Chapter 11
Nanobiotechnology: Scope and Potential for Crop Improvement

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1 Introduction

1.1 Nanotechnology

Nanotechnology is manufacturing at the molecular level—building things from nanoscale components, where unique phenomena enable novel applications. Nanos: Greek term for dwarf, Technology: visualize, characterize, produce and manipulate matter of the size of 1–100 nm (Ball et al. 2002). In layman's language, nanotechnology is the science behind the intentional creation, manipulation, and characterization of extremely small particles and macro molecules. Nanotechnology proposes the construction of novel nanoscale devices possessing extraordinary properties. The chemical, physical, and biological properties of materials differ in fundamental and valuable ways from those of individual atoms, molecules, or bulk matter (Nel et al. 2006). To get an idea of the size of particles that nanotechnology encompasses, consider some comparisons. A nanometer (nm) is one-billionth of a meter. A typical sheet of paper is about 100,000 nm thick, a red blood cell is about 2,000–5,000 nm in size, and the diameter of DNA is in the range of 2.5 nm. The size range of highest interest in the field of nanotechnology is from 1–100 nm (Maynard et al. 2006), so nanotechnology deals with matter that ranges from one-

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half the diameter of DNA up to 1/20 the size of a red blood cell. This size range is comparable to that of viruses and is one-fourth the wavelength of visible light. The beginning of nanoscience was mainly devoted to the study and fabrication of materials at the nanoscale, where much effort was dedicated to shrink the dimension of fabricated materials. It was the same time when the two basic fabrication approaches were defined: “bottom-up” and “top-down.” The bottom-up approach aims at building things by combining smallest possible building materials such as single molecules and atoms, which are held together by covalent forces. The advantage of the bottom-up design is that the covalent bonds that hold a single molecule together, are far stronger than the weak interactions that hold more than one molecule together. A top-down approach (also known as step-wise design) is essentially the moulding, carving, and fabricating of small materials and components by using bigger objects such as mechanical tools and lasers. Recently, application of nanomaterials prepared using techniques involving both the approaches have evolved. However, the bottom-up approach has far more practical and future applications. Thus, nanotechnology is a multi-disciplinary field that seeks to combine mature nanoscale technology of fields such as physics, biology, engineering, chemistry, computer science, and material science. Potential applications include agricultural production (plant and animal), food processing, and manufacturing in areas such as pathogen detection, food engineering, packaging, and equipment (Perez-de-Luque et al. 2009; Torney et al. 2007).

1.2 The “Nano-Bio” Interface

For last many years, nanoscale processes and structures have been optimized in order to govern the biosystems. Biologists have been working for several years at the molecular level, in the range of nanometers (DNA and proteins) to micrometers (cells). A typical protein such as hemoglobin has a diameter of about 5 nm, DNA’s double helix is about 2 nm wide, and a mitochondrion spans a few hundred nanometers (Whitesides et al. 2003). Consequently, the study of any subcellular entity can be considered “nanobiology”. Moreover, today, a living cell having its hundreds of nanomachines is considered to be the essential nanoscale fabrication system. Nano-sized molecular building blocks have been the basis of each and every biological system that cooperates to produce living entities. These elements have enlightened the imagination of nanotechnologists for many years, resulting in the birth of the new science “Nanobiotechnology: The combination of nano and biotechnology”.

Nanotechnology provides the tools and technology platforms for the investigation and transformation of biological systems, whereas biology offers inspirational models and bio-assembled components to nanotechnology (Fortina et al. 2005; Lowe et al. 2000; Bohr et al. 2002). The difference between “nanobiology” and “nanobiotechnology” exists in the technology part as anything that is “man-made” falls into the technology section of nanobiotechnology. Nanobiotechnology will lead to the design of entirely newclasses of micro- and nanofabricated devices and machines, for which the inspiration will be based on bio-structured machines.

1.3 Nanobiotechnology

The term "nanobiotechnology" was first given by Lynn W. Jelinski, a biophysicist at Cornell University, USA. Nanobiotechnology is a promising area that combines nanofabrication and biosystems to the benefit of both, for all applications of genomics, including mammalian, plants and microbials (Robinson et al. 2009). As mentioned earlier, there are two basic fabrication approaches to create nanostructures—top-down and bottom-up. In top-down approach, nanobiotechnology utilizes tools and methods of nano/microfabrication to manufacture nanostructures and nanodevices. However, the bottom-up approach exploits biological structures and processes via a collection of molecular tool kits of atomic resolution, to create novel functional materials, biosensors, and bioelectronics for different applications. Nanobiotechnology helps in achieving many essential goals that are rather difficult to achieve by other means. For instance, a DNA's ladder structure provides a natural framework for assembling nanostructures instead of fabricating silicon scaffolding for nanostructures as DNA's ladder provides highly specific bonding which brings atoms together to create a nanostructure. It provides the tools and technology for gathering information and designing novel devices to investigate questions related to the biological importance of the genomic information and its implementation in various fields, particularly medicine and agriculture. Applications of nanobiotechnology in agriculture are gradually moving from the theoretical possibilities into the applicable area and play an important role in improving the existing crop management techniques. Nanoscale devices with novel properties are capable of responding to different conditions by themselves, and therefore taking appropriate remedial action. These systems help in delivering chemicals in a controlled and targeted manner through genetic improvement of plants (Kuzma et al. 2007; Scott et al. 2007), delivery of genes and drug molecules to specific sites at cellular levels (Maysinger et al. 2007), and nano-array-based gene technologies for gene expressions in plants under stress conditions. The interest is increasing with suitable techniques and sensors for precision in agriculture, natural resource management, early detection of pathogens and contaminants in food products, smart delivery systems for agrochemicals like fertilizers and pesticides. Agrochemicals are conventionally applied to crops by spraying and/or broadcasting. In order to avoid the problems such as leaching of chemicals, degradation by photolysis, hydrolysis and microbial degradation, a concentration of chemicals lower than minimum effective concentration to reach the target site of crops is required. Hence, the nanocapsulated agrochemicals should be designed in such a manner that they hold all essential properties such as effective concentration, time-controlled release in response to certain stimuli, enhanced targeted activity and less ecotoxicity with safe and easy mode of delivery, thus avoiding repeated application (Green et al. 2007; Wang et al. 2007; Boehm et al. 2003; Tsuji et al. 2001). The best example is the reduction of phytotoxicity of herbicides on crops by controlling the parasitic weeds with nanocapsulated herbicides (Perez-de-Luque et al. 2009). Proper functionalization of nanocapsules ensures better penetration and allows slow and controlled release of active ingredients on reaching the target weed and also makes the concentrated active ingredients, safe and easy to handle. Besides these, plants and/or their extracts help in biological synthesis of some metallic nanoparticles which is more ecofriendly and gives a controlled synthesis with well-defined size and shape (Kumar et al. 2009; Sharma et al. 2009). Therefore, with increased advances made by using nanobiotechnology in the agricultural sector, it can be expected to become a major economic driving force and benefit consumers as well as farmers with no harmful effect on the ecosystem.

2 Types of Nanomaterials

Depending on their existence in nature, nanomaterial is a term that includes all nanosized materials, including natural, incidental and engineered nanomaterials.

2.1 Natural Nanomaterials

Natural nanomaterials have been in existence since the beginning of the earth' history, and still occur in the environment. Materials that are a result of natural process with a structure approximately 1–100 nm are called natural nanomaterials. For example, particles arising from volcanic eruptions, sea spray, and atmospheric gas-to-particle conversion. Many important functions of living organisms also take place at the nanoscale level. The human body uses natural nanoscale materials, such as proteins and other molecules, to control many systems and processes of the body.

2.2 Incidental Nanomaterials

Incidental nanomaterials are defined as the materials with a structure approximately 1-100 nm that are produced as a result of manmade industrial processes such as diesel exhaust, coal combustion, welding fumes, etc.

2.3 Engineered Nanomaterials

Materials that are purposefully manufactured with nanoscale dimensions (1 and 100 nm), can be termed as engineered nanomaterials. Engineered particles of very small dimension attract enormous interest of researchers and are of potential benefit to society due to their properties which are different from larger particles of the same chemical composition. Engineered nanomaterials have received a particular attention for their positive impact in improving many sectors of economy, including consumer products, pharmaceuticals, cosmetics, transportation, energy and agriculture (Nowack et al. 2007; Roco et al. 2003). The properties of engineered nanomaterials are essentially important due to their aggregation behavior and mobility in aquatic and terrestrial systems and also for their interaction with algae, plants and fungi (Enrique et al. 2008). Engineered nanomaterials can be categorized as:

- Carbon-based nanomaterials
- Metal-based nanomaterials
- Dendrimers
- Composites

2.3.1 Carbon-Based Nanomaterials

These types of nanomaterials mainly consist of carbon having the most common form of hollow spheres, ellipsoids, or tubes. Spherical and ellipsoidal carbon nanomaterials are referred to as fullerenes, while cylindrical ones are called nanotubes such as single-walled carbon nanotube (SWCNT) and multi-walled carbon nanotubes (MWCNT). These materials have various potential applications, such as improved films and coatings, stronger and lighter materials, and applications in electronics, agriculture and food (Remya et al. 2010). Recently, it was found that fullerenes may act as antioxidants, preventing lipid peroxidation induced by super oxide and hydroxyl radicals (Wang et al. 1999).

2.3.2 Metal-Based Nanomaterials

Metal-based nanomaterials have received considerable attention in science and technology in the last decade. These include quantum dots, gold, silver, palladium and metal oxides (TiO_2 and ZnO) nanomaterials. There is a huge scope for applying nanomaterials to plants for agricultural use (Liu et al. 2002; Pavel et al. 1999; Pavel et al. 2005; Joseph et al. 2006). TiO_2 nanoparticles have been found to induce spinach seed germination of aged seeds and its vigor (Zheng et al. 2005). It was also observed that the presence of TiO_2 nanoparticles increases the dry weight, chlorophyll synthesis, and metabolism in photosynthetic organisms. Due to the antimicrobial properties of engineered nanomaterials, the strength and resistance of plants to stress can be increased. Gene transfer by bombardment of DNA-absorbed gold particles has been successfully used to generate transgenic plants in a species-independent manner (Christou et al. 1988).

2.3.3 Dendrimers

Dendrimers are nanosized polymers composed of branched units having the capability to be customized to perform a specific chemical function. The surface of a dendrimer has numerous chain ends; this property could also be useful for catalysis. In addition, since three-dimensional dendrimers contain interior cavities into which other molecules could be placed, they may be useful for drug delivery.

2.3.4 Composites

The combination of two different nanomaterials or nanomaterials with bulk-type materials (Lin et al. 2007) is called composites. It can be of different morphologies such as spheres, tubes, rods and prisms (Yu-Nam et al. 2008). For example, nanosized clays are being used now-a-days for products ranging from auto parts to packaging materials, to enhance mechanical, thermal, barrier, and flame-retardant properties.

3 Preparation of Different Nanomaterials

The synthesis of metal and metal oxide nanomaterials is a growing research area due to their potential applications in the development of novel technologies. The scientific and technological importance of metal (Au, Ag, Pd etc.) and metal oxide (ZnO, $SiO_{2,}$ $TiO_{2,}$ etc.) nanomaterials has made them the subject of intensive research owing to their wonderful physical and chemical properties and also their important applications in physical, chemical and biological field such as in nanobiotechnology. Generally, nanomaterials are prepared by a variety of chemical methods, including sol gel, template method, wet chemical synthesis, electrochemical method, photochemical method, and sonochemical synthesis. However, eco-friendly and cost-effective procedures for the synthesis of nanomaterials are of great interest to biologists, chemists and materials scientists using non-toxic chemicals, environmentally benign solvents, and renewable materials. Currently, there is growing need to develop eco-friendly and body benign nanomaterials synthesis methods without the use of toxic chemicals in the synthesis protocols to avoid adverse effects in biomedical and agricultural applications. Recently, the green chemistry which aims at reducing or eliminating substances hazardous to human health and the environment, is becoming more and more important (Poliakoff et al. 2001, 2002). Various types of nanomaterials have been successfully prepared by our research group using green chemistry route.

3.1 Gold Nanoparticles

Gold nanoparticles have been synthesized by simple and cost-effective microwave irradiation processes with an irradiation time of 40–70 s.

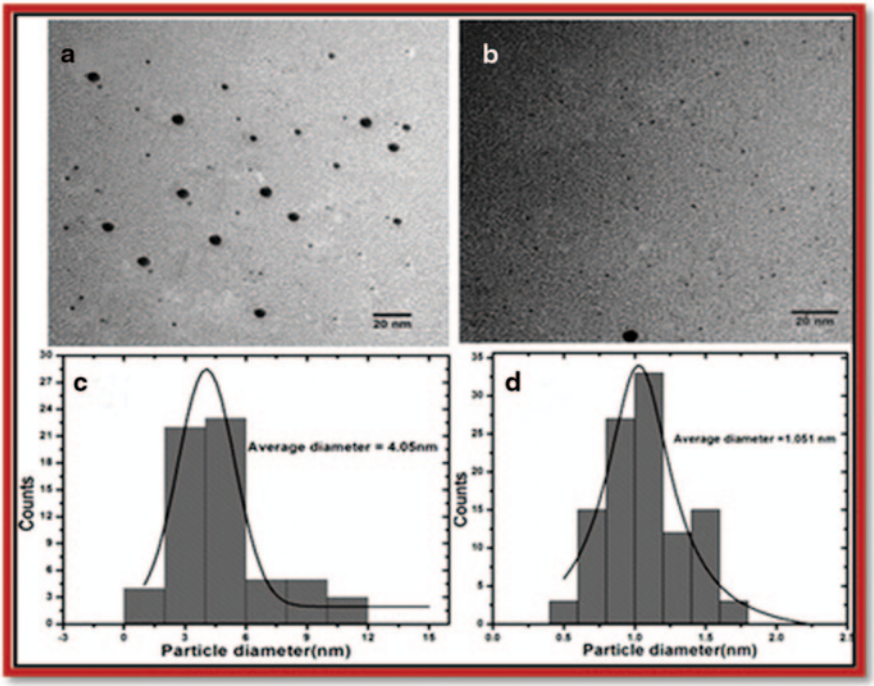
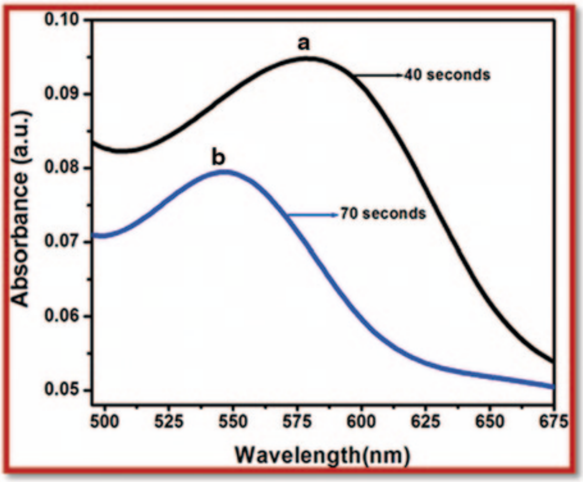


Fig. 11.1 Transmission Electron Microscopy (TEM) images of gold nanoparticles for **a** 40 s **b** 70 s and their corresponding size distribution for **c** 40 s **d** 70 s, respectively. (Adopted with kind permission from Arshi et al. 2011a)

Microwave irradiation is an efficient and distinct heating method, and has attracted interest of researchers owing to its unique features such as short reaction time, rapid volumetric heating, energy saving, environmental friendly and high reaction rate (Ela et al. 2009; Ahmed et al. 2011a, b). The synthesized gold nanoparticles were characterized by Transmission Electron Microscopy (TEM) and UV/Vis spectroscopy. The TEM images (Fig. 11.1) reflect stable and nearly spherical nanoparticles with an average diameter of 4.05 nm and 1.05 nm for samples at 40 s and 70 s irradiation time, respectively. Particle size calculated by using approximately 100 randomly selected individual nanoparticles from TEM micrograph shows that the size of the nanoparticles for 40 s and 70 s ranges from 1–10 nm (see Fig. 11.1a) and 1–2 nm (see Fig. 11.1b), respectively.

Figure 11.2 shows the UV/Vis absorption spectra of gold nanoparticles. Surface plasmon resonance peaking at 590 nm for 40 s and 560 nm for 70 s samples respectively, confirms the presence of gold nanoparticles. Generally, the broadness of the peak is a clear indicator of the size of the nanoparticles. There is a blueshift in the absorption peak to 560 nm which shows that the particle's size is decreasing as the heating time exceeds from 40 to 70s (Shahverdi et al. 2007).

Fig. 11.2 UV/Vis spectra of gold nanoparticles heated for **a** 40 s **b** 70 s. (Adopted with kind permission from Arshi et al. 2011a)



3.2 *Silver Nanoparticles*

We presented a simplest, cheapest and environmentally benign synthesis of silver nanocrystals using sugar as the reducing-cum-stabilizing agent in ambient conditions without any solvent. The structural analyses of the as-synthesized nanocrystals were performed using X-ray diffraction (XRD). The morphological study of the sample was done using Atomic force microscopy (AFM) and TEM. The optical study of the synthesized product was performed using UV/Vis spectroscopy.

Figure 11.3 shows the XRD pattern of the as-synthesized silver nanocrystals. The XRD pattern of silver nanocrystals showed a single phase nature with face-centered cubic (FCC) structure. No secondary phase was detected and the high intensity of peaks revealed the high crystalline nature of the as-synthesized silver nanocrystals. The average grain size calculated by using Debye-Scherrer formula (Cullity and Stock 2001) was found to be ~19 nm.

The UV/Vis absorption spectrum of the silver nanocrystals is shown in Fig. 11.4. A strong absorption peak at approximately 426 nm of nanosized silver nanocrystals was observed, which is the characteristic of the surface plasmon resonance of Ag materials. Figure 11.5a, b depict the topographical 2D and 3D AFM images of silver nanocrystals. It is clear from Fig. 11.5a, b that most of the grains are in the size ranging from 10–20 nm with average diameter of ~18 nm.

This is evidently in accordance with the results obtained by XRD. Figure 11.6a shows the TEM micrograph of homogeneous silver nanocrystals with particle size in the range of about 10–30 nm. It is clear from the TEM micrograph that the particles are nearly cubic in shape. A statistical distribution of particle size is shown in Fig. 11.6b, which shows average particle size of a silver nanocrystal to be about ~22 nm.

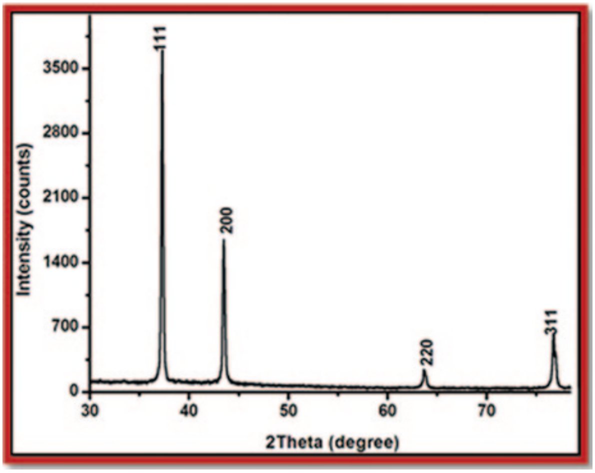


Fig. 11.3 XRD pattern of silver nanocrystals. (Adopted with kind permission from Arshi et al. 2011b)

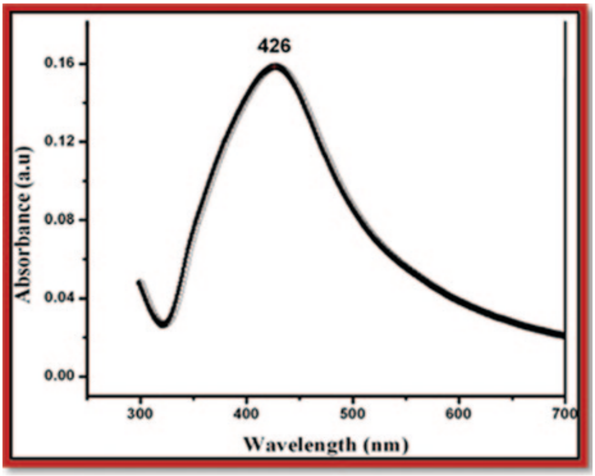


Fig. 11.4 UV-Vis spectrum of silver nanocrystals. (Adopted with kind permission from Arshi et al. 2011b)

3.3 ZnO Nanostructures

ZnO nanoflowers consisting of nanorods have been prepared using efficient, cost-effective and energy-saving microwave-assisted solution method. The as-synthesized nanorods were characterized by using XRD, FESEM and HRTEM measurements. These ZnO nanorods exhibit room temperature ferromagnetism which may be due to the presence of defects (oxygen vacancies) in the rods. A schematic representation of the possible growth mechanism of ZnO nanoflowers is shown in Fig. 11.7.

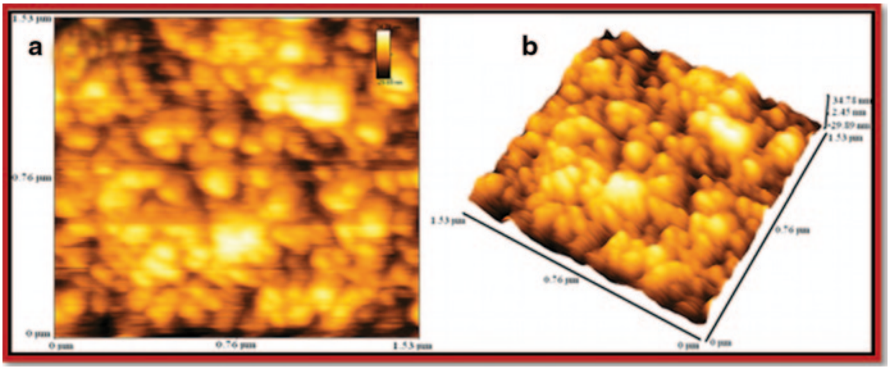


Fig. 11.5 AFM images of silver nanocrystals **a** 2D preview **b** 3D preview. (Adopted with kind permission from Arshi et al. 2011b)

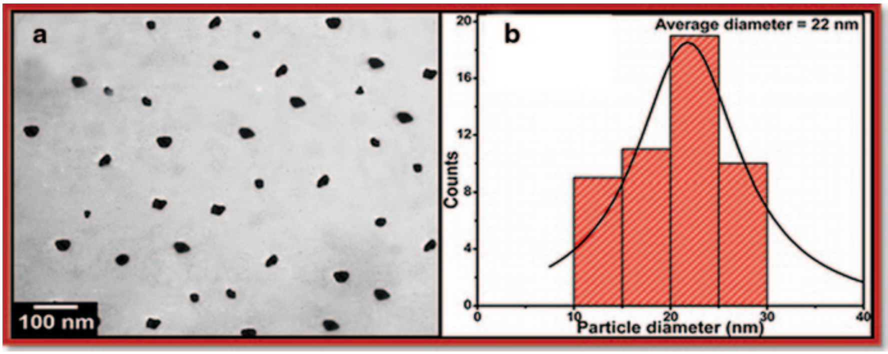


Fig. 11.6 **a** TEM image of silver nanocrystals **b** corresponding statistical size distribution histogram. (Adopted with kind permission from Arshi et al. 2011b)

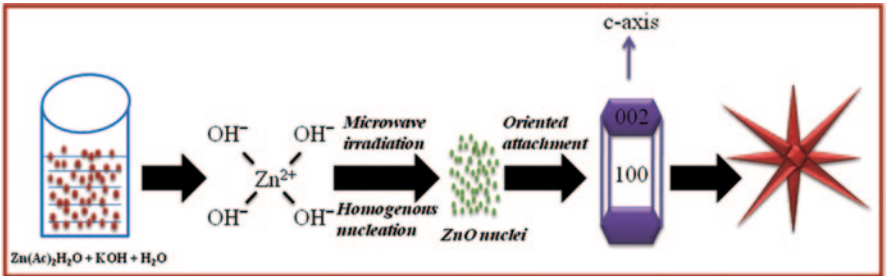


Fig. 11.7 Schematic diagram of the formation process of flowerlike ZnO nanostructures. (Adopted with kind permission from Ahmed et al. 2011a)

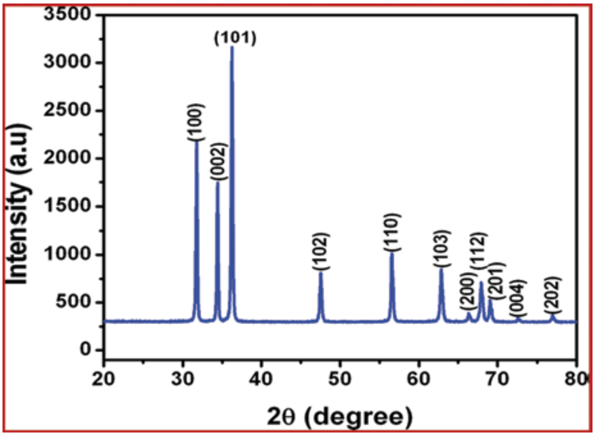


Fig. 11.8 XRD pattern of ZnO nanorods. (Adopted with kind permission from Ahmed et al. 2011a)

Figure 11.8 shows the typical XRD pattern of as-prepared ZnO nanopowder, which was indexed using POWDER-X software as the pure hexagonal phase ZnO with the lattice parameters a = 3.254 Å and c = 5.197 Å. No diffraction peaks from any other impurities are detected and the sharpness of the peaks implies the good crystallinity of the as-prepared ZnO nanorods. Figure 11.9a shows the typical FESEM images of the ZnO nanostructures. It can clearly be seen from the image that the as-synthesized ZnO nanorods are flowerlike clusters. Complementary morphological description is achieved through the TEM equipped with the SAED, as shown in Figs. 11.9b, c. The diameter of the nanorods is within 150–190 nm (tip diameter ~15 nm) with a length of about 2 μm. Figure 11.9c shows a typical TEM image of a single ZnO nanorod to confirm the crystal quality and growth direction. Well-resolved lattice spacing of 0.265 nm corresponding to the d spacing of the wurtzite ZnO (002) plane also indicates that the ZnO nanorod is of a single crystal in nature and referentially grows along the [001] direction (c-axis), which is further confirmed by the SAED pattern. Figure 11.9d shows the chemical composition of the nanorods determined by EDS. Only oxygen and zinc signals have been detected, which confirms that the nanorods are primarily ZnO.

4 Mechanism of Nanomaterials-Plants Interaction

Nowadays, a lot of attention is being given to the effect of different nanoparticles on plant growth and their metabolic functions. Plants cell walls, having a primary site for interaction, serve as a barrier for the entry of any external agent, including nanoparticles into plant cells. Major cell wall components include carbohydrates and proteins (Heredia et al. 1993; Knox et al. 1995), and these walls are semi-permeable in nature which permits the entry of small molecules and blocks the larger ones.

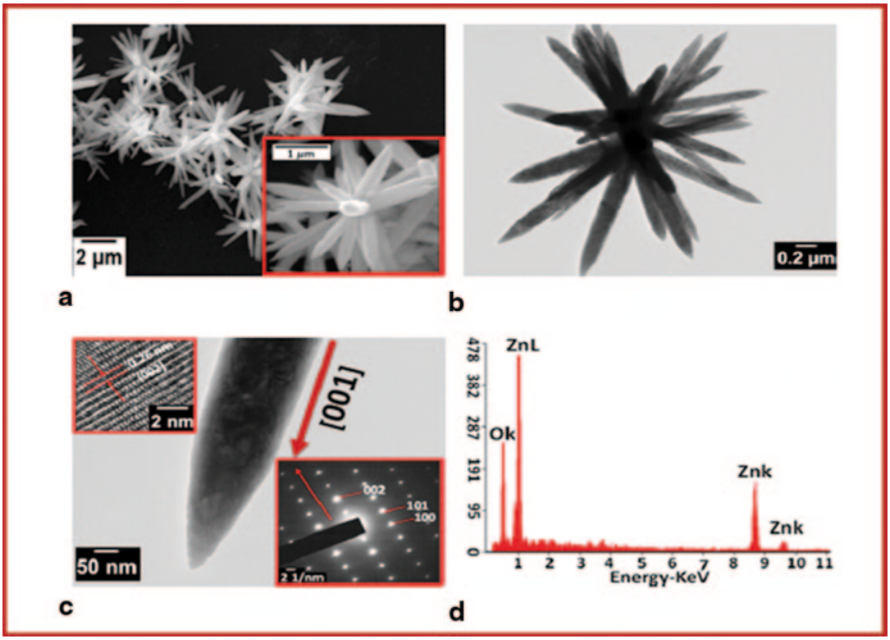


Fig. 11.9 **a** FESEM images of ZnO nanorods, TEM micrographs of **b** an individual ZnO flower and **c** focused image of a single ZnO nanorod. The upper left and lower right insets in **c** correspond to the HRTEM image and SAED pattern of a single nanorod, respectively, **d** EDS spectra of ZnO nanorods. (Adopted with kind permission from Ahmed et al. 2011a)

The pore diameter of the cell walls having a thickness ranging from 5 to 20 nm, determines its sieving properties (Fleischer et al. 1999; Fujino et al. 1998; Madigan et al. 2003; Zemke-White et al. 2000). Consequently, nanoparticles having a size smaller than that of the largest pore can easily pass through the cell wall and reach the plasma membrane. The enlargement of pores or induction of new cell wall pores might be possible upon interaction with nanoparticles, thus increasing the uptake of the nanoparticles through the cell wall. For example, ZnO nanoparticles have been reported to increase permeability and even create “holes” in bacterial cell walls (Brayner et al. 2006; Sondi et al. 2004; Stoimenov et al. 2002) with pore size similar to plant cell walls (Carpita et al. 1979). As the nanoparticles pass the cell wall, they reach the plasma membrane. This plasma membrane forms a cavity-like structure which surrounds the nanoparticles and pulls it into the cell during the endocytic process. The nanoparticles may also cross the cell membranes using embedded transport carrier proteins or ion channels. As the nanoparticles enter the cell, they may bind with different types of organelles (e.g., endoplasmic reticulum, Golgi, and endo-lysosomal system), and interfere with the metabolic processes at that site, possibly as a result of the production of reactive oxygen species (ROS) (Jia et al. 2005).

Plants also get exposed to nanomaterials in atmospheric and terrestrial environments. Nanomaterials present in air are attached to leaves and other aerial parts of plants while the soil-material-associated nanomaterials interact with the roots. Therefore, it is expected that the plants having higher leaf area indexes (LAI) also have a higher interception potential for air-borne nanomaterials, which in turn increase their entrance into trophic webs. Once the nanomaterials are applied on the leaf surface, they might penetrate the plants via the bases of trichomes or through stomatal openings and get translocated to different tissues. Due to stomata obstruction, the accumulation of nanomaterials in photosynthetic surfaces might provoke foliar heating which results in alteration of the gas exchange (Da Silva et al. 2006). This heating might produce changes in various physiological and cellular functions of plants (Da Silva et al. 2006).

5 Nanomaterials for Crop Improvement

5.1 Carbon-Based Nanomaterials

Carbon-based nanomaterials such as single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotube (MWCNTs), carbon buckyballs, etc., have found vast applications in the field of agriculture and food. Canas et al. (2008) reported the effects of functionalized SWCNTs and non-functionalized SWCNTs on root elongation of six different crop species, such as cabbage (Brassica oleracea), cucumber (Cucumissativus), carrot (Daucuscarota), onion (Allium cepa), lettuce (Lactuca sativa), and tomato (Solanumlycopersicum). They showed that the root elongation in onion and cucumber was enhanced by non-functionalized SWCNTs, and the interaction of both functionalized SWCNTs and non-functionalized SWCNTs with root surface, resulted in the formation of nanotube sheets on cucumber root surface, without entering into the roots. However, cabbage and carrot remained unaffected by either form of nanotubes. Furthermore, functionalized SWCNTs inhibited the root elongation of lettuce, while tomato was found to be most sensitive to non-functionalized SWCNTs with significant root length reduction, whereas a positive response has been shown on the seed germination and growth of tomato plants upon interaction with MWCNTs (Khodakovskaya et al. 2009). They showed that the presence of MWCNTs increased water uptake by seeds which in turn enhanced the germination process (Fig. 11.10a, b). Tomato seeds placed on medium with different concentrations of MWCNTs germinated on the third day, while the tomato seeds placed on regular MS (Murashige and Skoog) medium did not germinate at that time (Fig. 11.10b).

Similar positive effects of MWCNTs on seed germination and root growth of six different crop species—radish (Raphanussativus), rye grass (Loliumperenne), rape (Brassica napus), lettuce (Lactuca sativa), corn (Zea mays) and cucumber (Cucumissativus)—was also reported (Lin et al. 2007). Very recently, Remya et al. (2010) also reported the positive effects of both SWCNTs and MWCNTs on the germination of rice seeds and observed an enhanced germination for seeds germinated in the presence of nanotubes. But, the interaction of different nanomaterials with plants and their mechanism for genetic and molecular modification of plants

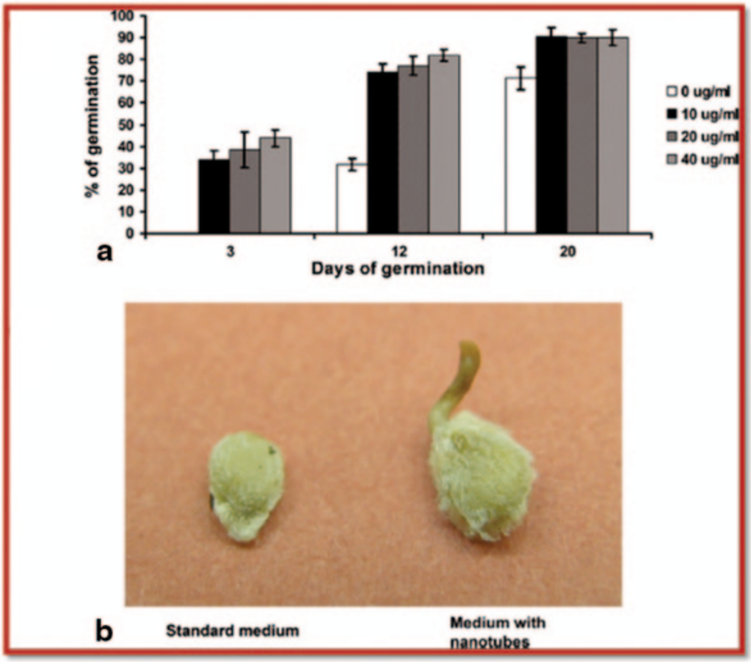


Fig. 11.10 Effect of CNTs on tomato seed germination. **a** Time of germination and germination percentages of seeds incubated with and without CNTs during 20 days. Seedlings with developed cotyledons and root system were recognized as fully germinated in this experiment. **b** Phenotype of tomato seeds incubated during three days without (*left*) or with (*right*) CNTs on MS medium. (Adopted with kind permission from Khodakovskaya et al. 2009)

are still unpredictable. The interaction of nanomaterials with plants differs with type and time of exposure to nanomaterials, so these facts should be kept in mind while performing nanotoxicity studies. Additionally, the orientation of nanotubes with respect to the plant cell wall might be important for their penetration, but the mode of entry of nanotubes through the cell wall remains mysterious which still needs more studies.

5.2 *Magnetic Nanomaterials*

Magnetic nanoparticles have received enormous attention because they allow specific localization of the particles to release their load, which plays a crucial role in the applications of nanoparticulate delivery for plants. Some research groups (Gonzalez-Melendi et al. 2008; Zhu et al. 2008; Corredor et al. 2009) have reported the uptake, translocation and specific localization of magnetic nanoparticles in pumpkin plants. No toxicity detected on plant growth which suggested that these kinds of

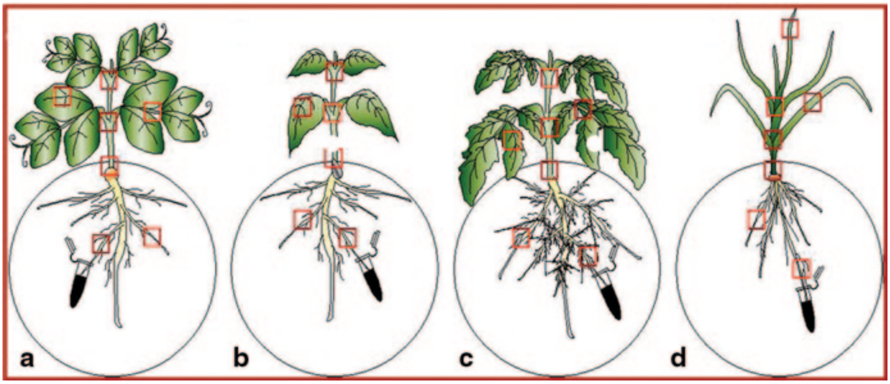


Fig. 11.11 Schematic representation of the Petri dish rizhotron with the four crops: **a** pea; **b** sunflower; **c** tomato; **d** wheat. Squares indicates sampling points of plant tissues. (Adopted from kind permission of Cifuentes et al. 2010)

nanoparticles are safe for nanoparticulate delivery in plants. Recently, genetic effect of ferrofluids has been a subject of great interest to nanobiologists as it leads to chromosomal aberrations in young plants (Racuciu et al. 2007a, 2009; Pavel et al. 1999, 2005). Racuciu et al. (2007b) analysed the influence of magnetic nanoparticles coated with tetramethylammonium hydroxide on the growth of *Zea mays* plant in early ontogenetic stages. They showed that these nanoparticles not only have chemical but also magnetic effect on the enzymatic structures in the different stages of photosynthesis. At low concentration of ferrofluid, the level of 'chlorophyll' was increased while higher concentrations of ferrofluid led to its inhibition. Also, the magnetic nanoparticles have the possibility to create some magnetic effects on the enzymatic entities involved in different photosynthetic and developmental stages. Therefore, in order to design the biotechnological tools for plant cultures, it is important to know the suitable ranges of ferrofluid concentration, so that a better yield of biochemical mutant types with improved photosynthetic pigment levels can be achieved. Zhu et al. (2010) reported that in an aqueous medium containing magnetite nanoparticles for the growth of Cucurbita maxima, particles can absorb, move and accumulate in the plant tissues. On the other hand, Phaseoluslimensis is not able to absorb and move particles. Therefore, different plants have different response to the same nanoparticles.

Cifuentes et al. (2010) studied the absorption and translocation of carbon-coated magnetic (iron) nanoparticles through the root in four crop plants (Fig. 11.11) belonging to different families—sunflower (*Helianthus annuus*) from the family Compositae; tomato (*Lycopersicum sculentum*) from the Solanaceae; pea (*Pisum sativum*) from the Fabaceae; and wheat (*Triticumae sativum*), from theTriticeae. They showed that after only 24 hours of exposure to the bioferro fluid, nanoparticles were able to leak into the vascular tissues of the tested crops (Fig. 11.12). This indicates that in order to get big amounts of nanoparticles inside the plant, the immersion of the roots into nanoparticle solutions is faster and more reliable than

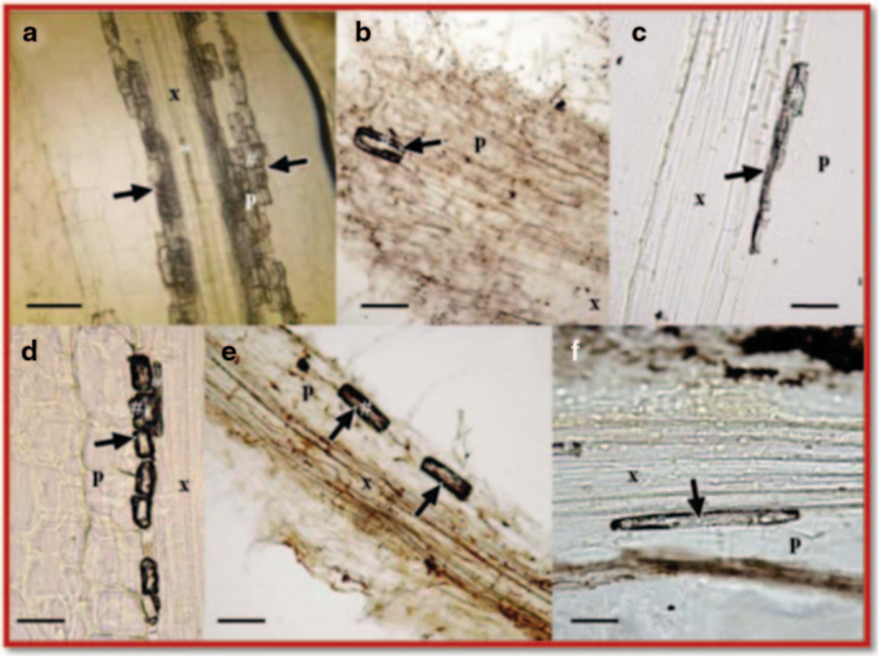


Fig. 11.12 Longitudinal sections of roots of pea (**a**, **d**), sunflower (**b**, **e**) and wheat (**c**, **f**). Arrows indicate accumulation of bioferrofluid in the cells; *, xylem-containing ferrofluid; #, parenchimatic cell-containing ferrofluid; p, parenchimatic cells; x, xylem vessels. Scale bars: (**a**) and (**f**), 50 µm; (**b**) and (**e**), 100 µm; (**c**) and (**d**), 25 µm. (Adopted with kind permission from Cifuentes et al. 2010)

applying the bioferrofluid through the leaves and aerial parts by pulverization or injection (Corredor et al. 2009; Ghosh et al. 2008).

For example, Pea roots accumulated higher contents of bioferrofluid (Fig. 11.12a) than sunflower or wheat which remain unchanged even after 48 h of exposure to bioferro fluid (Fig. 11.12d–f). This shows that pea roots could be more permeable to nanoparticle penetration. Therefore, the speed of absorption and distribution of the nanoparticles is faster in pea and wheat than in tomato and sunflower. This fast movement of the nanoparticles inside the plants can be an important factor in the development of nanoparticles as smart delivery systems inside the plants.

5.3 Metal-Based Nanomaterials

5.3.1 Gold Nanoparticles

In recent years, gold nanoparticles have been used in many biomedical and agricultural applications (Paciotti et al. 2004; Rosi et al. 2005; Peer et al. 2007; El-Sayed et al. 2006; Shukla et al. 2005; Arshi et al. 2011a). In most of these applications, it is essential that nanoparticles should pass cell plasma membranes either by endocytosis (Onelly et al. 2008) or by direct penetration to reach target cellular compartments. Onelly et al. (2008) reported the internalization of gold nanoparticles using tobacco protoplasts. In their report, they showed the penetration of gold nanoparticles into the protoplasts by endocytosis linking to different pathways upon their charge.Therefore, endocytosis appears as a reasonable way for internalization of nanoparticles. A recent report deals with penetration of gold nanoparticles through lipid membranes bypassing endocytosis (Lin et al. 2010). Their mean force calculations showed a significant gain of energy upon adhesion and penetration. In the case of penetration, it was found that defective areas were induced across the entire surface of the upper leaflet of the bilayer and a hydrophilic pore that transports water molecule was formed with its surrounding lipids highly disordered. It was also found that the increase in charge density of gold nanoparticles increased the level of penetration and membrane disruption. These findings suggest a way of controlling the gold nanoparticles–cell interactions by manipulating surface charge densities of gold nanoparticles to achieve designated goal in their biological applications, such as a delivery agent.

5.3.2 PalladiumNanoparticles

Since the geogenic background, a significantly lower concentration of palladium (Pd) than the concentration of other non-essential toxic elements such as mercury, lead or cadmium, Pd may not yet have affected biological systems. Earlier studies (Battke et al. 2008; Jo et al. 2009) showed a higher mobility and uptake rates of Pd in soil than platinum, e.g., in grass samples from roadsides. Battke et al. (2008) studied the uptake of Pd in barley and behavior of Pd nanoparticles in nutrient solutions used to grow plants, in order to develop a model of Pd exposure of plant systems. Their results showed that smaller and larger Pd particles were comparatively assessed and the Pd uptake, via the roots, depends on its particle diameter. Pd nanoparticles of smaller diameter cause significant effects on leaf length growth. As the concentration of Pd increased in the nutrient solution, leaf length decreased significantly with the increased variability of leaf lengths. Moreover, with increasing Pd concentration in the nutrient solution, leaves become rigid and slightly convoluted.

5.3.3 Silver Nanoparticles

Owing to their several antimicrobial functions, silver nanoparticles have been widely used to control various phytopathogens (Park et al. 2006; Min et al. 2009; Kim et al. 2009; Stampoulis et al. 2009; Arshi et al. 2011c). Harris et al. (2008) studied the uptake limits and the distribution of silver nanoparticles in *Brassica juncea* and *Medicago sativa*. They observed that *Medicago sativa* showed an increase in metal uptake with a corresponding increase in the substrate of metal concentration and exposure time as compared with *Brassica juncea*. Study of hydroponic solution mended with Ag nanoaprticles for the seed germination and root growth of zucchini plants, showed no negative effects, whereas on prolonging their growth in the presence of Ag NPs, a decrease in plant biomass and transpiration was observed (Rehm et al. 1997).

5.4 Metal Oxide-Based Nanomaterials

5.4.1 ZnO Nanoparticles

Zinc (Zn) is one of the necessary micronutrients required for the optimum growth of plants. It plays an important role in driving many metabolic reactions within the plants. Zn is also a part of several other enzymes such as super oxides dismutase and catalase, which prevent oxidative stress in plant cells. Growth and development would stop if specific enzymes were not present in the plant tissues (Vitosh et al. 1994). Important role of Zn can be decided as it controls the synthesis of indole acetic acid (IAA), a phytohormone which significantly regulates the plant growth. It also helps in the chlorophyll synthesis and carbohydrate formation (Adiloglu et al. 2002) and enables the plants to withstand lower air temperatures. Zn helps in the biosynthesis of cytochrome, a pigment and maintains the plasma membrane integrity, and the synthesis of leaf cuticle. Additionally, the enhancement of Zn nutritional status helps in reducing harmful heavy metals uptake which hinders their toxicity in plants, such as Cd (Pandey et al. 2010). Pandey et al. (2010) studied the effect of ZnO nanoparticles consisting of oxygen vacancies on the seed germination and root growth of *C. arietinum* seeds. They observed that due to oxygen vacancies, the oxygen deficient, i.e., Zn-rich ZnO nanoparticles increased the level of IAA in roots (sprouts), which in turn indicated the increase in the growth rate of plants.

Due to the presence of very small quantity of Zn, the amount of Zn utilized byplant is very less, this ensures a controlled delivery of Zn to the plant, hence the excess of Zn that may toxicate the plant is avoided. Furthermore, its excess amount would least spoil the soil quality as ZnO is an eco-friendly and bio-friendly material which can be used as a green reagent.

5.4.2 TiO_2 Nanoparticles

Owing to its photocatalytic nature, TiO_2 nanoparticles under light are able to generate an oxidation–reduction reaction, and produce superoxide anion radical and hydroxide (Crabtree et al. 1998; Hong et al. 2005a). Photosterilization by TiO_2 nanoparticles improves the growth and development of plants. Hong et al. (2005b) reported the effects of TiO_2 nanoparticles (rutile phase) on the photochemical reaction of chloroplasts of Spinaciaoleracea. They found that the treatment of TiO_2 nanoparticles enhanced the Hill reaction and chloroplasts activity, which accelerated FeCy

Fig. 11.13 Effect of nanoanatase TiO_2 on the growth of spinach. The picture was taken after four weeks of cultivation. Lane 1 Control, lane 2 treated with bulk-TiO_2, lane 3 treated with nano TiO_2 (anatase). (Adopted with kind permission from Linglan et al. 2008)

reduction and oxygen evolution. Furthermore, noncyclic photophosphorylation activity was higher than that of cyclic photophosphorylation activity. The possible reason, according to author, could be due to the penetration of TiO_2 nanoparticles into the chloroplast and their oxidation-reduction reactions which accelerate electron transport and oxygen evolution. TiO_2 nanoparticles (anatase) have also been found to induce spinach seed germination and plant growth (Fig. 11.13) by regulating the germination of aged seeds and their vigor indexes (Linglan et al. 2008; Yang et al. 2006). An increase of these indexes was observed at 0.25–4 % TiO_2 nanoparticles treatments. Furthermore, it was observed that during the growth stage, the presence of TiO_2 nanoparticles increased the dry weight, chlorophyll synthesis, and metabolism in photosynthetic organisms. These results confirmed that the nanometer-size particles have remarkable effects on physiological processes. These positive effects are assumed to be due to the antimicrobial properties of nanoparticles, which in turn can increase strength and resistance of plants to stress. Additionally, nanoparticles could also sequester nutrients on their surfaces and thus serve as a nutrient stock to the organisms, especially those nanoparticles having high specific surface area. The authors also reported that the effects of bulk-TiO_2 particles were not significant. In another report, it was shown that TiO_2 nanoparticles (anatase) improved plant growth by enhanced nitrogen metabolism (Yang et al. 2007) which promotes the absorption of nitrate in spinach, and helps in accelerating the conversion of inorganic nitrogen into organic nitrogen, consequently increasing the fresh and dry weights. Other studies also showed the effects of nitrogen photoreduction on the improved growth of treated spinach plant (Mingyu et al. 2007). It has also been reported that

TiO_2 nanoparticles (anatase) enhanced antioxidant stress by decreasing the accumulation of superoxide radicals, hydrogen peroxide, malonyldialdehyde content and increase the activities of superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase and thus increase the evolution oxygen rate in spinach chloroplasts under UV-B radiation (Lei et al. 2008).

6 Conclusion and Future Scenario

Nanobiotechnology being studied since several years is still in the early stages of advancement, however, the development is multi-directional and spreading rapidly. Moreover, the increasing interest in nanobiotechnology has attracted enormous attention which led to the rapid development of commercial applications involving utilization of manufactured nanomaterials for crop improvement. In order to reduce the collateral damage in plants, nanomaterials are proved to be a promising tool to distribute pesticides and fertilizers in a controlled manner. In the framework of plant–pathogen interaction, nanomaterial-based tools and their efficient transportation to specific sites provides novel solutions for the plants treatment. As compared to bulk materials, size of nanoparticles plays key role in the behavior, reactivity and toxicity of nanoparticles. With these characteristic, it is obvious to discover both positive and negative effects of nanoparticles on plants. Therefore, for assessing toxicity and trophic transport of nanoparticles, an indepth understanding of plant interactions with the nanoparticles is very important. Recently, Sabo-Attwood et al. (2011) reported that gold nanoparticles, AuNPs, enter plants through size-dependent mechanisms, translocate to cells and tissues and cause biotoxicity. We also need to be very careful of the presence of engineered nanoparticles in our environment which may be of potential risk to the ecosystem. Recently, Dey et al. (2011) have studied the effect of nanomullite (NMu) and their metal-amended derivatives on the growth of mung bean plants and found that the metal-amended NMu exerts adverse effects on the growth and biomass production of plants compared to NMu. The plant system can also be used to test the phytotoxicity of the nanoparticles as Ma et al. (2010) have investigated the phytotoxicity of four rare earth oxide nanoparticles—nano-CeO(2), nano-La(2)O(3), nano-Gd(2)O(3) and nano-Yb(2)O(3)—on seven higher plant species (radish, rape, tomato, lettuce, wheat, cabbage and cucumber) by means of root elongation experiments. Their results were helpful in understanding phytotoxicity of rare earth oxide nanoparticles (Ma et al. 2010). Recently, the phytotoxic and genotoxic effects of ZnO nanoparticles on garlic (*Allium sativum* L.) have also been reported (Shaymurat et al. 2011).

Can metal nanoparticles be a threat to microbial decomposers of plant litter in streams is a big question for which we need to be worried. Recently, Pradhan et al. (2011) have suggested that the extensive use of nanometal-based products can increase the chance of their release into aquatic environments, which can pose a risk to aquatic biota and the associated ecological processes. If there is a possibility to distribute and guide the well functionalized nanoparticles all over the plant vascular system to targeted sites, the subsequent unloading of chemicals (fungicides, insecticides, etc.) can be achieved by using these nanoparticles. Plant cell-nanoparticles interaction modifies the plant gene expression and its biological pathways, which consequently affects plant growth and development.

It is well known that nanobiotechnology industry is spreading rapidly; nevertheless, there is a crucial urgency to perform further studies on the subject. Hence, future work is needed to evaluate how the nanoparticles penetrate and are transported within the plants, and also the mechanism of intracellular internalization to explore the potential use of nanoparticles. However, in spite of the fact that plants have the capability to endure the presence of nanoparticles inside their tissues, an important issue that arises is what happens when such nanoparticles move into the food chain.

Exploitation of the biological machinery of nature for designing a 'smart' biomaterial such as forisomes could also be used to develop stress tolerant plants. Forisomes are spindle-like bodies that are composed of ATP-independent, calcium-powered, mechanically active proteins which are present in sieve tubes in legumes (Tuteja et al. 2010a–c). When legumes experience mechanical injury, forisomes disperse and occlude sieve tubes to hinder leakage of photoassimilates or invasion of phytopathogens (Tuteja et al. 2010c). The interesting properties of the forisomes could be exploited in biomimetics and in nanobiotechnological devices (Shen et al. 2005; Knoblauch et al. 2004a, b; Peters et al. 2008). The overexpression of forisomes in crops may also lead to the development of the insect injury resistant plants.

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