Nanotechnology in Agriculture

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S. Saranya, R. Aswani, A. Remakanthan,
and E. K. Radhakrishnan

1.1 Introduction

Agriculture is always an important sector as it offers the raw materials for food and feed industries. The agricultural products come across in our lives in a number of ways such as food, fuel, furniture, textiles, and feedstock. However, agricultural productivity is very much challenged by the insufficient space, diseases, and changes in agro-climatic conditions. This demands the need to adopt modern technologies which focuses on improved agricultural production (Yunlong and Smit 1994). The sustainable growth of agriculture can greatly be accelerated by new, smart, and innovative techniques like nanotechnology (Tilman et al. 2002; Prasanna and Hossain 2007; Ditta 2012; Mishra et al. 2014).

Nanotechnology is a promising field of interdisciplinary research. The term "nanotechnology" was popularized by Professor Taniguchi et al. (1974) (Bulovic et al. 2004). Nanotechnological developments have resulted in advanced instrumentation to isolate and characterize nanomaterials in a precise way (Adams et al. 2005; Bonnell and Huey 2001; Gibney 2015). Nanoparticles have remarkable properties which make them to have applications in different fields like electronic, medicine, pharmaceuticals, engineering, and agriculture. The materials that have less than 100 nm size are known as nanoparticles (NPs) (Thomas et al. 2012). Fundamental characters and physico-chemical properties of NPs are different from those of the corresponding bulk material. Biologists and chemists are actively engaged in the synthesis of organic, inorganic, metal, and hybrid nanoparticles with unusual optical, physical, and biological activities (Thomas et al. 2012; Nanjwade et al. 2011). Nanomaterials are synthesized by two basic methods, the top-down and bottom-up approaches.

S. Saranya · A. Remakanthan
Department of Botany, University College, Thiruvananthapuram, Kerala, India

R. Aswani · E. K. Radhakrishnan (✉)
School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, India
e-mail: radhakrishnanek@mgu.ac.in

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Techniques such as polarized optical microscopy (POM), scanning electron microscopy (SEM), transmission electron microscopy/high-resolution transmission electron microscopy (TEM/HRTEM), scanning tunnelling microscopy (STM), and atomic force microscopy (AFM) are used for the morphological characterization of nanoparticles. The composition and the nature of materials are studied by energy dispersive X-rays (EDX) analysis, X-ray diffraction (XRD), Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), particle size analyzer, and dynamic light scattering (DLS) (Babu et al. 2016; Bacc et al. 2006).

More than 1300 commercial nanomaterials, with potential applications, are currently available. The synthesis of nanomaterials (NMs) with specific composition, size, and properties has expanded their effective application in various fields including agriculture. Nanomaterials used in agriculture may be of natural origin or engineered particles. Engineered nanomaterials (ENs) can roughly be categorized into inorganic, organic, and combined materials which include surface-modified clay. Metals, metal oxides, salts, carbon nanotubes, fullerenes, and carbon black have broad applications. Lipid-based NMs containing micelles and liposomes have remarkable stability. Protein-based NMs are often developed from molecules having self-assembling property (Puri et al. 2009). The size to shape features can influence the characteristics of the nanoparticles (Yang and Ma 2010; Khan et al. 2017).

Application of engineered NMs (ENMs) has been demonstrated to enhance the earlier plant germination as well as plant production (Servin and White 2016). Some plants are efficient in uptaking and accumulating engineered nanomaterials. The interaction of plant cell with the ENs can lead to the modulation of plant gene expression and associated biological pathways, which eventually affect the plant growth and development. Effect of ENs on various plant species can vary with the stages of plant growth, method, and duration of exposure (Panpatte et al. 2016). At the same time, this can also be influenced by the shape, size, chemical composition, concentration, surface structure, aggregation, and solubility of ENs. Some of the engineered nanomaterials are also reported to enhance the growth of many plants (Miralles et al. 2012). Carbon-based nanomaterials are more hydrophobic, and this property enhances their ability to interact with several organic substances. Single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs) are the most studied nanotubes. Fullerene particles with size lower than the pore diameter of cell wall could simply pass through and reach the plasma membrane. Its further transmission has been indicated by the fullerene aggregation in leaves (De La Torre-Roche et al. 2012).

Various cultural methods including the use of fertilizers and pesticides are used to enhance the crop yield, but these have been demonstrated to have serious and even life-threatening aftereffects. Hence there is an urgent need to upgrade the agricultural practices and methods with new-generation technologies. Here comes the relevance of application of nanotechnology in agriculture. Various nanotechnological methods are shown to have applications in agriculture to enhance the productivity. These involve development of nano-formulations of agrochemicals for crop protection, toxicity identification through nanobiosensors, plant genetic manipulation mediated through nanodevices, and smart and effective diagnosis of plant diseases. The genetic material and protein delivery with the help of nanoarray are also shown to have applications in crop engineering, drug delivery, pathogen detection, and environmental monitoring (Pandey et al. 2010; Mc Loughlin 2011; Jacobson et al. 2005; Mir et al. 2018).

The nano-based methods have become easier with the development of bio-based methods for the synthesis of metal nanoparticles (Au, Ag, Fe, Pt, Ti, Zn, Mg, etc.) (Delfani et al. 2014; Dimkpa et al. 2017; Kharissova et al. 2013). Biological materials such as plant extracts, sugars, polyphenols, vitamins, and microorganisms have been widely used as reducing and capping agents to generate stable and biocompatible nanoparticles with enhanced longevity (Parsons et al. 2007; Kalaiarasi et al. 2010; Rai et al. 2011).

The use of nanowires, nanofilters, nanofibrous mats, and quantum dots (QDs) to resist plant pathogens and as interactive agrochemicals provides new opportunities in agriculture and related fields. QDs have unique spectral properties and hence have been used as new-generation fluorophores in bioimaging and biosensing (Bakalova et al. 2004). QDs at low concentration were revealed to have no detectable toxicity for seed germination and seedling growth (Das et al. 2015). QDs can also be utilized for live imaging in plant root systems to verify the known physiological processes (Hu et al. 2010; Das et al. 2015). The use of gold nanorods to transport plant growth regulator 2,4-D has been demonstrated to have significant influence on the regulation of tobacco cell culture growth (Nima et al. 2014). Even though chemically synthesized nanoparticles have been reported to have varying toxicity, such issues for agricultural applications can be minimized by using biofabricated nanoparticles. One emerging area of such application is the myconanotechnology, where fungi can be harnessed for the production of nanomaterials or nanostructures with distinct shape and size. Here, the functional reducing agents, metabolites, and enzymes produced by fungi can convert the toxic ions into less toxic nanomaterials. Mycosynthesis of triangle-shaped intracellular gold nanoparticles (20–35 nm) has been reported using the endophytic fungus *Aspergillus clavatus* isolated from *Azadirachta indica* (Verma et al. 2011). Several other species of *Aspergillus*, including *Aspergillus niger* (Gade et al. 2008, 2010, 2011), *A. fumigatus* (Bhainsa and D'souza 2006; Navazi et al. 2010), *A. flavus* (Vigneshwaran et al. 2006, 2007; Moharrer et al. 2012), *A. oryzae* var. *viridis* (Binupriya et al. 2010), and *A. terreus* (Li et al. 2012), have been reported as promising candidates for the fabrication of silver and gold NPs. Most importantly, the biofabricated NPs have been demonstrated to have reduced toxicity compared to chemically produced NPs (Sanchez-Mendieta and Vilchis-Nestor 2012; Varma 2012; Órdenes-Aenishanslins et al. 2014; Moharrer et al. 2012). Nanoparticles (NPs) and nanomaterials (NMs) have been shown to be an effective alternative. The development of nanofertilizers proves it to be more efficient than traditional fertilizers. For managing challenges with stress tolerance and nutritional quality in crops, the use of nanofertilizers is shown to have promising future.

Nanotechnology can have significant impact on precision farming which focuses on maximizing output (i.e., crop yields) through minimal chemical input (i.e., fertilizers, pesticides, herbicides, etc.) through monitoring of environmental factors and targeted action (Hussein et al. 2005). This enables the restriction of accumulation of agrochemicals in the soil and water (De-Lugue and Rubiales 2009; Rickman et al. 2003). Computers, global satellite positioning systems, and remote sensing devices are used in the precision farming to measure the highly localized environmental factors. This will be helpful to know whether crops are growing at maximum productivity or precisely identify the nature and location of problems. This technology helps to minimize the agricultural waste and environmental pollution and offers effective methods to maintain the soil health and conditions (Raliya et al. 2017; Duhan et al. 2017). Nanotechnological approaches can greatly enhance the functioning of precision farming.

Growing plants in soil-free medium is called hydroponics and has been widely used to grow crop plants (Seaman and Bricklebank 2011). Nanotechnology has also been described to improve functioning of hydroponic system (Schwabe et al. 2013). As there is a big demand for the fast, reliable, and low-cost systems for the identification, monitoring, and diagnosis of various agricultural issues, nanotechnology can have significant application in this sector (Vidotti et al. 2011; Sagadevan and Periasamy 2014). However, the application of chemically synthesized nanomaterials is considered to have more toxicity; hence green nanotechnology-based methods have more demand (Prasad et al. 2014).

1.2 Nano-Based Products

1.2.1 Nanofertilizers

Nanofertilizers involve materials which are modified at the nanoscale. Nanofertilizers generally include nano zinc, titanium dioxide, silica, and iron. Studies on the uptake, biological fate, and toxicity of several metal oxide NPs like Al_2O_3, TiO_2, CeO_2, FeO, and ZnO have been studied intensively in the present decade for agricultural production (Dimkpa 2014; Zahra et al. 2015; Zhang et al. 2016). Usually fertilizers are applied into the soil as surface application or applied after mixing with water. Majority of these fertilizers become unavailable to plants as they are lost as run-off leaching resulting in pollution (Wilson et al. 2008). This indicates the technological need for the development of smart fertilizer or nanofertilizer. The nanomaterial-based formulations are remarkable due to its higher surface area, higher solubility, ability to induce systemic activity because of its smaller particle size, higher mobility, and lower toxicity when compared to conventional fertilizers (Sasson et al. 2007). On the other hand, due to the huge ratio of surface area to volume, NPs possess very good transduction properties which offer its application in analysis of agricultural products (Kandasamy and Prema 2015). Thus, the nanoscale particles have numerous advantages and applications in agriculture when compared to available methods.

Different types of slow release fertilizers (SRF) and controlled release fertilizers have been generated with synthetic or biopolymers. Polymeric nanoparticles have also been used as coating material for biofertilizer to make it resistant to desiccation

(De Rosa et al. 2010; Perlatti et al. 2013). Several nanomaterials have been studied for their nanofertilizer properties which include carbon-based nanoparticles, TiO_2, urea hydroxyapatite, iron oxide, zinc oxide, and $nSiO_2$ nanoparticles (Subbaiah et al. 2016; Kottegoda et al. 2017). A practical problem faced during the pesticide application in the field is the settlement of its components in the spray tank and clogging of spray nozzles. However, nano-sized fungicide (~100 nm, Banner MAXX, Syngenta) was demonstrated to prevent spray tank filters from clogging, and additionally there was no need for mixing as it did not settle down in the spray tank due to their smaller size (Robinson and Zadrazilova 2010). Other advantages with its use include increased mobilization of nutrients, maintenance of soil conditions and microbial population which ultimately favor the improved crop yield, production of high nutrient food, and sustainability. Significant increase in yield has been demonstrated with the foliar application of nanofertilizer (Tarafdar et al. 2012a, b, c; Ghormade et al. 2011). Hence there is significant demand to develop nano-formulations containing all the desired essential nutrients in a suitable proportion. Micronutrient availability to crops can be tremendously improved with the application of nanotechnology. Both micronutrients and fertilizers at nanoscale are shown to enhance soil health (Jose and Radhakrishnan 2018).

Titanium oxide nanoparticle application in soybean has been described to result in drastic enhancement of chlorophyll content and nitrate reductase with enhanced water absorption and improved anti-oxidant system (Kataria Sunita et al. 2019). Increased diosgenin biosynthesis has also been reported in fenugreek with the application of silver nanoparticle which indicates the ability of nanoparticle treatment to enhance secondary metabolite production (Jasim et al. 2017). Increased growth of spinach plant has been noted with the help of titanium oxide nanoparticles through the improved metabolism of nitrogen and photosynthetic rate (Zheng et al. 2005). Zinc oxide nanoparticle application has been shown to promote seed germination, seedling vigor, early flowering, and higher leaf chlorophyll content with increased stem and root growth in peanut (Prasad et al. 2012). These indicate the promises of nano-based methods for the improvement of crop productivity. Silica nanoparticles ($nSiO_2$) were also found to support plant growth under various abiotic and biotic stresses (Kannan et al. 2014). At the same time, TiO_2 nanoparticles have been shown to influence seed germination of tomato (*Lycopersicum esculentum*) with significant improvement in mean germination time, seed germination index, seed vigor index, and seedling fresh weight and dry weight (Siddiqui and Al-Whaibi 2014; Mingfang et al. 2013). Engineered carbon nanomaterials have also been reported to influence the plant growth and development by increasing the root length, seed germination, and biomass production (Khot et al. 2012).

Many commercial nanofertilizers are now available which include NanoGroTM, Nano Green, Master Nano Chitosan Organic Fertilizer, TAG NANO (NPK, PhoS, Zinc, Cal, etc.), Biozar Nano-Fertilizer, and Nano Max NPK Fertilizer (Fig. 1.1). However, the toxic concern of nano-sized materials has not been addressed so far to explore its full application.

Fig. 1.1 Commercial nanofertilizers. (Modified from Prasad et al. 2017)

1.2.2 Nanopesticides

Insect pests cause severe problems in agricultural fields and also in its products. Hence nanotechnology can have a key role in the reduction of insect pests and host pathogens (Khota et al. 2012). The expanding developments in nanoencapsulated pesticide formulations have been demonstrated to have slow releasing properties with enhanced solubility, specificity, permeability, and stability (Kookana et al. 2014; Bhattacharyya et al. 2016). These benefits are mainly achieved through the protection of encapsulated active components from premature degradation or increasing their pest control efficacy for a longer period (Chhipa 2017). Nanoencapsulated pesticide formulations demand reduced dosage of pesticides and hence human exposure, and the resulting issues are minimal which make it to be environmentally friendly for crop protection (Nuruzzaman et al. 2016). Chemical companies are recently promoting nanoscale pesticides for field application (Gouin 2004).

Nanopesticides can contain particles of pesticidal active ingredients or engineered structures with useful pesticidal properties (Fig. 1.2). The objectives of nano-formulations are generally the same as that of other pesticide formulations.

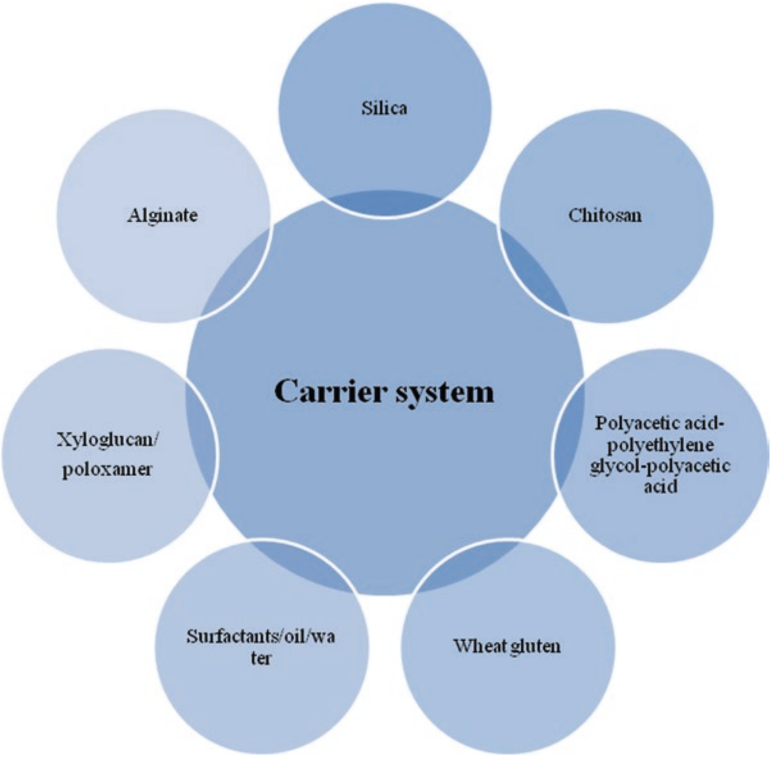

Fig. 1.2 Nanopesticides and herbicides carrier. (Modified from Prasad et al. 2017)

These include enhancement of solubility of poorly soluble active components, release of the active ingredients in a slow or targeted manner, and protection from premature degradation. Nanoencapsulation also helps to increase the stability, delivery, and bioavailability of nutrients and agrochemicals.

1.2.3 Micro- and Nanoencapsulations

Encapsulation is defined as the process in which the given object is surrounded by a coating or embedded in homogeneous or heterogeneous matrix, and this process produces capsules with potential uses (Rodriguez et al. 2016). The benefits of encapsulation include protection of substances from adverse environments, controlled release, and precision targeting (Ezhilarasi et al. 2012; Ozdemir and Kemerli 2016). The capsules in macroscale are developed through macroencapsulation, whereas the micro- and nanoencapsulation give particles with micro- and nanoscale size range (Ozdemir and Kemerli 2016). Vesicular systems where the substances are confined to a cavity consisting of an inner core enclosed by a polymeric membrane are referred to as nanocapsule (Couvreur et al. 1995). Currently, micro- and NPs are getting significant attention for the delivery of drugs, for the protection and increased bioavailability of food components, for food fortification, and also for the self-healing of several materials (Nair et al. 2010; Ozdemir and Kemerli 2016). The development of nanoencapsulated materials for its delivery to targeted tissues will make possible effective delivery of several biologically active compounds (Pohlmann et al. 2008).

1.2.4 Nanoemulsion

The term "nanoemulsion" has been used to describe the complex systems which include oil phase, surfactant, and water, which are optically isotropic and kinetically stable colloidal solution with droplet size (20–200 nm) (Anton and Vandamme 2011; Gutierrez et al. 2008). Nowadays, nanoemulsions are becoming the subject of many studies because of their wide range of particle sizes, and this has contributed to more branches of potential uses and applications (Salim et al. 2011). Nanoemulsions can encapsulate desired components within their droplets, which can reduce its chemical degradation. The nanoemulsion-based pesticides are recently being studied for its potential application.

1.2.5 Nanosensors

Smart sensors, which are developed by nanotechnology, are the powerful tools to track, detect, and control pathogens in plants and animals (Elibol et al. 2003; Rai et al. 2012). The sensitivity and performance of biosensors can be improved by using nanomaterial and also through new signal transduction technologies (Sagadevan Sagadevan and Periasamy 2014; Kwak et al. 2017). Biosensor methods are currently being developed as screening tools in field analysis (Tothill 2011; Jianrong et al. 2004). Many nanotechnology-based biosensors are at various stages of its development (Fogel and Limson 2016). This is due to the development of methods for the modifications of tools and procedures to fabricate, measure, and image nanoscale materials. The NMs such as NPs, CNT, magnetic NPs, metal (cobalt, gold, silver, etc.), and QDs have been actively demonstrated for their applications in biosensors. Integration of chemical, physical, and biological devices to work together as nanoscale sensor has promising potential to detect small amounts of chemical contaminant, virus, or bacteria in agricultural and food systems. Microelectronics and nanotechnology have been combined to develop tiny sensors that can help the farmers in the early detection of post-harvest grain spoilage. Sensors with several chips have already been developed for the detection of insect or fungus responsible for the spoilage, changes in carbon dioxide, and ongoing deterioration of stored grains. Once the specific cause of spoilage is identified, suitable treatment can be made to rectify the problem (Neethirajan et al. 2010). The biosensor system is also an ideal tool for online monitoring of organophosphate pesticides and nerve agents (Liu and Lin 2006). The nanosensors could be distributed throughout the field to identify the soil conditions and crop growth and also for the evaluation of presence of pollutants in the environment (Scott and Chen 2013). With the introduction of new signal transduction technologies to biosensors, its performance can be improved and will also lead to cost reduction (Sertova 2015). Nanotechnology-enabled devices and its linkage to GPS system will enable real-time monitoring of agricultural production.

1.3 Nanoparticle-Mediated Gene Delivery

Micrometer-sized calcium alginate beads with encapsulated plasmid DNA molecules with reporter gene are the biobeads. Biobeads have a possibility for efficient transformation in plants (Takefumi et al. 2002). The use of nanoscale materials for such applications is fast growing due to its high sensitivity and immediate response. Drug delivery systems with liposomes and NPs have become very popular in nanotechnology (Gayathri and Vasudevan 2010). Application of mesoporous silica NPs (MSNPs) has been suggested to be useful in the delivering of chemicals and DNA into isolated plant cells (Lin et al. 2007; Yi et al. 2015). MSNPs which are chemically coated can act as containers for the gene delivery to the plants. The coating stimulates the plant to take the particles through the cell wall. Mesoporous silica NPs are shown to deliver DNA into tobacco protoplasts (Torney et al. 2007). A novel gene delivery method has been developed in plants using poly(amidoamine) dendrimer NPs, and using this successful delivery of green fluorescent protein-encoding plasmid DNA into turf grass cells has been achieved (Shcharbin et al. 2009; Astruc 2012). NP-mediated gene delivery in plants has great importance in plant nanobiotechnology. The gene gun transfer method followed by capping the gene with gold nanoparticles has been shown to result in successful expression in tobacco and maize tissues, and this makes use of simultaneous and target-specific transfer of DNA and the effector molecule (Martin-Ortigosa et al. 2014).

1.4 Nanomaterials in Plant Tissue Culture

The wide range of applications of NPs in plant tissue culture involve elimination of microbial contaminants from explants, callus induction, organogenesis, somatic embryogenesis, somaclonal variation, genetic transformation, and secondary metabolite enhancement. By integrating the concept of nanotechnology into plant tissue culture techniques, synthesis, purification, and yield of desired plant-derived compounds can be improved. Such approaches can have significant industrial applications as many phytochemicals are used as medicinal products. The incorporation of ZnONPs into the MS medium has been described to result in contamination free cultures (Helaly et al. 2014). The combination of AgNPs and thymol has been shown to inhibit microbial growth in *Cynodon dactylon* (Taghizadeh and Solgi 2014). The incorporation of Au NPs into basal MS medium has demonstrated to improve the percentage of seed germination and seedling growth in *Arabidopsis* *thaliana* (Kumar et al. 2014). The Ag NPs (Agrovit, a commercial product) have been shown to have the potential hermetic effect on shoot regeneration of *Vanilla planifolia* in a temporary immersion bioreactor system (Spinoso-Castillo et al. 2017). Au NPs and Ag NPs individually or in combination enhanced the callus proliferation of *Prunella vulgaris* also (Faizal et al. 2016). The incorporation of Ag NPs in tobacco has been shown to be helpful to minimize the damage caused by cellulolytic enzymes during protoplast isolation (Bansod et al. 2015). NMs can have influence on somaclonal variation, and several studies have demonstrated the phytotoxicity of NPs at higher concentration level. The applications of Au and AgNPs have resulted in enhanced somaclonal variation in *Linum usitatissimum*. Rate of occurrence of somaclonal variations was higher in both calli and regenerated shoot in medium supplemented with Au and AgNPs (Kokina et al. 2013). NMs can also influence the secondary metabolite production in tissue culture. The shape of Ag NPs is shown to enhance the effective production of anthocyanins in *Arabidopsis* (Syu et al. 2014). The concentration of ZnONPs in the MS medium has been shown to increase the accumulation of specific bioactive compounds in *Lilium ledebourii* (Chamani et al. 2015). In recent studies, Ag NPs have also been investigated in detail for its role in increasing the content of artemisinin in hairy root cultures of *Artemisia annua* and on atropine production in hairy root cultures of *Datura metel* (Zhang et al. 2013; Shakeran et al. 2015.)

1.5 Plant Protection and Pathogen Detection

The methods for detection and identification of plant pathogens currently depend upon a wide range of technologies and skills, ranging from traditional culturing and taxonomic skills to modern molecular methods. Nanotechnology provides a wide range of opportunity to develop new products to manage pests (Singh et al. 2010; Saurabh et al. 2015). Nano- based sensors offer improved detection of pathogens in plants (Bacc et al. 2006). Nano-chips are popular for their specificity in locating single-nucleotide changes in bacteria and viruses (Lopez et al. 2009). The use of fluorescence silica nanoparticle in combination with antibody has also shown to detect *Xanthomonas axonopodis*, the causative agent of bacterial spot disease in *Solanaceae* plants (Yao et al. 2009). Application of nano-gold-based immune sensors has been demonstrated to detect Karnal bunt disease of wheat with the help of surface plasmon resonance (SPR) (Singh et al. 2010). Modified gold electrode with copper nanoparticle has been studied to monitor salicylic acid in oil seeds (Wang et al. 2010).

The advances in nanofabrication and characterization methods have made the technology to understand properly the plant disease management (Sharon et al. 2010; Patel et al. 2014; Singh et al. 2015). Precision agriculture integrated with smart sensors will allow enhanced productivity in crops by providing accurate information to farmers. The nanofabricated xylem vessels which biomimic the capillary action are capable of shedding insight into the colonization and film development along with the subsequent movement and recolonization by the xylem-inhabiting bacteria (Jose and Radhakrishnan 2018). This will open up new methods to identify and demonstrate plant beneficial organisms. Nanotechnological sensors, techniques, and mode of sensing need to be expanded for the detection of pathogens and their products or monitoring of physiological changes in plants.

The nanotechnological application for plant protection and production of food can have significant contribution to deal with global food security challenges. Nanotechnology has the prospects to change the current approaches in agricultural and food industry by introducing effective methods for the treatment of plant diseases, rapid pathogen detection, and improvement of nutrient absorption power of plants (Lamsal et al. 2011). NPs possess attracted recognition in biological studies owing to their low toxicity, biocompatibility, and unique optical properties. Nanobiosensors and other well-organized delivery systems will also help the agriculture for the management of plant pathogens. Nanobiotechnology techniques are efficient to detect, control, and remediate pollutants by acting as sensors. Further development in green nanotechnology will enable reduction of potential risks. However, the toxic aspect of nanomaterials has not been addressed so far to explore its full application.

1.6 Conclusions

The current chapter described the nanotechnological approaches to enhance crop productivity and yield. This in turn provides a deep understanding on various nanoparticles and its preparation methods for various agricultural applications. Hence the chapter helps to develop advanced and effective nano-formulations for sustainable agricultural practices.

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