZnO as compared to chelated bulk ZnSO4. Similar findings were reported by Raliya and Tarafdar (2013) on shoot length, root length, root area, and plant biomass in cluster bean (*Cymopsis tetragonoloba*), when 10 ppm ZnO NPs were foliar-sprayed on leaf of 14-day-old plant. Significant improvement was observed in shoot length (31.5 %), root length (66.3 %), root area (73.5 %), and plant biomass (27.1 %) over control in 6weekold plants because of the treatment with ZnO NPs (Fig. 9.9; Table 9.3).

Fig. 9.9 Effect of ZnO NPs on growth of cluster bean (6 weeks old). Plant treated with *n* ZnO-nano zinc oxide at 10 ppm concentration exhibited maximum growth as compared to O ZnO-ordinary zinc oxide and control (adapted from Raliya and Tarafdar 2013)

Table 9.3 Effect of nano-ZnO and ordinary- ZnO on some phenological parameters of 6 weeks aged cluster bean plants (adapted from Raliya and Tarafdar 2013)

Treatment	Shoot length (cm)	Root length (mm)	Root area ($mm^{2)}$	Dry biomass (g^{-1})
Control	44.53	720.23	809.30	10.47
Ordinary ZnO	47.73	835.20	1241.47	11.60
Nano-ZnO	58.57	1197.70	1404.30	25.33
LSD ($p = 0.05$)	0.10	0.09	0.03	0.15

In another study, Kisan et al. (2015) examined the effect of nano-ZnO on the leaf physical and nutritional quality of spinach. The spinach plants were sprayed with varying concentrations (0, 100, 500, and 1000 ppm) of ZnO NPs after 14 days of sowing. At the time of maturity (45–50 days), the leaf physical parameters such as leaf length, leaf width, and leaf surface area were noted and nutritional parameters such as protein, carbohydrate, fat, and dietary fiber contents in leaf samples were determined. When 500 and 1000 ppm concentration of ZnO NPs were sprayed, increase in leaf length, width, surface area, and color of spinach leaves were recorded with respect to control. Similarly, elevated levels of protein and dietary fiber contents were observed in plants treated with ZnO NPs at the concentration of 500 and 1000 ppm in comparison with control leaf samples of spinach. It was proposed that the nanozinc oxide has a potential to be used as a biofortification agent for the improvement of protein and dietary fiber contents of spinach leaves and thereby reduces malnutrition.

Morales et al. (2013) assessed the impact of cerium oxide nanoparticles (CeO_2 NPs) on cilantro (*Coriandrum sativum*) plants grown in organic soil. Cilantro seeds were germinated, and plants were grown in organic soil treated with 0–500 mg kg^{-1} CeO_2 NPs for 30 days and analyzed by biochemical assays and spectroscopic techniques to determine the CeO_2 uptake, variations in macromolecules, and catalase (CAT) and ascorbate peroxidase (APX) activity. At 125 mg kg^{-1} concentration of CeO_2 NPs, plants produced longer roots and shoots, had a higher biomass production, and significantly increased in catalase activity in shoots and ascorbate peroxidase activity in roots. Furthermore, CeO_2 NPs downregulated the production of these defensive enzymes and altered the carbohydrates in shoots, signifying its role in changing the nutritional properties of cilantro. Thus, this study demonstrated the fertilizing effects of CeO_2 NPs, which helped plants to grow better. Although CeO_2 NPs produce stress in cilantro plants, at the same time they have antioxidant activity. In fact, CeO_2 NPs induce conformational changes within the plant (in the components of roots), visible in the spectra by vibrational shifting, but do not induce chemical reactions and substantial changes. From all these findings, positive effects of CeO_2 NPs on the development of plants were depicted.

In another study, Rico et al. (2014) examined the impact of CeO_2 NPs on agronomic traits, yield, and nutritional parameters in wheat. Wheat was grown in soil administered with 0, 125, 250, and 500 mg of $nCeO_2$ kg^{-1} (control, $nCeO_2$-L, *n*CeO_2-M, and *n*CeO_2-H, respectively). The cultivated grains and tissues were studied for contents of minerals, fatty acids, and amino acids. Results revealed that *n*CeO_2-H improved plant growth, shoot biomass, and grain yield by 9.0, 12.7, and 36.6 %, respectively, relative to control Fig. 9.10). *n*CeO_2 modified S and Mn storage in grains. *n*CeO_2-L modified the amino acid composition and increased linolenic acid by up to 6.17 % but decreased linoleic acid by up to 1.63 %, compared to control. These findings evidenced the potential of CeO_2 NPs to modify crop physiology and food quality. *n*CeO_2 treatment caused a 6-day delay in spike formation and physiological maturity in wheat compared to control. Li et al. (2011) suggested that the extended period for spike formation and physiological maturity might be the reason for improved yield in wheat. More recently, it has been reported by Marchiol et al. (2016) that plants treated with *n*CeO_2 and *n*TiO_2 had a longer vegetative period than the control. This fact as such may not be undesirable. In fact, a longer vegetative phase may support higher biomass and grain yield as plants have comparatively more time to produce more photosynthetically active leaves and therefore more photosynthates (Dofing 1995).

The impact of nano-SiO_2 on the characteristics of seed germination was studied in tomato. Results revealed that the treatment with *n*SiO_2 significantly enhanced seed germination potential. Exposure of *n*SiO_2 increased seed germination percentage, mean germination time, seed germination index, seed vigor index, seedling fresh weight, and dry weight. An increase in germination parameters by the use of *n*SiO_2 may be inductive for the growth and yield of plants. Nonetheless, the present findings offer a scope to search out the mechanism of interaction between nanosilica and plants, since *n*SiO_2 could be used as a fertilizer for the crop improvement (Siddiqui and Al-Whaibi 2014).

The use of nanofertilizers in agriculture is an important approach to enhance agronomic production and ensure global food and nutritional security (Liu and Lal 2015; Servin et al. 2015). In the context of applicability of nanoparticles as nanotoxicants or nanonutrients, Liu et al. (2016) stated that manufactured NPs were not

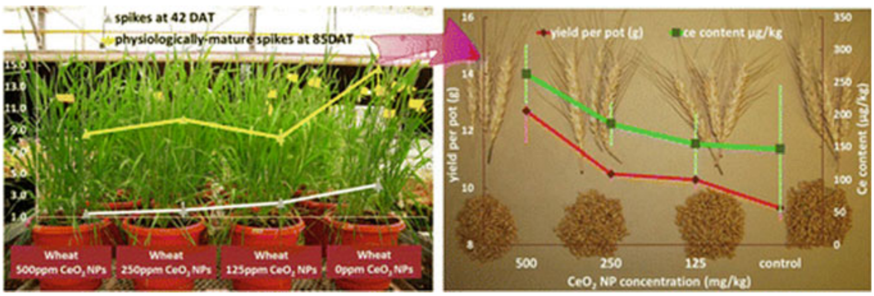

Fig. 9.10 Yield response of wheat to different concentrations of *n*CeO_2. Wheat was cultivated to grain production in soil amended with 0, 125, 250, and 500 mg of *n*CeO_2 kg^{-1} (control, *n*CeO_2-L, *n*CeO_2-M, and *n*CeO_2-H, respectively). NCeO_2-H resulted in improved plant growth, shoot biomass, and grain yield by 9.0, 12.7, and 36.6 %, respectively, as compared to control. Ce content in roots increased with increased *n*CeO_2 concentration (adapted from Rico et al. 2014)

at all times more toxic than other chemical species comprising the same elements. MnOx NPs and FeOx NPs stimulated the growth of lettuce seedlings by 12–54 % and were found less toxic than their ionic counterparts. Fe or Mn NPs can significantly improve plant growth and has promising role as nanofertilizers for increasing agronomic productivity (Liu et al. 2016).

9.2.4 Iron Oxide Nanoparticle in Combination with Irradiation

The effect of the presowing laser irradiation (He–Ne) combined with different concentrations of iron NPs on growth and yield of pea was investigated (Al Sherbini et al. 2015). Leaf area, dry weight per plant, chlorophyll content, Fe and Mn concentration, pod protein, pod number, and yield per feddan (0.42 ha) were determined. Research findings indicated that treatment of seeds with He–Ne laser irradiation for 10-min exposure time combined with 300 ppm iron oxide NPs improved all the tested parameters significantly. It was concluded that separate or combined He–Ne laser irradiation at 10 min and 300 ppm of iron oxide NPs gave the best growth parameters and the highest yield, compared to the control.

9.3 Nanoparticle-Mediated Enhancement of Secondary Metabolites

A plant cell produces two types of metabolites: Primary metabolites are involved directly in growth and metabolism, viz. proteins, carbohydrates, and lipids, whereas secondary metabolites are considered as the end products of primary metabolism, viz. phenolics, flavonoids, alkaloids, resins, quinones, essential oils, lignins, tannins, steroids, terpenoids, etc.

Plant secondary metabolites are organic substances that are not directly involved in the normal plant growth, development, or reproduction; rather, they play some vital role in various signaling cascades, defense mechanism against microorganisms, etc. Secondary plant products are considered for their vital role in the survival of the plant in its ecosystem, time and again protecting plants against pathogen attack, insect attack, mechanical injury, and other types of biotic and abiotic stresses (Hartmann 2007). It has been documented in various research articles that most of these plant secondary metabolites have some beneficial role in the human body, so these are considered as phytomedicines. Secondary metabolites, also known as natural products or phytochemicals, are responsible for medicinal properties of plants to which they belong. Classification of secondary metabolites is based on the chemical structure, composition, their solubility in various solvents, or their biosynthetic pathway. They are mainly classified into three major groups: terpenoids, alkaloids, and phenolics (Kabera et al. 2014).

Plants offer a great diversity of bioactive small molecular metabolites that are potentially valuable as pharmaceuticals, nutraceuticals, and agrochemicals. Plant crude extract contains various novel bioactive constituents such as phenolics, flavonoids, alkaloids, resins, quinones, steroids, and terpenoids, which are responsible for the reduction of ionic compounds to bulk metallic NPs (Aswathy Aromal and Philip 2012). Primary and secondary metabolites are also reported to be involved in the synthesis of ecofriendly nanosized particles.

The complete level of secondary metabolites is generally low in many medicinally important plants. In the search for alternatives to enhance the production of desirable medicinal compounds in plants, nanotechnological approach, specifically ENPs are found to have great potential as a supplement to traditional agriculture.

Nowadays, researchers are developing novel techniques which facilitate the plants in the improvement of its innate functions. Nanoparticles are empowered with unique physicochemical properties and have the potential to boost the plant metabolism (Giraldo et al. 2014). Galbraith (2007) and Torney et al. (2007) reported the use of engineered NPs to deliver DNA and chemicals into plant cells. This research area offers new possibilities in plant biotechnology to target-specific genes' manipulation and expression in the specific cells of the plants.

9.3.1 Enhancement of Secondary Metabolites Through Nanotreatment In Vivo

Several strategies have been conducted to improve the yields of secondary metabolites also known as natural products or phytochemicals in plants. Only few studies reported the enhancement of secondary metabolite on treatment with NPs under in vivo condition, whereas the effects of different NPs have been reported on plant growth and metabolic function (Nair et al. 2010; Krishnaraj et al. 2012). The same concentration of individual NP may cause effects in diverse directions and ranges on different variables. Hence, selection of appropriate concentration of nanoparticle is essential for recognizing higher benefits for a target agroeconomic trait.

9.3.1.1 Enhancement of Phytomedicines

Kole et al. (2013) observed varied effects of seed treatment with five concentrations of fullerol on the content of five phytomedicines in bitter melon fruits. Contents of two anticancer phytomedicines, namely cucurbitacin B and lycopene, were enhanced by 74 and 82 %, at 9.88 and 47.2 nM fullerol, respectively. Antidiabetic phytomedicines, charantin, and insulin contents were augmented up to 20 and 91 %, when the seeds were treated with 4.72 and 9.88 nM fullerol, respectively (Fig. 9.11).

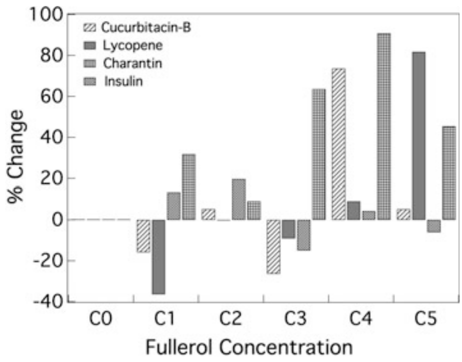

Fig. 9.11 Effect of fullerol at five concentrations (C_1–C_5) in comparison with control (C_0) on changes (in %) in the contents of four phytomedicines in bitter gourd. C_0 denotes control (without fullerol), C_1–C_5 denote five fullerol concentrations (0.943, 4.72, 9.43, 9.88, and 47.2 nM, respectively) (adapted from Kole et al. 2013)

9.3.1.2 Enhancement of Gum and Resins

Significant improvement in the gum content and its viscosity was reported in cluster bean seeds at crop harvest when the leaf of 14-day-old plant was foliar-sprayed with 10 mg L^{-1} ZnO NPs. Improved growth parameters and gum content might be due to adsorption of NPs on plant surface and taken up by the plants through natural nano- or microscale openings (Raliya and Tarafdar 2013).

9.3.1.3 Enhancement of Essential Oil

Amuamuha et al. (2012) recorded the effect of varying concentrations and time of nanoiron foliar application on the essential oil of pot marigold. Four concentrations (0, 1, 2, and 3 gL^{-1}) of iron NPs were used for spraying at different stages (foliar application at stem initialize, flowering, and after the first and second harvest). Significant influence of spraying time (growth stage) on the essential oil percent was observed at the first harvest and the essential oil yield at the third harvest. Similarly, nanoiron concentrations showed significant effect on the yield of essential oil at the first harvest. The highest percentage (1.573 %) of essential oil was reported when nanoiron was applied at the early stage (stem initialized) led to the maximum yield of essential oil (2.397 kg ha^{-1}) in the flower. The lowest essential oil percentage (0.981 %) was recorded when nanoiron was applied at later stages (after the second harvest).

9.3.2 Enhancement of Secondary Metabolites Through Nanotreatment in Vitro

Plant kingdom is the potential source of agrochemicals, flavors, and pharmaceuticals, known as secondary metabolites that have several economic advantages. In this regard, plants can be considered to be the best, non-polluting chemical factories. The chemical industries are making enormous efforts to synthesize these products, but the success rate is still limited. Usually, secondary metabolites, a rich source of pharmaceuticals with defensive properties, are synthesized by plants when exposed to different elicitors and/or inducer molecules (Zhao et al. 2005a, b). An "elicitor" can be defined as chemicals or bioagents from various sources which initiates or progresses biosynthesis of specific compounds responsible for physiological and morphological changes in the target living organism, when provided in very low concentrations to a living cell system. In plants, the elicitors can trigger the physiological and morphological changes and phytoalexin accumulation (Zhao et al. 2005a, b). Nowadays, various biotic and abiotic elicitors are practiced to trigger and concentrate the secondary metabolites and cell volume in suspension culture (Rao and Ravishankar 2002).

Among the various strategies available to increase the levels of metabolite of interest, application of elicitors in suspension culture is mostly trusted and practiced strategy. Elicitors in a precise concentration can be administered at desirable time to the suspension culture, resulting in achieving the highest levels of metabolite in a short span of time (Mulabagal and Tsay 2004).

The phenomenal surface characteristics of NP attribute to its extraordinary and unique properties. By increasing the number of atoms on surface, there is an increase in total free energy, resulted in the alteration of material characteristics. Nanoparticles have the potential to be used as novel effective elicitors in plant biotechnology for the elicitation of secondary metabolite production (Fakruddin et al. 2012). Many researchers have studied the role of NPs as elicitors (Aditya et al. 2010; Asghari et al. 2012; Sharafi et al. 2013; Zhang et al. 2013; Ghanati and Bakhtiarian 2014; Raei et al. 2014; Ghasemi et al. 2015; Yarizade and Hosseini 2015). Effect of NPs on enhancement of secondary metabolites is furnished in Table 9.4. A number of studies have supported the possible role of NPs as elicitors for enhancing the expression level of genes related to the production of secondary metabolite (Ghasemi et al. 2015; Yarizade and Hosseini 2015).

Nanoparticles have successfully offered a new strategy in enhancing the secondary metabolite production. But still an in-depth and consolidate insight in research is required to elucidate the effects of NPs in production mechanisms of secondary metabolite production in medicinal plants.

Table 9.4 Effect of nanoparticles on enhancement of secondary metabolites

Elicitors	Plant cell culture	Elicited product	References
AgNPs	*Taxus chinensis* (Chinese yew)	Paclitaxel	Choi et al. (2001)
	Salvia miltirorrhiza (Chinese sage)	Tanshinone	Zhang et al. (2004), Zhao et al. (2010)
	Saussurea medusa (Saw-wort)	Flavonoid jaceosidin Hispidulin	Zhao et al. (2005a, b)
	Bacop amonnieri (Brahmi)	Total phenol content	Krishnaraj et al. (2012)
	Artemisia annua (Sweet sagewort)	Artemisinin	Zhang et al. (2013)
	Datura metel (Datura)	Tropane alkaloids atropine	Shakeran et al. (2015)
CoNPs	*Artemisia annua (Sweet sagewort)*	Artemisinin	Ghasemi et al. (2015)
	Calendula officinalis (Marigold)	Saponin	Ghanati and Bakhtiarian (2014)
Fullerol	*Momordica charantia* (Bitter melon)	Cucurbitacin B, lycopene, charantin, and insulin	Kole et al. (2013)
TiO_2NPs	*Aloe vera*	Aloin	Raei et al. (2014)
	Cicer arietinum (chick pea)	Phenolic and flavonoid compounds	AL-Oubaidi and Kasid (2015)
ZnONPs	*Hypericum perforatum* (St John's wort)	Hypericin Hyperforin	Sharafi et al. (2013)
FeO_xNPs	*Hypericum perforatum* (St John's wort)	Hypericin Hyperforin	Sharafi et al. (2013)

9.3.2.1 Enhancement of Terpenoids

Artemisia annua is a medicinal plant that produces artemisinin as one of the secondary metabolites, which is a sesquiterpene lactone. Artemisinin is used against malaria parasite (*Plasmodium falciparum* and *P. vivax*) (Snow et al. 2005), for treating different types of cancers such as leukemia, colon cancer, breast cancer, and small carcinomas in lungs (Lei et al. 2011). Artemisinin is produced in very low quantity in *A. annua*. Thus, there is a hike in the price of medicines made from artemisinin, particularly for people in developing countries, where malaria is widely prevalent. Being very expensive, it is not economical to synthesize it chemically. Till now, scientists have not achieved a commercial method to enhance artemisinin content in spite of its known valuable medicinal properties (Ferreira et al. 1995). The study by Zhang et al. (2013) highlighted the potential of nanosilver particles as a novel and effective elicitor in plant biotechnology for the production of plant secondary metabolites. Exposure of Ag-SiO_2 core–shell nanoparticles (Ag NPs) resulted in increased artemisinin content in the hairy root culture of *A annua.* Recent investigations have reported the potential of lipid nanoparticles for parenteral delivery and the augmentation of antimalarial potential of artemether, a derivative of artemisinin (Aditya et al. 2010). Influence of nanocobalt on the expression level of involved genes and content in *Artemisia* was examined (Ghasemi et al. 2015). Nanocobalt particles were used for the elicitation of artemisinin in the cell suspension culture of *A. annua*. qRT-PCR and HPLC were used for quantification of the expression levels of *SQS* and *DBR2* genes and artemisinin content in cell suspension culture, respectively. For this purpose, different concentrations (0.25, 2.5, and 5 mg L^{-1}) of nanocobalt particles were used and samples were analyzed after 8, 24, 48, and 72 h. The maximum increase (2.25-fold, i.e., 113.35 mg g^{-1} dw as compared to control) in artemisinin content was recorded when cells were exposed to 5 mg L^{-1} nanocobalt for 24 h. At the same time, suppressed expression of *SQS* and *DBR2* genes was observed. This decline in the expression of *SQS* and *DBR2* genes might be the cause of enhanced production of artemisinin content by high concentrations of the nanocobalt particles. The mechanism of the impact of nanocobalt on enhancing artemisinin content will be unstated with the expression analysis of all genes involved in artemisinin production (Ghasemi et al. 2015). However, to increase the production of a metabolite, enhancing the expression of particular one gene is not sufficient.

Yarizade and Hosseini (2015) examined the effect of nanocobalt and nanozinc (0, 0.25, 0.5, and 1 mg L^{-1}) on the expression levels of *ADS*, *DBR2*, *ALDH1*, and *SQS* genes at 8, 24, 48, and 72 h after treatment in the hairy root culture of *A. vulgaris*. It was reported that cobalt NP at 0.25 mg L^{-1} caused the maximum expression for all genes under investigation, whereas nanozinc particles at 1.0 mg/L caused the maximum gene expression. It was the first report for the use of NPs for increasing the expression level of genes related to artemisinin production. Potential application of nanozinc and nanocobalt oxide as elicitor to increase artemisinin production in biologic systems such as hairy roots was suggested. Nanocobalt was recommended as the better elicitor compared to nanozinc, since concurrent to the increase in the *ADS* upregulation; subsequently, it downregulates its antagonist, the *SQS* gene (Yarizade and Hosseini 2015).

Baldi and Dixit (2008) stated a slight increase in the artemisinin content of artemisia cell suspension upon the addition of yeast extract. This increase was credited to the presence of metal ions Co^{2+} and Zn^{2+}. The mechanism of nanoparticles as elicitors for enhancement in secondary metabolite content is still unrevealed, and more research is required (Zhao et al. 2005a, b).

9.3.2.2 Enhancement of Phenols

Aloe vera is an important medicinal plant from Aloaceae family with African origin. Among 300 *Aloe* species, *A. vera* is considered as an important medicinal plant in many countries (Reynolds 2004; Hasanuzzaman et al. 2008). *A.vera* contains different secondary metabolites, and the most important of them is aloin which is an anthraquinone. Aloin is the active component having medicinal property, displays antimicrobial activity against some bacteria and fungi, and possesses healing ability of skin burns, ulcer, and cutaneous injuries. Raei et al. (2014) investigated the effects of different abiotic elicitors including nano-Ag, nano-TiO_2, NH_4NO_3, and sucrose on cell suspension culture of *A. vera*. The induced calli by elicitors was collected at five intervals (6, 24, 48, 72, and 168 h) and have been analyzed by HPLC. Increased aloin production was recorded in 48 h after elicitation with Ag NPs, but this level was declined gradually with time and reached the control level. The decline might be related to the feedback of aloin on the gene expression, and increased production of aloin is the reason for reduced gene expression (Raei et al. 2014). TiO_2, when used as nanoelicitor, could increase the aloin content in 48 h after elicitation but reduced to a lower level, 8.8 %, than the control. The decline might be related to the toxic effect of nano-TiO_2 in the culture medium or impact of that NP on gene expression. However, both (nano-Ag and TiO_2) of the nanoelicitors enhanced the aloin content 48 h after treatment but after that decreased gradually.

In another study, Krishnaraj et al. (2012) investigated the effect of biologically synthesized SNPs on plant growth metabolism in *Bacopa monnieri* (Linn.) (Brahmi). Total phenol content was assayed in various parts of the plants grown in hydroponic solution, and enhanced total phenol content was recorded in plants treated with Ag NPs. Results revealed that treatment with biologically synthesized Ag NPs exerted a slight stress condition on the growth and metabolism of *B. monnieri*, and therefore, rise in phenol content is one of the mechanisms to mimic mild stress condition.

Enhancement of Polyketides

Hypericum perforatum is a well-known medicinal plant (Deltito and Beyer 1998). Extract of *H. perforatum* is widely used to treat mild-to-moderate depression (Dias et al. 1998). Hypericin and hyperforin are naphthodianthrones and prenylated acylphloroglucinols, respectively, placed under polyketides. Numerous elicitors for the production of hypericin and hyperforin in cell cultures of *H. perforatum* have been examined. Iron- and zinc-nano oxides were used as elicitors for the first time by Sharafi et al. (2013). Different concentrations of zinc- and iron-nano oxides (0, 50, 100, and 150 ppb) were used for the treatment, and samples were analyzed after 72 h. Hypericin and hyperforin were detected, identified, and quantified in cell suspension cultures of *H. perforatum* by HPLC. It was reported that zinc- and iron-nano oxides (100 ppb) promoted the hypericin and hyperforin production in cell suspension culture. In the cultures stimulated by zinc-nano oxide, the hypericin and hyperforin production reached to the maximum (7.87 and 217.45 μg g^{-1} dry weight, respectively), which were 3- and 13-fold higher than the control. The amount of hypericin and hyperforin was increased from 2.07 and 16.27 μg g^{-1} dry weight to 11.18 and 195.62 μg g^{-1} dry weight in cultures treated with iron-nano oxide. The cell cultures treated with zinc- and iron-nano oxides showed increased hyperforin production as compared to the hypericin production. It can be suggested that NPs can be appropriate candidates for elicitation studies of in vitro secondary metabolite production.

Jasmonate (JA), an important stress hormone, triggered various plant defense responses, along with the biosynthesis of defensive secondary metabolites (Menke et al. 2009). Nanoparticles may play an important role in regulating the expression of genes for jasmonate production in treated cells. Induced jasmonate production may be responsible for enhanced production of hypericin and hyperforin. Plant cell wall might act as a barrier for entry of any external materials including NPs. But with diameters less than the pore diameter of the cell wall, nanoparticles can pass through and reach the plasma membrane. They may also cross the membrane by using transport carrier proteins or ion channels. The NPs may bind with different organelles or interfere with the metabolic processes. Studies on the uptake mechanism, transportation, and binding sites of NPs in plant cells are required to elucidate the elicitation mechanism of these in vitro applied NPs for the enhancement of secondary metabolite production. However, higher concentrations of zinc- and iron-nano oxides (150 ppb) showed negative effects on hypericin and hyperforin production (Sharafi et al. 2013).

Enhancement of Flavonoids

Flavonoids and isoflavonoids are the most popular groups of secondary metabolites found in plants. Many legume seeds have been reported to be rich sources of these secondary metabolites (Heiras-Palazuelos et al. 2013). AL-Oubaidi and Kasid (2015) demonstrated the increased production of secondary metabolite (phenolic and flavonoid compounds) in gram on exposure to TiO_2 NPs under in vitro condition. Secondary metabolite contents in the callus were estimated qualitatively and quantitatively using HPLC and compared with the mother plant. TiO_2 NPs at varying concentrations (0.5, 1.5, 3, 4.5, 6) mg L^{-1} were used for an effective increase in secondary metabolites. The results revealed that the secondary metabolite concentration from callus embryo of gram increased to highly significant level at the concentrations of 4.5 and 6.0 mg L^{-1}. The HPLC outcomes confirmed the elevation in the secondary metabolite level under the effect of the TiO_2 NPs when compared with the mother plant.

In a very recent report, Khan et al. (2016) examined the effect of nine types of metal nanoparticles including monometallic and bimetallic alloy nanoparticles [Ag, Au, Cu, AgCu (1:3), AgCu (3:1), AuCu (1:3), AuCu (3:1), AgAu (1:3), AgAu (3:1)] on total phenolic and flavonoid contents in milk thistle plant. The sterilized seeds were soaked in NPs suspensions for 2 h and allowed to grow under in vitro condition. The experiment was conducted for 6 weeks, and samples for total phenolic and flavonoid contents were collected on weekly interval. NPs suspensions affected total phenolic and flavonoid contents in the plant in a different way. It was observed that the amount of phenolics and flavonoids did not show any correlation with the total dry mass of the plant. However, duration of the experiment significantly affected the amount of total flavonoids and phenolics in milk thistle. After 21 days presoaking of seeds in bimetallic alloy, enhanced whereas monometallic NPs suspensions, reduced phenolics and flavonoids content in milk thistle plantlets. After 28 days, Au and Cu NPs caused maximum total phenolic and flavonoid accumulation in milk thistle plants. Therefore, maximum effect on secondary metabolites was recorded with monomatellic NPs. Mainly three factors (size, surface area, and composition of NPs) played a significant role either singly or in combination.

Enhancement of Saponins

The effects of SNPs and methyl jasmonate (MeJA) on secondary metabolites of marigold were studied (Ghanati and Bakhtiarian 2014). When plants were exposed to SNPs, chlorophyll and carotenoid content decreased by 30–50 %, while MeJA increased both of these contents, whereas when plants were treated with 0.4 mM SNPs and 100 μM MeJA, saponin content in the plants augmented by 177 %. Significant reduction in the viability of HeLa cells was recorded when exposed to the extracts of marigold, and this decline was more evident in the plants exposed to MeJA and SNPs.

Enhancement of Phenyl Propanoids and Terpenoids

Aromatic constituents are derived from phenylpropane hydrocarbons. The major identified components of fennel oil are phenyl propanoids and terpenoids. Fennel is annual or biennial aromatic plant, which is widely grown in Mediterranean and some tropical regions, and it is used for herbal drug preparations. One of the major compounds of fennel volatile oil is *trans*-anethole, the amount of which is the major governing factor for the quality of fennel volatile oil (Billia et al. 2002; Gurdip et al. 2006; Chaouche et al. 2011).

Bahreini et al. (2015) analyzed the phytoconstituents of in vitro grown fennel plantlets in normal and nanoelicited (TiO_2 and SiO_2) conditions. A significant difference was observed among the metabolites of normal and elicited conditions. The major components of normal plant were anethole, fenchone and limonene and decane. Some identified constituents of TiO_2-elicited plant extract were dodecane, phytol, and phenol 2,4 *bis* (1,1 dimethyl ethyl), and the most frequent compound was octane. In plants elicited with SiO_2, benzoic acid, jasmonic acid, and hexadecanoic acid were detected as elicited plant components and the major compound was pyrrolidinone. Some of other accumulated metabolites, which appeared by elicitor inductions such as phytol and benzoic acid, can be used as pharmaceutical and industrial precursors (Bahreini et al. 2015).

9.4 Conclusions and Future Perspectives

There is a demanding need for agriculture to produce more output with less input. We are on the edge of time where we have to adopt modern agriculture techniques and technologies as conventional agricultural practices will not be sufficiently able to feed an ever-increasing population with changing climate, depleting resources, and shrinking landscape. Among most recent technical improvements in the field of agriculture, nanotechnology holds an eminent position in remodeling agriculture and food production to fulfill the demands in an efficient and cost-effective way.

Nanotechnology being studied since the last few decades is still in its premature phase of development. However, the whole course of action is very broad and being popularized day by day. Nanotechnology in combination with biotechnology has led to the rapid development of marketable formulations involving deployment of artificially designed nanoparticles for crop improvement. To restrict the indiscriminate use of excess pesticides and fertilizers in plants, nanoparticles are proved to be a gifted tool of this age. Many nanoparticles have been proved to have beneficial role in the case of plant biomass and yield improvement, whereas some nanoparticles have been reported to have a deleterious role regarding reactivity and toxicity in plants. Hence, we are supposed to be very careful during screening and selection of ENPs. Otherwise, they could become the source of potential threat to the whole ecosystem. The interactions between plant cell and nanoparticles modify gene expression in the plants that regulate the overall process of plant growth and development. Nanomaterials that could be used for accelerated plant growth give a new research insight for areas such as biofuels for which the total biomass is important for the final production and yield. Effect of nanoparticles depends on the experimental conditions, and it would change (positive effect to negative or vice versa) if condition varies. Such aspects would include the type of plants and nanomaterials, concentrations of the nanostructures, as well as their chemical and biologic surface functionalizations (Khodakovskaya et al. 2009). Racuciu and Creanga (2007) reported the stimulating effect on growth of maize plants when exposed to low concentrations of aqueous ferro fluid, while its higher concentrations induced an inhibitory effect. However, the future perspectives on nanobiotechnological approaches for the improvement of plant productivity will depend on an in-depth understanding of the molecular mechanisms accountable for the activation of germination potential of seed and plant growth when exposed to complex ENMs (Khodakovskaya et al. 2012).

Nowadays, nanobiotechnology industries are growing very rapidly; however, there is an urgent need to perform profound studies in this field in order to develop comparatively safe and ecofriendly nanoparticles in the long run. The widespread assessment of these ENPs in agri-food sector should also be carried out for public acceptance to prevent them from the unlike challenges as were faced by genetically modified organisms worldwide. The impact of nanotechnology in farmers' field is just in the beginning, but expectations for nanotechnology to help meet the challenges related to food productivity, environment sustainability, and even fossil fuel are still high.

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