



A review on recent developments of nano-biotechnology: An effective and promising pathway for crop production

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Abstract

The emerging of two novel techniques, nanotechnology and biotechnology has resolved many concerns that prevail with the traditional strategies of agriculture. The agricultural sector has many problems achieving the desired yield in products because of pathogens, pesticides, herbicides, temperature, soil moisture, among others. Nanotechnology based agricultural systems have developed with a worthy to manage crop production using drug-carrier and a controllable drug targeting and releasing system as it can enhance the quality of agriculture production. Through advancement in nanotechnology, there are a number of state-of-the-art techniques available including applications of several types of nanoparticles as protectants and carriers in the form of 'nanopesticides'. Direct applications of nanotechnology in agriculture include delivery of agrochemicals and nutrition, pesticides, nano-scale carriers, nanosensors, detection of nutrient deficiencies etc. Nowadays nano-fertilizers are increasingly used as alternates to bulk fertilizers and reduce pollution of soil and water by different agrochemicals. Nano-fertilizers facilitate the slow and steady release of nutrients and thereby reduce the loss of nutrients and enhance the nutrient use efficiency. Genetic Engineering is another promising strategy which can be used nanotechnology to make the production of transgenic crops much easier than through conventional process. The rapid growth of the both techniques may ensure the sustainable agriculture system and food security.

Keywords: Crop production, nanotechnology, nanobiotechnology, agriculture

Introduction

Over the last two decades, a significant amount of research has been carried out on nanotechnology emphasizing its numerous applications in agriculture sectors ^[1, 2, 3]. Fertilizer application plays a pivotal role in increasing the agricultural production; however, the excessive usages of fertilizers irreversibly alter the chemical ecology of soil, further reducing the available area for crop production. Sustainable agriculture entails a minimum use of agrochemicals that can eventually protect the environment and conserve different species from extinction. Notably, nanomaterials enhance the productivity of crops by increasing the efficiency of agricultural inputs to facilitate site-targeted controlled delivery of nutrients, thereby ensuring the minimal use of Agri-inputs. In addition, the development and exploitation of nanosensors in precision farming, to measure and monitor crop growth, soil conditions, diseases, uses and penetration of agrochemicals and environmental pollution have substantially improved the human control of soil and plant health, quality control and safety assurance contributing much to sustainable agriculture and environmental systems ^[2].

On the other hand, biotechnology uses the techniques of biology in order to manipulate molecular, genetic and cellular processes to develop products and services and it can be used in diverse fields from medicine to food and agriculture ^[4]. Having said that, the Nanobiotechnology is the integration of nanotechnology, biotechnology and also chemical and physical processing, material science and system engineering with the aim of obtaining new products previously modified at nanoscale that may have improved conditions with respect to the conventional materials ^[5] Due

to the new chemical, physical and biological properties of nanomaterials and nanoparticles, they have been rapidly exploited in many areas such as agriculture, food, medicine, pharmacy, electronics, material science and energy sectors among others ^[4, 6].

Plants remain the primary source of nourishment for humans, and food quality determines the health of majority of the people. Staple foods are usually high calorific foods, consumed regularly in high quantity, which becomes dominant part of a standard diet in a community. As a result, there is high correlation between staple foods and nutrition of its consumers, especially among the rural and poor communities who rarely have other sources of nutrient supplementation. Lack of essential micronutrients in food is common in such areas. These have become a global issue with serious adverse effects ^[7, 8, 9]. Indeed, the assistance of nanobiotechnology in plant protection products has exponentially increased, which may assure increased crop yield. Moreover, the major concern in agricultural production is to enable accelerated adaptation of plants to progressive climate change factors, such as extreme temperatures, water deficiency, salinity, alkalinity and environmental pollution with toxic metals without threatening existing sensitive ecosystems ^[10].

Nano-biotechnology engineering is the cutting-edge track of research that supports the development of high-tech agricultural fields by offering a wider specific surface area crucial for the sustainable development of agriculture systems ^[11, 12]. Therefore, nanobiotechnology can not only reduce the uncertainty, but also coordinate the management strategies of agricultural production as an alternative to conventional technologies. In many instances, agro-

nanobiotech innovations offer short-term techno-fixes to the problems faced in modern industrial agriculture. The present review summarizes the applications of nanobiotechnology in agriculture, which may ensure the sustainability of agriculture and environment for effective and promising crop production.

Synthesis of nanoparticles

Nanoparticles are typically synthesized from a top-down or bottom-up approach. A bottom-up approach relies on nucleating atomic-sized materials into the eventual nanoparticles. Top-down methods, where a bulk material is

physically broken down to make smaller molecules, include milling, laser ablation, and spark ablation (Figure 1). The synthesis of specific nanomaterials with the correct and homogeneous size is not easy processes which require unique skill and facilities. Several methods for the synthesis of NPs are employed, such as the following.

Chemical reduction method is used to synthesize copper nanoparticles by reducing copper salt ^[13]. Microemulsion or colloidal method is an efficient procedure to synthesize NPs from microemulsion or group of micelles (oil in water or O/W in the presence of hydrophobic surfactants) by mixing an appropriate quantity of water, oil, and surfactant ^[14].

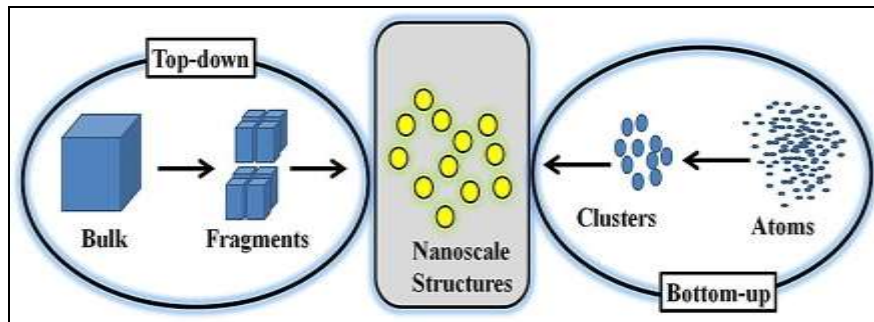


Fig 1: Synthesis of nanoparticles *via* top-down and bottom-up pathways. (Adapted from <https://www.nanoscience.com/techniques/nanoparticle-synthesis>)

Many metallic nanoparticles (e.g., silver (Ag), aluminum (Al), titanium dioxide (TiO₂), copper (Cu), cadmium sulfide (CdS), etc.) are synthesized according to this method ^[15]. Microwave method has become widespread and is a simple procedure for synthesizing copper NPs ^[16]. Polyol-based crystalline NPs have been produced through this method ^[17]. Electrochemical method is another type of procedure attracting many researchers because of its simplicity, high-purity product, less cost, user- and environmental friendly attribute, etc. In this procedure, NPs are accumulated at the interface of electrode and electrolyte, e.g., synthesis of copper NPs 40–60 nm in size. Nanoparticles produced from microorganisms: Several research studies have shown that large particles of materials are possible to convert into nanoscale particles using “vast and natural factories” of microbes. There are a significant number of NPs that have been synthesized using different types of microorganisms. For example, (1) silver NPs have been synthesized using many microorganisms such as *Escherichia coli* etc. ^[18]. Nanoparticles from plants: NPs phytosynthesized using natural extracts of various plants are yet again considered as one of the cost-effective and eco-friendly substrates. One of the widely utilized NPs is silver NPs, which are synthesized with the support of many plant extracts, including *Ocimum tenuiflorum*, *Centella asiatica*, *Syzygium cumini* ^[19], *Urtica urens* ^[20], etc., in the presence of a silver nitrate solution.

Nanobiotechnology applications for sustainable crop production

Nano and biotechnology now comes into play, as innovative technologies that may have the solutions to many of the issues that the humans and agricultural industry have been facing. The model nanomaterials for agricultural applications are supposed to have the following properties: 1. providing actual concentration and controlled release of fertilizers or pesticides in response to certain conditions (TiO₂ Nano particles used as plant fertilizer for Mung bean

to enhance crop production) ^[21]; 2. Improved targeted activity ^[22] and, 3. Lower Eco harmful with safe and relaxed transport.

Nano-farming of agriculture

Nanotechnology has emerged as a vibrant technique in agriculture when conventional agricultural practices have failed to achieve a yield increase for the rapidly growing world population. Furthermore, this provides an ecosystem-friendly technique by reducing the application of pesticides, water usage, and the overall cost of crop production for a sustainable and fastidiousness agricultural system (Figure 2). There are two main features to be considered when nanomaterials are used in plant disease management ^[23] : 1. Synthesis of nanomaterials, which deals with the conversion of relevant materials to nanosized particles, between 1 nm and 100 nm; 2. Effective use of nanomaterial for a specific interest or purpose, alone or mixed with some other relevant materials.

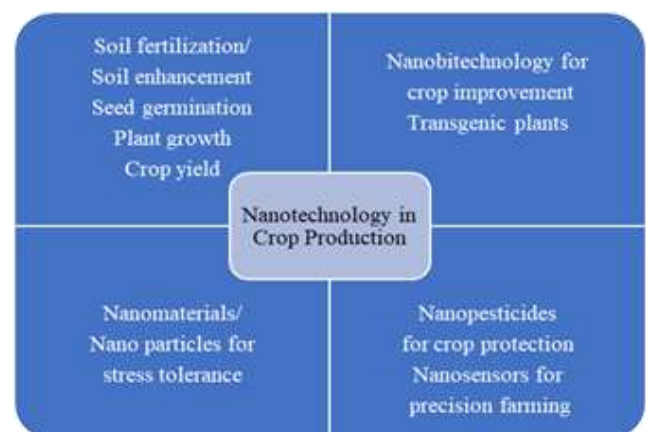


Fig 2: Application of nanotechnology in crop production

Nanofertilizers

Farmers have resorted to the use of conventional fertilizers at high rates and long periods of time, which is the main anthropogenic factor that has caused the eutrophication of coastal and freshwater ecosystems [24]. In that way, different approaches have been developed to deal with this issue. One of them is the creation of nanofertilizers that are either nanomaterials that can supply nutrients to the plant, or those that act as enhancers of the traditional fertilizers, but do not directly provide the nutrients [24]. For example, nanoparticles made out of hydroxyapatite, TiO₂, SiO₂, or even carbon nanotubes [25]. Another strategy has been the development of biofertilizers, in which beneficial microorganisms are used that are able to convert organic matter into compounds that are nutritional for the plant and, at the same time, maintain the soil fertility and its natural habitat [26]. Nevertheless, this approach has a few drawbacks, like short shelf life, lack of suitable carrier materials, high temperature and desiccation sensitivity [26]. The success of using nanomaterials as fertilizers in plant growth depends on the species of the plants and some other factors such as the size, concentration, composition and chemical properties of nanomaterials [27]. The vast knowledge of the fields of biology, biotechnology, material science, and engineering is key to development of new technologies needed to expand the field on nano-agriculture for efficient crop production.

Nanopesticides

The application of nanomaterials to control plant diseases is an innovative and smart process. This has made evolvement of number of strategies for application of nanoparticles. Further, this may evidence the exact influence and potential forecasts in forthcoming decade with the advancement of application features of nanotechnology. Moreover, it is assessed that nearly 90% of applied pesticides are wasted or lost throughout or subsequently in the agriculture system [28]. As a consequence, there is an increased enthusiasm to change to the less cost and high performance pesticides, which are minimum or less detrimental to the environment. Hence, the nano size materials in the form of particles, carbon tubes, capsules, cups etc. are used in number of ways to manage many important plant diseases which cause a significant yield loss annually. Furthermore, nanotechnology can provide benefits to pesticides, like (a) reducing toxicity, (b) enhancing the shelf-life, (c) accelerating the solubility of poorly water soluble pesticides, (d) improving site-specific uptake into the target pest, etc. [29,30]. All of these could ensure a green environment with a positive impact. Hence, similar or improved results of the application of chemical pesticides or other cultural or biological methods could be obtained by a direct application of NPs to seeds, roots, and leaves while minimizing the disadvantages of the abovementioned traditional methods. Although a direct application of NPs would be significantly effective, the beneficial microbial population or nontarget organisms that are found surrounding the root zone of plants will be affected much when nanoparticles are introduced straight to the soil. However, a control release of various chemicals for different purposes of plants is possible through NPs as carriers especially when plants are under any stress conditions such as flood [31]. Metal-based nanoprotectants or NPs, such as silver, gold, copper, titanium oxide, zinc oxide [32], and carbon nanoparticles [33], and other NPs function as

common nanocarriers, like silica [34], chitosan [35], solid lipid nanoparticles (SLN) [36], and layered double hydroxides (LDH) [37] nanoparticles have been utilized for plant disease management.

There are two major processes through which the advantages of application of nanomaterial or NPS can be obtained in order to estimate the possibility and usage of NPS in plant disease controlling, especially regarding the phytonematodes management: 1. Direct effect on pathogens when NPs are applied alone and used as protectants or applied in the form of nanopesticides by formulating nanomaterials using nanocarriers for insecticides, fungicides, herbicides, and RNA interference (RNAi); 2. Effect of nanomaterials or NPs on the physiological and biochemical activities of pathogens/microorganisms since nanomaterials are highly reactive substances due to their high surface-to-volume ratio. Many research studies have been carried out under the plant protection strategies to assess the damage caused by the nanomaterial on phytopathogens like bacteria, fungi, viruses, nematodes etc. The potential application of engineered nanomaterials in agriculture is also ascertained in disease and weed management. Inorganic NPs, such as ZnO, Cu, SiO₂, TiO₂, CaO, MgO, MnO and AgNPs play important roles in various arena of plant protection including microbial activity and bacterial diseases [38, 39]. For example, ZnO nanoparticles have recently been shown to provide effective growth control of *Fusarium graminearum*, *Penicillium expansum*, *Alternaria alternata*, *F. oxysporum*, *Rhizopus stolonifer*, *Mucor plumbeus* and *A. flavus* as well as pathogenic bacteria *Pseudomonas aeruginosa* [38, 40]. Nano-Cu application was found to be more effective against *Phytophthora infestans* compared to currently available non-nano Cu formulations in tomato [41].

Nanobiosensors

The nanobiosensors are considered to be smart sensors in the agriculture sector and they are considered as a powerful tool for track detect and control animal and plant pathogen, mainly because the devices help in the detection of chemical contaminants, virus and bacteria in agricultural systems, nutrient content and plant stress [42]. Some specific applications of these sensors in agriculture are the MEMS (Micro Electro Mechanical Systems) that through microelectronic circuits are capable of sensing and monitoring temperature and moisture in soil, to diagnose oil disease (caused by infecting soil microorganisms, such as viruses, bacteria and fungi) via the quantitative measurement of differential oxygen consumption in the respiration of good microbes and bad microbes in the soil. Also biosensors are developed, using photosystem II, known to bind several groups of herbicides and chemicals, among others [43]. Through the development of new devices, farmers and population will be benefited in aspects such as economic gain, reduced environmental pollution and lower labor costs [44]. Physical and chemical new properties of nanoscale materials are also exploitable in the development of biosensors. Nanomaterials are used in biosensors with the purpose of improve their sensitivity and efficiency through new signal transduction technologies [45]. Nanosensors with immobilized bioreceptor which is selective for target analyte molecules are called nanobiosensors. In other words, this is a modified version of a biosensor that incorporates a biological derived sensitized element linked to a physical-

chemical transducer. This device integrates a biological element with an electronic component to yield a measurable signal and in the other hand, the biological recognition is through the transducer process [45].

The use of each type of nanobiosensor depends on the analyte, for example, biosensors are used for the detection of phenols, polyaromatic compounds, halogenated pesticides and volatile organic compounds (VOCs) while electrochemical sensors can be used as chemical gas sensors [46]. Their applications include detection of analyte like urea, glucose, pesticides, also the monitoring of metabolites and detection of various microorganisms/ pathogens [43]. Regarding the latter, one example is a novel microbial detection technology based on silica nanoparticles has been developed in the last years.

Nanomaterials as disinfectants

Elimination of contaminants (such as bacteria, fungi, and viruses) is the first step of any successful investigation on in vitro plant tissue culture. One of the major problems of in vitro plant culture techniques is chronic contamination by microorganisms [47]. Frequent application of conventional antibiotics and chemotherapeutic agents may be phytotoxic or may retard plant tissue growth [47]. In this regard, using AgNPs (with the size range of 35 nm) solution after surface sterilization has showed acceptable influence on the control of bacterial infection without any adverse effects on the growth characteristics of regenerated plantlets. However, there are some reports on ineffectiveness of AgNPs on fungal contamination, likely due to AgNPs size. Antifungal activity of AgNPs is size dependent, the smaller is the better [47]. Although several methods are used to produce silver nanoparticles (AgNPs), the inclusion of toxic chemical substances cannot be avoided in their common method of synthesis, the chemical approach [48]. Therefore, a considerable number of research is being carried out to synthesize them using plant extracts as the biological base so as to maintain “clean,” “nontoxic,” “harmless,” and “eco-

friendly green chemistry.” There are quite a lot of journal papers that claim to reveal that plant extracts have nematicidal and nematostatic properties [49]. A potential and effective nematicide can be made from plant extracts when they are formulated into metal-based NPs. Silver NP (AgNP) is one of the most utilized nanomaterial that has emerged as a superior product to control phytonematodes. Silver NPs possess sufficient conductivity, have a good catalytic attribute with pronounced antimicrobial activity, and are chemically stable [49]. Despite the many advantages of nanotechnology relating to plant disease management, there are certain risks associated with the application of this technique in the environment. Importantly, the phytotoxic character of nanomaterials should be assessed carefully before releasing them for commercial purposes. Nanomaterials can not only influence pathogenic nematodes, but even more, they can interfere with the growth, development, and reproduction of host plants. Hence, a detailed study is essential to assess their influence or impact during the seed germination, seedling, and flowering stages of plants.

Nano-Biotechnological approaches of crop improvement and production

Biotechnology plays a major role in the crop improvement and production in several ways (Figure3). Genetic engineering has evolved as a promising field to manage pest and diseases through gene cloning and gene modification of host plants. There are many information on transgenic plants against pathogenic microorganisms or any other pathogenic organisms such as phytonematodes etc. or insects as to express insecticidal genes such as *Bt*, trypsin suppressor, lectins, plantibodies, ribosome inactivating proteins, secondary plant metabolites, vegetative insecticidal proteins, etc. [50]. Besides, there is a gene transfer technique to transfer especially *Bt* Cry genes to microbes, such as fungi associated with disease-causing nematodes, which helps the nematodes to complete their life cycle [51].



Fig 3: Roles of Nanobiotechnology in precision agriculture

Plant–pathogenic microbes interaction provides many avenues to manipulate host plants against the disease

causing pathogens associated with plants. The main objective of the manipulation of plants by transferring

resistance gene/s against pathogenic microorganisms in them and controlling all possible physiological and biological activities of pathogens due to the expression of those genes. The basic concept of 'gene for gene' interaction plays a significant role corresponding to the 'resistance gene (R) in the host and an avirulence (Avr) gene' in the pathogenic microbes. As a result, there will be a series of defense responses (hypersensitive response (HR)) causing the death of cells (necrosis) at the site of infection, or the responses may have an impact on and alter normal physiological and biological reactions, such as reproduction, digestion, metabolic processes, etc. There are many natural resistance genes found as single gene or polygenic manner which were used to produce transgenic plants against phytonematodes [52].

Nanobiotechnology offers a new set of tools to manipulate the genes using nanoparticles, nanofibers and nanocapsules. Properly functionalized nanomaterials serve as transportation and could carry a larger number of genes as well as compounds able to trigger gene expression or to control the release of genetic material throughout time in plants [43]. Particle bombardment is used as a popular tool in transgenic formation than the *Agrobacterium* mediated transformation [47]. There are several nanoparticles (NPs) such as gold NPs which are efficient than the normal gold particles in delivery of DNA during transformation because of their less requirement of quantity for use and minimal damage to plant tissue or cells. Similarly, carbon nano tubes (CNTs) are considered to be an effective gene delivery system that can be applied in transgenic plant production. In addition to these vectors, nano-fibers or nanovesicles in the form of cationic liposomes which have ability to bind with negatively charged DNA and thereby facilitating smooth transformation of targeted DNA or gene in to the tissue through plasma membrane. These can be persuaded further through nanoparticulate cationic polymers as alternatives. Due to their permanent cationic charges interact electrostatically with negatively charged DNA to form complexes (lipo- or polyplexes) which is more stable due to the protection of DNA from nuclease degradation [26]. Moreover, a newly introduced mesoporous silica nanoparticles (MSNs) precisely manipulate gene expression in single cell level [53]. Similarly, 'bio-beads' are used to encapsulate the plasmid DNA carrying the reporter gene for precise expression [54]. Therefore, nanobiotechnology based genetic manipulation make the process of producing transgenic crop much easier than the conventional method.

Conclusions

Nanotechnology consider a novel key to growing agricultural production through implementing nutrient efficiency, improve plant protection practices, also, nanotechnology may have real solutions for various agriculture problems like improved crop varieties, plant protection, detect diseases and monitor plant growth (As shown in the figure 3). Nanotechnology offers generous visions for the development agricultural sector through advanced applications and the probability of products and increases global crops production volume to feed the world population in next decades. Promising results and applications are already being developed in the areas of nano nutrients, implement crop productivity, protect plants (herbicides and pesticide), nano-packing and Nano sensors. Since nanobiotechnology is use to develop advanced tools

in agriculture, the toxicity of nanoparticles is a recurrent theme in many research studies. Integrating "sustainable development" and considering the "environmental impact" are the challenges that nanotechnology has for the use of new resources within agriculture

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