Chittaranjan Kole · D. Sakthi Kumar
Mariya V. Khodakovskaya *Editors*

Plant Nanotechnology

Principles and Practices

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Editors
Chittaranjan Kole
Bidhan Chandra Agricultural University
Mohanpur 741252 West Bengal
India

and

Jacob School of Biotechnology and Bioengineering
Sam Higginbottom Institute of Agriculture, Technology and Sciences
Allahabad 211007
India

D. Sakthi Kumar
Graduate School of Interdisciplinary New Science, Bio Nano Electronics Research Center
Toyo University
Kawagoe
Japan

Mariya V. Khodakovskaya
Department of Applied Science
University of Arkansas at Little Rock
Little Rock, AR
USA

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Preface

Nanotechnology and nanomaterials are increasingly imparting its great influence in our life and environment. During the last two decades, significant amount of research has been conducted in nanotechnology focusing on their application in electronics, energy, mechanics, and life sciences including plant sciences. The impact of nanotechnology and nanomaterials is inevitable in the field of agriculture, and many researches are evidencing their potential in improving the food and agricultural systems through different approaches resulting in the enhancement of agricultural output and development of new food and food products, etc.

The early research investigations in this direction documented absorption, translocation, accumulation, and effects of nanomaterials, mostly metal-based and carbon-based, in several plants including crops. Many of these research studies evidenced for the potential utility of nanomaterials in crop improvement as demonstrated by enhanced germination and seedling parameters in rice, maize, wheat, alfalfa, soybean, rape, tomato, radish, lettuce, spinach, onion, pumpkin, and cucumber; and also enhanced nitrogen metabolism, chlorophyll content, and activities of several enzymes leading to enhanced photosynthesis in maize, soybean, peanut, tomato, and spinach.

There are many investigations reported on nanomaterial-induced improvement in agronomic traits including yield, biomass content, and content of secondary metabolites by direct treatment in soybean, bitter melon, and rice indicating the ability of the nanomaterials in modifying genetic constitution of plants. Nanomaterials have exhibited promise in targeted gene delivery for developing atomically modified plants—a safer and acceptable strategy in contrast to genetic engineering. Interestingly, generational transmission of nanomaterials has been documented in rice and bitter melon.

The usage of these nanomaterials can ultimately land in our food cycle and so a careful study and analysis is pertinent regarding their usage before putting these materials in actual use.

The spurt in the research in this interdisciplinary field that involves primarily the fusion of nanotechnology and plant science may lead to the creation of a new field as “Plantnanomics.”

Nanomaterials have also exhibited promise for precise and environmentally safe application of fertilizers and plant protection chemicals using nanoformulations besides plant disease management using nanosensors and nano-based diagnostic kits.

Some concerns have been raised about potential adverse effects of nanomaterials on biological systems and environment although carbon-based nanomaterials, in general, have been found to be safe in many instances.

The book “Plant Nanotechnology” comprises 15 chapters. Chapter 1 clearly lays out the foundation of the book by providing the overview of the concepts, strategies, techniques, and tools of nanobiotechnology and its promises and future prospects. Before using the nanomaterials, we should know its physical and chemical properties. Based on the properties, we can decide the use of the materials in different applications. Chapter 2 deals with the physical and chemical nature of the nanoparticles. After characterizing the nanomaterials, we can employ them in intended applications in plants. While doing that we should know how it could be applied and how we could detect and quantify the uptake of the nanomaterials, translocation, and accumulation. Chapter 3 is devoted to provide the information about the quantification of uptake, translocation, and accumulation of nanomaterials in plants.

For application of any materials anywhere, we should have a clear-cut knowhow, such as how it can be applied and what are the different ways. Chapter 4 describes various methods for using nanomaterials. After the usage of the nanomaterials, naturally we have to look for their impact on plants. The earlier indication of their impact can be assessed by the germination, seedling parameters, and physiological attributes. Chapter 5 deals with the assessment of the impact of nanomaterials on plant growth and development. Chapter 6 provides the information on the effects of nanomaterials on plants with regard to physiological attributes.

After laying a very good foundation toward the characterization and application of nanomaterials and their impact, in general, in plants, we are discussing on the response of plants to nanoparticles at molecular level including changes in gene expression (Chap. 7), and movement and fate of nanoparticles in plants and their generational transmission (Chap. 8).

Recent researches have shown that nanomaterials can be used for the improvement of yield of crops and quality. This finding will lead to the application of nanomaterials in agriculture. For shedding light on the use of nanomaterials in agriculture for different applications, Chap. 9 has been incorporated to elucidate the potential of nanomaterials for the enhancement of yield, plant biomass, and secondary metabolites. A highly promising application potential of nanomaterials for delivery of genetic materials has been deliberated in Chap. 10. Application of agrochemicals including fertilizer and plant protection chemicals using conventional methods leads to less effectivity and even pollution of plant products, soil, water, and air. In contract, use of nanomaterials can lead to precise and targeted delivery of these chemicals. Utilization of nanoparticles for delivery of fertilizers and for plant protection has been deliberated in Chap. 11 and Chap. 12, respectively. We have included another chapter (Chap. 13) to discuss the impact of the nanomaterials in soil-plant systems.

Use of nanomaterials can arouse the concern of safety of their usage with regard to human health and environment. This concern led us to include the Chap. 14 that deals with the concerns of hazards of nanomaterials to human health and environment and also critical views on compliances.

As mentioned earlier, nanotechnology and nanomaterials are increasingly finding their application in the field of agriculture; time has come for the policy makers and researchers to think and depict a road map for the use of nanotechnology in future. Chapter 15 has been specially designed for enumerating on the future road map for plant nanotechnology.

The fifteen chapters of this book have been authored mostly by different teams of scientists dealing with various aspects related to the concepts, strategies, techniques, and tools of plant nanotechnology focusing on the application potential and also on concern for nanotoxicity. Hence, some overlapping contents, particularly on a few fundamental aspects of nanomaterials including their types, natures, and impacts, are obvious. However, the responsibility lies on us as the editors for such redundancy and for addressing them in the future editions of this book.

We believe that our book "Plant Nanotechnology" provides a very precise discussion pertinent to the application of nanotechnology and nanomaterials in plant sciences so that by reading the book, any student, researcher, or policy maker can appreciate the potential and the tremendous application value of this approach and can have a precise and clear idea as to what is going on in this field.

We express our sincere thanks to the 23 scientists beside us for their chapters contributed to this book and their constant cooperation from submission of the first drafts to revision and final fine-tuning of their chapters commensurate with the reviews.

Finally, we wish to extend our thanks to Springer Nature and its entire staff particularly Dr. Christina Eckey and Dr. Jutta Lindenborn involved in publication and promotion of this book that will hopefully be useful to students, scientists, industries, and policy makers.

Mohanpur, India — Chittaranjan Kole
Kawagoe, Japan — D. Sakthi Kumar
Little Rock, USA — Mariya V. Khodakovskaya

Contents

Abbreviations

μ-XANES	Micro-X-ray absorption spectroscopic near-edge structure
μ-XRF	Micro-X-ray fluorescence
2,4-D	2,4-Dichlorophenoxyacetic acid
2-DE	Two-dimensional electrophoresis
3D	Three-dimensional
ADS	Amorpha diene synthase
AES	Atomic absorption spectrometry
AF4	Asymmetrical flow-field flow fractionation
AFM	Atomic force microscopy
AgNP	Silver nanoparticle
Al_2O_3	Aluminum oxide
ALDH	Aldehyde dehydrogenase
APX	Ascorbate peroxidase
ARGOS	Auxin-regulated gene involved in organ size
AuCapped-MSN	Mesoporous silica nanoparticle closed end with gold nanoparticle
AuNP	Gold nanoparticle
AuNR/Ag	Plasmonically active nanorods based on gold cores and silver shells
BET	Brunauer–Emmett–Teller
BP	Bulk particle
BSE	Backscattered electron
BY-2	Tobacco bright yellow-2 cell line
CAT	Catalase
CB	Carbon based
CB NP	Carbon-based nanoparticle
CEC	Cation exchange capacity
CeO_2	Cerium dioxide
CeO_2 NPs	Cerium oxide nanoparticles
Cfu	Colony-forming unit

CLSM	Confocal laser scanning microscopy
CM	Confocal microscopy
CNM	Carbon-based nanomaterial
CNT	Carbon nanotube
CPN	Conjugated polymer nanoparticle
CPS	Counts per second
CSCNT	Cup-stacked carbon nanotube
CS-Se NP	Chitosan-modified selenium nanoparticle
DBR2	Double-bond reductase
DDE	Dichlorodiphenyldichloroethylene
DF-STEM	Dark-field scanning electron microscopy in transmission mode
DLS	Dynamic light scattering
Ebeam	Electron beam
EDAX	Energy dispersive analysis of X-rays
EDX	Energy dispersive X-ray spectrometer
EELS	Energy loss spectroscopy
EM	Electron microscopy
ENM	Engineered nanomaterial
ENP	Engineered nanoparticle
EPA	European Parliament
ER	Endoplasmic reticulum
EXAFS	Extended X-ray absorption fine structure
Fe_3O_4	Magnetite
FEG	Field emission gun
FFF-ICP-MS	Field flow fractionation inductively coupled plasma mass spectrometry
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FITC	Fluorescein isothiocyanate
FS	Fullerene soot
FTIR	Fourier-transformed infrared
GA	Gum arabic
GC	Gas chromatography
GLP	Germin-like protein
GMO	Genetically modified organism
GO	Graphene oxide
GPS	Global positioning satellite
GSH	Glutathione
HA	Humic acid
HAP	Hydroxylapatite
HPLC	High-performance liquid chromatography
HRTEM	High-resolution transmission electron microscopy
HS-AFM	High-speed atomic force microscopy
ICDD	International Center for diffraction Data
ICP	Inductively coupled plasma
ICP-MS	Inductively coupled plasma mass spectrometry

ICTA	International Center for Technology Assessment
IDMS	Isotope dilution mass spectrometry
IgG	Immunoglobulin G
In_2O_3	Indium oxide
JCPDS	Joint Committee on Powder Diffraction Standards
LaB6	Lanthanum hexaboride
LA-ICP-MS	Laser ablation inductively coupled plasma mass spectrometry
LC-ESI-MS/MS	Liquid chromatography electrospray ionization tandem mass spectrometry
LM	Light microscopy
MB NP	Metal-based nanoparticle
MeJA	Methyl jasmonate
miRNA	Micro-RNA
MNM	Manufactured nanomaterial
MS	Mass spectrometry
MSN	Mesoporous silica nanoparticle
MSNS	Mesoporous silica nanoparticle system
MWCNT	Multi-walled carbon nanotube
$NaBH_4$	Sodium borohydrate
nAg	Silver nanoparticle
Nano Fe_2O_3	Nano ferric oxide
$nCeO_2$	Cerium dioxide nanoparticle
NDEA	N-nitroso-diethylamine
NGS	Next-generation sequencing
NM	Nanomaterial
NOM	Natural organic matter
NOx	Nitric oxides
NP	Nanoparticle
NR	Nanorod
NS	Nanosphere
NSS	Nanosized Ag–silica hybrid
NT	Nanotechnology
$nTiO_2$	Titanium dioxide nanoparticle
OES	Optical emission spectrometry
OPO	Optical parametric oscillator
PCD	Programmed cell death
PHSN	Porous hollow silica nanoparticle
PIP	Plasma membrane intrinsic protein
PIXE	Particle-induced X-ray emission
PLA	poly(L-lactide)
POX	Peroxidase
PR	Pathogenesis-related
PVP	Polyvinylpyrrolidone

QD	Quantum dot
qRT-PCR	Quantitative real-time polymerase chain reaction
RAPD	Random amplified polymorphic DNA
RER	Rare earth element
ROS	Reactive oxygen species
SA	Salicylic acid
SADS	Selected area (electron) diffraction
SAR	Systemic acquired resistance
SE	Secondary electrons
SEM	Scanning electron microscope
SERS	Surface-enhanced Raman spectroscopy
SiO2	Silicon dioxide
SIP	Small and basic intrinsic protein
SNP	Silver nanoparticle
SOD	Superoxide dismutase
SP-ICP-MS	Single particle inductively coupled plasma mass spectrometry
SPION	Super paramagnetic iron oxide nanoparticle
SPR	Surface plasmon resonance
SQS	Squalene synthase
SR	Synchrotron radiation
Sr31	Wheat stem rust gene
STEM	Scanning electron microscopy in transmission mode
SWCNH	Single-walled carbon nanohorn
SWCNT	Single-walled carbon nanotube
TEM	Transmission electron microscopy
TiO_2	Titanium dioxide
TIP	Tonoplast intrinsic protein
TMA-OH	Tetramethyl ammonium hydroxide
TMAPS/F-MSNs	N-trimethoxysilylpropyl-N,N,Ntrimethylammonium chloride-labeled MSNs
TNB	Temple northeastern Birmingham
TPEM	Two-photon excitation microscopy
TSC	Trisodium citrate
TUNEL	Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling
TXM	Transmission X-ray microscopy
Ug99	Uganda99 (race)
USEPA	United States Environmental Protection Agency
USDA	United States Department of Agriculture
XANES	X-ray absorption near-edge structure
XAS	Synchrotron X-ray absorption spectroscopy
XRD	X-ray diffraction

ZnO	Zinc oxide
ZnO NP	Zinc oxide nanoparticle
$ZnTiO_3$	Zinc titanate

Chapter 1
Plant Nanotechnology: An Overview on Concepts, Strategies, and Tools

Joydeep Banerjee and Chittaranjan Kole

Abstract Nanotechnology is the branch of science dealing with manipulation of matter on an atomic, molecular, or supramolecular level. Application of nanoparticles is of great scientific interest due to diverse applications of nanotechnology in the field of life sciences, medicine, electronics, and energy. Since the last couple of decades, several research groups worked on the application of nanoscience in the field of agriculture. Efficient utilization of agrochemicals and manipulation of several physiological parameters of plants are key research areas of agriculture nanotechnology. This introductory chapter presents a brief glimpse on the present global scenario of research on plant nanotechnology and several pros and cons of nanoscience in the fields of plant sciences particularly agriculture.

Keywords Nanoparticles · Agriculture · Nanotechnology · Germination · Translocation · Accumulation · Yield · Agrochemicals · Physiology · Gene expression · Safety issues

J. Banerjee
Survey Selection and Mass Production, Bidhan Chandra Agricultural University, Mohanpur 741252, West Bengal, India

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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1.1 Nanoparticles and Nanotechnology

The materials that are lesser than 100 nm, at least one dimension, are referred to as nanomaterials. Hence, the nanoparticles can be zero-dimensional (all dimensions are at nanoscales), one-dimensional (fine rod-shaped), two-dimensional (ultrathin films), or three-dimensional (of any shape) based on their manipulation of matter (Bernhardt et al. 2010; Tiwari et al. 2012). Hence, nanotechnology is the study of different nanoparticles, which are available in 1–100 nm range, at least in one dimension (Love et al. 2005). Nanoparticles categorized on the basis of dimensions, which are not confined to the nanoscale range, are presented in Fig. 1.1. During the last two decades, a significant amount of research has been conducted in nanotechnology focusing on their applications in electronics, energy, medicine, life sciences including plant sciences (Mnyusiwalla et al. 2003; Nair et al. 2010). In the field of agriculture, nanotechnology has been used to improve the food and agricultural systems through different approaches including enhancement of agricultural output, development of new food products, and conservation of foods (Chen 2002). In the course of time, the experiences in the field of nanotechnology facilitated the development of genetically modified crops, chemicals for protecting the plants from biotic stresses, better weed management, and improvement of precision farming techniques. The chapters of this book deliberate on the achievements so far made in plant nanotechnology and the safety issues as well as prospects for fundamental and applied research.

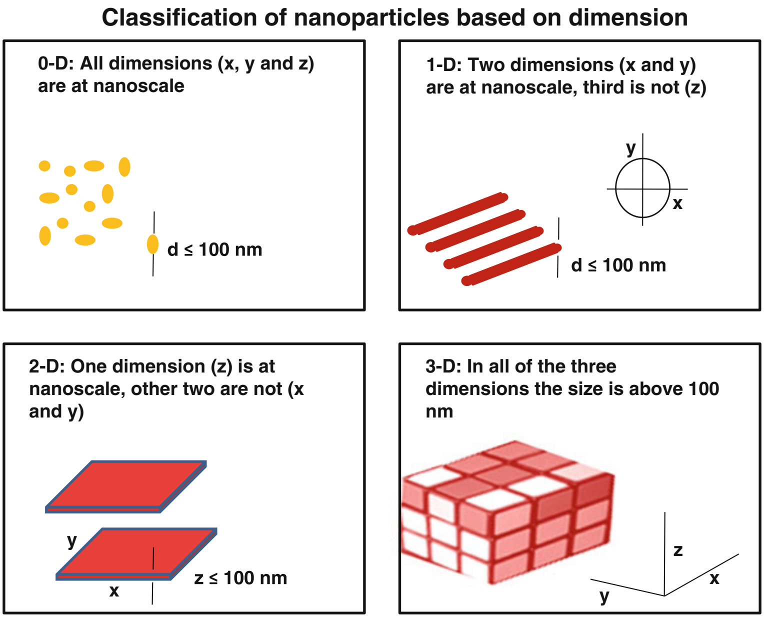

Fig. 1.1 Classification of nanoparticles based on dimension. Four different types of nanoparticles viz., zero dimentional (0-D), one dimentional (1-D), two dimentional (2-D) and three dimentional (3-D) have been mentioned with appropriate diagram

1.2 Use of Nanoparticles in Agriculture, Medicine, and Environment

In the field of agriculture and medicine, the use of nanoparticles (NPs) was found to be effective to combat biotic stresses, to increase the efficacy of agrochemicals including pesticides, and to manage the weeds in a better and eco-friendly manner. To control various bacterial and fungal pathogens, the silver NPs (Ag NPs) were found to be very effective (Nair et al. 2010). To control pathogenic *Candida* species, application of Ag NPs was found to be effective at the concentrations below their cytotoxic limit compared to that of the ionic silver against the tested human fibroblasts (Panáceka et al. 2009). Similarly, silver-based NPs were more effective against gram-negative bacteria compared to the gram-positive bacteria and the larger surface-to-volume ratio was the main reason for the effectiveness of these smaller particles (Singh et al. 2008). Similar to the Ag NPs, silica-based NPs have been widely used in medical as well as agricultural industries. Gold-coated silica has been used for the treatment of benign as well as a malignant tumor. Additionally, lipophilic nano-silica has been used for the treatment of chicken malaria and nuclear polyhedrosis virus infestation of silkworm (*Bombix mori*) (Barik et al. 2008). Other studies documented that the use of surface-modified hydrophobic nano-silica is absorbed into the cuticular layer of the insects and subsequently causes damages to the protective wax layer causing the death of the insects by desiccation (Nair et al. 2010). Such pesticides are not only safer to the plants but also less harmful to the environment compared to the chemical pesticides.

In agricultural field, different agrochemicals are used as fungicides, insecticides, pesticides, or herbicides either by spraying or by broadcasting at various growth stages of plants. A significant amount of the applied chemicals is lost due to various means such as leaching of chemicals, degradation of chemicals by photolysis, hydrolysis, and by microbial degradation. A field study was conducted in cotton plants infested with aphid for estimating the efficacy of nanosphere (NS) formulations compared to a classical suspension used as a reference. The results indicated that compared to the classical suspension, the NS formulations were slower regarding the speed of action and sustained release, but NS formulations were better for enhancing the systemicity of the active ingredient and for improving the penetration through the plant (Boehm et al. 2003). Hence, nano-encapsulated agrochemicals should be designed in such a way that the active ingredients will be released efficiently with improved solubility, stability as well as effectiveness, and finally enhanced targeted activity and reduced ecotoxicity will be achieved. In a similar approach to controlling obnoxious and parasitic weeds, nanocapsule herbicide could be used effectively to reduce the phytotoxicity as mentioned by earlier researchers (Perez-de-Luque and Rubiales 2009). Another class of nanoparticles, namely porous hollow silica nanoparticles (PHSN), was found to provide shielding protection to pesticides from degradation due to UV light exposure (Li et al. 2007). Another study on wheat demonstrated the slow release of fertilizer for regulated, responsive, and timely release of active ingredients using nano- and subnanocomposites (Zhang et al. 2006; Nair et al. 2010). Very recently, a slow-release fertilizer hydrogel nanocomposite has been prepared by free radical polymerization of sodium alginate, acrylic acid, acrylamide, and clinoptilolite using N, N′-methylene bisacrylamide as a crosslinker and ammonium persulfate as an initiator (Rashidzadeh et al. 2014). Additionally, it was found that the swelling of the hydrogels was pH dependent, and the swelling in different salt conditions was significantly lower than the values in distilled water. Moreover, another group showed that plasmonically active nanorods linked with 2,4-D, an auxin growth regulator, can enhance the growth of tobacco (*Nicotiana tabacum*) cells (Nima et al. 2014). In this way, although different NPs are being studied in the agricultural industries, their uptake, accumulation as well as their impact on the yield and different yield attributing characters should be analyzed in detail.

1.3 Types of Nanoparticles and Their Relative Merits

Based on their origin, nanoparticles (NPs) are of three types, namely natural, incidental, and engineered NPs (Monica and Cremonini 2009). Naturally occurring NPs are existing since the beginning of the Earth, and those are available in volcanic dust, lunar dust, terrestrial dust storms, mineral composites, photochemical reactions, forest fires, simple erosion, etc. Incidental NPs are generated mostly by a man-made industrial process like petrol/diesel exhaust, coal combustion, welding fumes, industrial exhausts, etc. (Buzea et al. 2007). Engineered NPs can be categorized into five types including carbon-based NPs (CB NPs), metal-based NPs (MB NPs), magnetic NPs, dendrimers, and composite NPs. Carbon-based NPs include fullerene (C_{70}), fullerol [$C_{60}(OH)_{20}$], single-walled carbon nanotubes (SWCNTs), multiwalled carbon nanotubes (MWCNTs), and single-walled carbon nanohorns (SWCNHs), while MB NPs include gold (Au), silver (Ag), copper (Cu), and iron (Fe)-based nanomaterials. In addition to that, different types of metal oxide-based NPs, such as TiO_2, CeO_2, FeO, Al_2O_3, and ZnO, are extensively studied in agriculture and medical sciences. Magnetic NPs can be manipulated using a magnetic field, and such particles commonly consist of Fe, cobalt (Co) and nickel (Ni) and their compounds. Among different magnetic NPs when ferrite (an iron oxide Fe_2O_3) particles become smaller than 128 nm, they become superparamagnetic (Lu et al. 2007). Dendrimers are nano-sized polymers built from branched units, and they are typically symmetrical around the core part, and mostly, they adopt a spherical three-dimensional structure. Composite NPs are either the combination of different NPs, or the combination of NPs with larger bulk-type materials and those include hybrids. In addition to that, the core–shell nanoparticles are prepared using two or more materials, e.g., silica/inorganic, silica/polymer, or polymer/inorganic combinations. Composite NPs possess improved solubility, easier functionalization, and decreased toxicity compared to the single-component materials (Lin and Xing 2007; Janczak and Aspinwall 2012). NPs are available in different shapes such as spheres, tubes, rods, and prisms.

Among the CB NPs, the significance of fullerene C_{70} and fullerol in agricultural sciences has been extensively studied and reviewed by some researchers (Lin et al. 2009; Kole et al. 2013) and it has been found that these two types of CB NPs get readily accumulated in plants (Rico et al. 2011). An interesting study on rice documented that the individual fullerene C_{70} NPs were possibly entering plant roots through osmotic pressure, capillary forces, pores on cell walls, and intercellular plasmodesmata, or via the highly regulated symplastic route (Lin et al. 2009), whereas another study on onion, *Alium cepa*, reported that the application of hydrophobic fullerenes C_{70}—Natural organic matter in onion cell suspensions—caused negligible NPs uptake by the cells due to blockage of cell wall pores (Chen et al. 2010). In contrast to the fullerenes, C_{70}—another CB NP (SWCNT)—was found to penetrate the cell walls and cell membranes of tobacco cells (Liu et al. 2009). It was demonstrated that CNT can activate water channels in roots as well as seeds and enhance seed germination/plant growth (Khodakovskaya and Biris 2009; Khodakovskaya et al. 2011; Villagarcia et al. 2012; Lahiani et al. 2013). Likewise, another study on tobacco cells demonstrated that the application of MWCNTs in a wide range of concentrations (5–500 μg/mL) could enhance the cell growth significantly compared to the control conditions and a correlation was found between the activation of MWCNT-treated cell growth and the up-regulation of some major genes involved in cell division/cell wall formation and water transport (Khodakovskaya et al. 2013). Lahiani et al. (2013) showed that NPs could successfully activate germination of valuable crops including soybean (*Glycine max*), maize (*Zea mays*), and barley (*Hordeum vulgare)* after deposition of MWCNTs on seed surfaces. Later on, another group confirmed the promising capabilities of carbon nanohorns, another group of CB NPs, in activating the germination of different crop seeds and enhancing growth of plant organs (Lahiani et al. 2015). Furthermore, it was also documented that MWCNTs could improve the water uptake in wheat (*Triticum aestivum*), maize, peanut (*Arachis hypogea*), and garlic (*Allium sativum*) seeds possibly through the creation of new pores (Srivastava and Rao 2014). In contrast to the positive findings on the application of CNTs, another study depicted inhibitory effect on root elongation in tomato (*Solanum lycopersicum*) but enhanced root elongation in onion and cucumber (*Cucumis sativa*) (Cañas et al. 2008). Other studies also evidenced the toxic effect of MWCNTs in plant cells, and application of MWCNTs was found to be deleterious due to the accumulation of reactive oxygen species (ROS) and subsequently decreased cell proliferation and cell death (Tan and Fugetsu 2007; Tan et al. 2009). Based on the positive as well as negative effects of CB NPs, it can be stated that the response of plants or plant cells to NPs varies with the plant species, stages of growth, and the nature of the NPs. Further research on nanosciences is needed to reveal the most efficient and useful combinations of NPs for the betterment of agriculture.

Biogenic nanocrystallines such as Fe, manganese (Mn), zinc (Zn), Cu, Co, selenium (Se) have been extensively used in the agricultural sector due to their participation in different redox processes and their presence in many enzymes as well as complex proteins. Out of these metals, Fe, Cu, and Co with variable valences are highly bioactive in nature and their application in soybean was found to show positive role in germination, growth, and production in a dose-dependent manner (Ngo et al. 2014). Similarly, the application of silver nanoparticles showed their positive impact on germination, biotic stress tolerance, and other physiological parameters of plants (Nair et al. 2010; Savithramma et al. 2012; Sharma et al. 2012). Also, some reviews suggested the importance of typical metals such as gold (Au), platinum (Pt), and palladium (Pa) in agriculture, biosciences, and pharmacology (Abhilash 2010; Agrawal and Rathore 2014). An excellent review has documented the plant uptake, translocation, accumulation as well as toxicity of different NPs including those belonging to metal oxide/hydroxide category, namely TiO_2, ZnO, CeO_2, $Ni(OH)_2$, and Fe_3O_4 (Rico et al. 2011). Although some studies have been carried out on the beneficial role of various metal oxides including CuO, TiO_2, ZnO, $CuZnFe_2O_4$, Fe_3O_4, Fe_2O_3, the adverse effects of some of those metal oxide NPs on soil microbial community and soil structure have also been identified (Frenk et al. 2013). Hence, it is important to research on plant type and soil conditions before applying any specific type of NPs and further experimentation is needed in that regard.

1.4 Impacts of NPs on Germination and Seedling Parameters in Various Crops

Application of NPs was found to have positive as well as negative impact on seed germination and in different stages of growth and development. Khodakovskaya and her group demonstrated the ability of MWCNTs to penetrate tomato seed coat and activate seed germination (Khodakovskaya and Biris 2009; Khodakovskaya et al. 2011). Later, the same group documented that tomato plants grown in soil supplemented with MWCNTs were able to produce two times more flowers and fruits compared to plants grown in control soil (Khodakovskaya et al. 2013). Further studies showed that the positive effect of MWCNTs on germination and growth of corn, soybean, and barley seedlings was reproducible between crop species (Lahiani et al. 2013). An in-depth study was carried out on wheat, maize, peanut, and garlic for knowing the effect of MWCNTs on seed germination and plant growth (Srivastava and Rao 2014). Seeds exposed to nanotubes showed three to four times faster sprouting compared to the controlled condition, and after about 5–10 days of exposure to MWCNTs, a significant enhancement was detected in the plant growth and biomass production of the treated plants compared to the control one. It is to be noted here that the same study also showed evidence on the detrimental effects of MWCNTs at higher doses. Another study on tomato documented the inhibition of root elongation after application of CNTs (Cañas et al. 2008). Application of nanosized TiO_2 (10 ppm concentration) on wheat showed lowest germination time compared to the control condition, while the shoot as well as seedling length was found to be sufficiently higher after application of 2–10 ppm nanosized TiO_2 compared to control and bulk TiO_2-treated plants (Feizi et al. 2012). In addition, it was stated that the higher concentrations of TiO_2-based NPs had inhibitory effect or not any effect on wheat. Similarly, another study reported that the application of nano-TiO_2 in proper concentration accelerated the germination of aged spinach (*Spinacia oleracea*) seeds and enhanced vigor (Zheng et al. 2005). A different study on chickpea (*Cicer arietinum*) demonstrated that the application of hydroxylapatite (HAP) nanorod resulted in better germination and enhanced plant growth. The best performance was observed in presence of 1 mg/ml Hap-nanorod compared to control and other doses (Bala et al. 2014). Soybean seeds treated with superdispersive iron, cobalt, and copper nanocrystalline powders at zerovalent state under laboratory condition showed improved germination frequencies compared to the control condition (Ngo et al. 2014). In addition to that, the application of extra low dose (not more than 300 mg of each metal per hectare) of nanocrystalline powders in field experiment was found to have improvement in different aspects of plant growth and development such as chlorophyll content, number of nodules/root, number of pods/plant, pods weight, 1000-grain weight, and crop yield. Similarly, another study on soybean reported improved germination and growth parameters after application of nano-SiO_2 and nano-TiO_2 mixtures (Lu et al. 2002). Ag NPs are one of the widely used engineered NPs. A comprehensive study was carried out for knowing the effects of Ag NPs on germination and growth on 11 species of wetland plants including *Lolium multiflorum*, *Panicum virgatum*, *Carex lurida*, *C. scoparia*, *C. vulpinoidea*, *C. crinita*, *Eupatorium fistulosum*, *Phytolacca americana*, *Scirpus cyperinus*, *Lobelia cardinalis,* and *Juncus effusus* belonging to six different families, and it was found that different species showed differential response to germination (Yin et al. 2012). Additionally, the root growth was found to be affected more compared to the leaf growth after exposure to Ag. Exposure of tobacco plants to different concentrations of Al_2O_3 (0, 0.1, 0.5, and 1 %) documented that as the exposure to NPs increased, the average root length, average biomass, and leaf count of the NP- exposed plants were significantly decreased compared to the control samples (Burklew et al. 2012). Along with the various reports on the detrimental effect of various NPs on germination and plant growth, some studies reported the genotoxic effect of some NPs. Random amplified polymorphic DNA analysis was carried out for knowing the DNA damage as well as mutations caused by NPs, and it was found that after exposure to CeO_2 NPs on soybean plants, four new bands were detected at 2000 mg L^{-1}, and three new bands were found at 4000 mg L^{-1} treatment (López-Moreno et al. 2010). Another report documented the copper oxide NP-mediated DNA damage in some terrestrial plants. In that study, under controlled condition, strong plant growth inhibitions were recorded for radish (*Raphanus sativus*), perennial ryegrass (*Lolium perenne*), and annual ryegrass (*Lolium rigidum*) and in addition, some oxidatively modified, mutagenic DNA lesions (7,8-dihydro-8-oxoguanine; 2,6-diamino-4-hydroxy-5-formamidopyrimidine; and 4,6-diamino-5-formamidopyrimidine) were found to be accumulated in significant amount under laboratory conditions (Atha et al. 2012). Further experimentation is needed for understanding the probable impacts of NPs in biological systems as well as on their physiological aspects. Some of the chapters of this book are going to address those specific questions in detail.

1.5 Effects of Nanoparticles on Gene Expression

The effect of different NPs on gene expression of animals, human as well as plants has been studied by many workers (Khodakovskaya et al. 2011; Poynton et al. 2011; Lee et al. 2012; Kaveh et al. 2013; Lahiani et al. 2013). Some studies documented that after exposure to nanoparticles, the gene expression of superoxide dismutase (SOD) was altered along with other enzymes in the animal as well as in plant system (Lee et al. 2012; Kaveh et al. 2013; Siddiqi 2014). In addition to that, higher concentration (1 %) of Al_2O_3 nanoparticle stress was found to show significant up-regulation of a number of micro-RNA genes including miR395, miR397, miR398, and miR399 (Burklew et al. 2012). These findings might be analyzed in great detail to understand the global gene expression profiling after the application of NPs. Out of these miRNAs, especially miR398 was found to possess a significant relation to SOD expression (Sunkar et al. 2006; Dugas and Bartel 2008), whereas other miRNAs were involved in other stresses (Sunkar 2010). Microarray-based gene expression analyses were carried out in Arabidopsis (*Arabidopsis thaliana*) for knowing the nanoparticle-specific changes in gene expression after exposure to ZnO, TiO_2, and fullerene soot (Landa et al. 2012). The study reported that after exposure to ZnO and fullerene soot (FS), mostly the biotic (wounding and defense to pathogens) and abiotic stress (oxidative, salt, and water deprivation) responsive genes were up-regulated, whereas ZnO-exposure was responsible for down-regulation of genes involved in cell organization and biogenesis but FS-exposure leads to down-regulation of genes involved in electron transport and energy pathways. Interestingly, after exposure to TiO_2, most of the expressional changes (up-regulation and down-regulation) were detected for genes, which were responsive to abiotic and abiotic stimulus. Another study on Arabidopsis was done by microarray for knowing the changes in gene expression after exposure to AgNPs as well as Ag^+ (Kaveh et al. 2013). Among the up-regulated genes, a major part was associated with the response to metals and oxidative stress (such as cation exchanger, cytochrome P450-dependent oxidase, SOD, and peroxidase), whereas the down-regulated genes were responsive to pathogens and hormonal stimuli such as genes involved in systemic acquired resistance, ethylene signaling, and auxin-regulated gene involved in organ size (ARGOS). On the other hand, among the differentially expressed genes in response to AgNPs only, most remarkable up-regulation (>4.0 fold) was detected in two salt stress-related genes (*AT3G28220* and *AT1G52000*), one gene codes for myrosinase-binding protein (AT1G52040) involved in biotic stress, three genes engaged in the thalianol biosynthetic pathway (*AT5G48010*, *AT5G48000*, and *AT5G47990*), and a gene responsive to wounding (*AT2G01520*). Although it is clear from the above discussions that the exposure of Arabidopsis to ZnO, FS or AgNPs causes similar type of changes in gene expression (Landa et al. 2012; Kaveh et al. 2013), the mechanisms of phytotoxicity are highly specific to the type as well as concentrations of NPs. Interestingly, germins and germin-like proteins belonging to cupin superfamily were found to be involved in various biotic as well as abiotic stresses (Dunwell et al. 2008) and some of the members of this superfamily possessed SOD activity (Dunwell et al. 2008; Banerjee et al. 2010). Very recently, an interesting study on Indian mustard (*Brassica juncea*) showed a correlation between copper oxide nanoparticles induced growth suppression and enhanced lignification as well as modification in root system. It is worthy to mention that a germin-like protein from rice (OsGLP1) was found to have some relation to plant height and SOD-mediated cell wall reinforcement (Banerjee and Maiti 2010; Banerjee et al. 2010). If the proteins belonging to cupin superfamily members are involved in nanoparticle-regulated cascades, there will be a new area of research for understanding such complex plant signaling networks involving various stresses. A variety of NPs was found to have effects on gene expression in plant system as well as in animal systems including humans, and NPs are able to express distinct bioactivity and unique effects with different biological systems. For assessing the potential health risks after exposure to NPs, luciferase reporter system has been used for understanding the gene expression profiles in response to NPs (Ding et al. 2012). Further work is needed in model organisms to specifically identify the signaling cascades or to determine the regulation of a set of genes by specific NPs in a dose-dependent manner.

1.6 Translocation and Accumulation of Nanoparticles in Plant Tissues and Organs

Due to rapid progress in the field of nanosciences and wide applications of nanomaterials (NMs) in medical sciences as well as in agriculture, some researchers started analyzing the potential impacts of NMs along with their translocation and accumulation in tissues. The first study on the uptake, accumulation, and translocation analyses of magnetite (Fe_3O_4) nanoparticles was carried out on pumpkin (*Cucurbita maxima*) (Zhu et al. 2008). The study revealed that the iron oxide NPs (Fe_3O_4) were taken up by pumpkin roots and subsequently translocated through plant tissues. In addition to that, it was also found that almost 45.5 % of fed nanoparticles were accumulated in roots and about 0.6 % of the nanoparticles were detected in leaves. In contrast to that, application of same NPs on another crop, lima bean (*Phaseolus limensis*), did not show any uptake and transport of the NMs as revealed by same researchers.

Among the CB NMs, fullerene C_{70} and fullerols were mostly found to be taken up as well as accumulated in plants (Rico et al. 2011; Kole et al. 2013). An interesting study on uptake and translocation of CB NPs on rice (*Oryza sativa*) established that fullerene C_{70} was easily taken up by roots and transported to shoots compared to MWCNTs (Lin et al. 2009), possibly due to the relatively larger size of MWCNTs than fullerenes. Additionally, in the roots of mature plants, no C_{70} was detected, explaining robust transport of NPs from root to shoot. SWCNTs, another CB NPs, were found to show gradual findings regarding its penetration to plant cells (Liu et al. 2009; Shen et al. 2010). Some study on Bright Yellow (BY-2) cells reported that the water-soluble SWCNTs (<500 nm in length) were able to penetrate the intact cell wall and the cell membrane through fluidic phase endocytosis, whereas another study on cucumber documented no uptake of SWCNTs by the roots upon exposure to CB NPs for 48 h (Cañas et al. 2008). Little is known about the quantity of NPs being delivered inside plant tissues due to less availability of detection methods. Dr. Green and his group showed the ability of the microwave-inducing heating technique to quantify tubular structure CB NPs inside plant tissue (Irin et al. 2012). This method was followed to quantify SWCNHs inside different crop roots system (Lahiani et al. 2015) and MWCNTs inside different plant tissues (Irin et al. 2012).

Application of an aqueous colloidal solution of NaYF4:Yb,Er nanocrystals during watering was found to show uptake and transport of nanocrystals from roots to leaves in moth orchid (*Phalaenopsis* spp.) and Arabidopsis (Hischemoller et al. 2009). Probably that was the first report on uptake kinetics and that illustrated the potential penetration routes of NPs in plant tissues. The route of penetration of the nanocrystals at different period of times in different plant tissues was carried out using confocal laser scanning microscopic analyses. The uptake and accumulation of Cu NPs, Ag NPs, and metal NPs have been described in some recent reviews (Ma et al. 2010), and it was found that the higher application of Cu NPs resulted in higher uptake and accumulation under laboratory condition. Another review has nicely described the uptake and accumulation of metal oxide NPs as well as metal NPs in plant systems (Rico et al. 2011).

Other than the CB NPs, the magnetic NPs (Fe_3O_4) were detected in roots, stems, and leaves of pumpkin plants and the uptake was found to be dependent on the growth medium (Zhu et al. 2008). Among the metal oxide-based NPs, an ultra-small TiO_2 (<5 nm) complexed with Alizarin red S nanoconjugate was found to show uptake and translocation in Arabidopsis plants (Kurepa et al. 2010). The study also documented that the mucilage released by the roots of Arabidopsis formed a pectin hydrogel capsule surrounding the root, which either facilitated or inhibited the entry of TiO_2 complexed with Alizarin red S or sucrose. In contrast to that, another study on maize roots did not show any uptake of TiO_2 NPs (30 nm) probably due to the larger size of the NPs than the pore diameters (Asli and Neumann 2009). Other studies also documented that polysaccharides in mucilage might adsorb and inactivate toxic heavy metals in root rhizosphere and ultimately enhanced the accumulation of aluminum (Watanabe et al. 2008). The uptake and accumulation study of another metal oxide NPs (ZnO) by soybean seedlings demonstrated that at 500 mg L^{-1} concentrations, the uptake of the NPs (8 nm) was significantly higher possibly due to lesser aggregation, whereas at higher concentrations (1000–4000 mg L^{-1}), the passage of NPs through the cell pore walls was difficult probably due to agglomeration, and that caused reduced uptake and accumulation (López-Moreno et al. 2010).

It has been found that after application of CeO_2 at 4000 mg L^{-1}, the concentration of Ce (mg kg^{-1} dry weight biomass) significantly varied between soybean, alfalfa (*Medicago sativa*), maize, and tomato. The concentrations of Ce in corn, soybean, tomato, and alfalfa were found to be approximately 300, 462, 4000, and 6000 mg kg^{-1} dry weight biomass, respectively. The differences in concentrations could be explained by the variations in the root microstructures and the physical as well as chemical interactions between the NPs and root exudates in the rhizosphere of respective plant species (Rico et al. 2011). Due to advancement in the field of nanotechnology, some of the present research papers and review articles are focusing on the shape, size, structure, chemical composition, and surface chemistry of NPs for understanding the nanoparticle aggregation in the environment and subsequently the accumulation and transport of NPs in living systems (Hotze et al. 2010; Albanese et al. 2012). Further research is needed in this context for knowing the uptake capacity and permissible limit of different NPs in agriculture and food industry.

This book is going to describe the physio-chemical properties of different NPs, their merits as well as demerits, the detection, and quantification of NPs along with their involvement in uptake, accumulation, and translocation. Additionally, the chapters of this book will focus on the use of NPs and their impacts on germination, growth, and other physiological aspects as well as yield and quality components. Some of the sections will describe the modern understanding of the gene expressional changes caused by NPs and different modes of transmission of NPs. Later chapters will focus the importance of NPs for gene delivery, fertilizer delivery, and various agrochemicals applications along with their involvement in plant protection. At last but not the least, the possible merits and demerits of various NPs, the effects of NPs on soil, plant and environments and the prospects and policies for nanosciences will be considered.

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Chapter 2
Physical and Chemical Nature of Nanoparticles

Sanmathi Chavalmane Subbenaik

Abstract Nanoparticles have some specific features, including physical properties, chemical properties, merits, and demerits, which have drawn much attention for their application in nanobiotechnology. This chapter explains the state of the art of different properties of nanoparticles and their potential beneficial roles. In addition, this chapter discusses on the research on nanoparticles essentiality for plants and describes the current knowledge concerning the key nanoparticles with important studies for their future applications.

Keywords Nanoparticles · Physiochemical nature · Merits and demerits

2.1 Introduction

Nanoparticles in general refer to particles having internal structural measurement or external dimensions within the size range of a few nanometers, preferable up to 100 nm size. According to the European Committee for Standardization, nanomaterials are defined as the materials with any external dimension at the nanoscale, or that possess nanoscale internal or surface structures. Nanoscale describes the size range from approximately 1–100 nm (ISO/TS 27687: 2008) (Lövestam et al. 2010). It is most frequently used as a specific size description (usually <100 nm, though sometimes <50 nm), and this book chapter will use the term nanoparticle to refer to particles of <100 nm.

Nanoparticles have been developed for use in the area of agriculture (Nair et al. 2010; Campos et al. 2014), where they can increase the efficiency and productivity of crops. To properly assign the mechanisms for the application of nanoscale materials in plants, their synthesis and characterization must be well understood. Scientists have many methods to synthesize NPs of different size, shape, and

S.C. Subbenaik (✉)
Nano Research Facility (NNIN-NSF), School of Engineering and Applied Science, Washington University in St. Louis, St. Louis, MO 63130, USA
e-mail: sanmathi.nrf.wustl@gmail.com

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surface properties. The major synthesis routes are liquid phase, gas-phase, and biological methods (Klaus et al. 1999; Konishi et al. 2007; Raliya and Tarafdar 2012; Mittal et al. 2013). The main liquid phase syntheses of inorganic NPs are coprecipitation, solgel processing, micro-emulsions, hydrothermal or solvothermal methods, template synthesis, and biometric synthesis (Cushing et al. 2004). The biological method can be approached for synthesis of NPs, which is rapid and cost-effective. (Gilaki 2010; Raliya and Tarafdar 2012). Besides these synthesis methods, the gas-phase synthesis methods are of interest because they allow elegant way to control process parameter in order to be able to produce size-, shape-, and chemical composition-controlled nanostructures, and also can be used to prepare the large quantity of NPs (Jiang et al. 2007; Thimsen et al. 2008).

Nanoparticles are of two types: non-engineered and engineered NPs. Non-engineered NPs present in the environment are derived from natural events such as terrestrial dust storms, erosion, volcanic eruption, and forest fires (Nowack and Bucheli 2007). Engineered NPs (ENPs) are intentionally produced by man using many different materials, such as metals (including Au, Ag, Zn, Ni, Fe, and Cu) (Fedlheim and Foss 2001), metal oxides (TiO_2, Fe_2O_4, SiO_2, CeO_2, and Al_2O_3) (Fernández-García and Rodriguez 2011), nonmetals (silica and quantum dots) (Ehrman et al. 1999), carbon (graphene and fullerene) (Endo et al. 2013), polymers (alginate, chitosan, hydroxyethylcellulose, polyhydroxyalkanoates and polyhydroxyalkanoates, and poly-E-caprolactone) (Paques et al. 2014) (Rao and Geckeler 2011), and lipids (soybean lecithin and stearic acid) (Ekambaram et al. 2012).

Engineered NPs are able to enter into plants cells and leaves and also can transport DNA and chemicals into plant cells (Galbraith 2007; Tripathi et al. 2011; Raliya et al. 2015). The unique physical and chemical properties of nanoparticles could boost plant metabolism (Nair et al. 2011; Brew and Strano 2014). Here, we describe the physical and chemical nature of the NPs and compare their merits and demerits during application. Figure 2.1 shows the different physical and chemical nature of NPs.

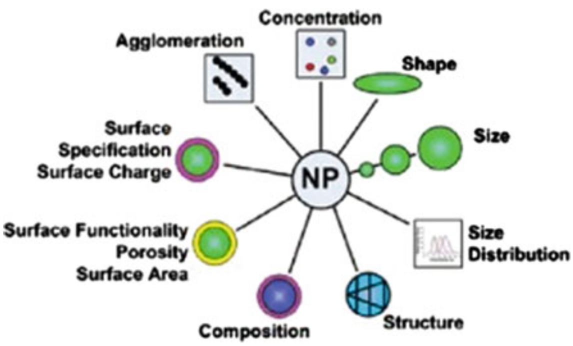

Fig. 2.1 Physical and chemical nature of nanoparticles