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Priyanka Kumari
Department of Genetics and Plant Breeding, Birsa Agricultural University, Ranchi, Jharkhand, India

Swapnil
Department of Genetics and Plant Breeding, Birsa Agricultural University, Ranchi, Jharkhand

Jenny Priya Ekka
Department of Genetics and Plant Breeding, Birsa Agricultural University, Ranchi, Jharkhand, India

SK Tirkey
Department of Genetics and Plant Breeding, Birsa Agricultural University, Ranchi, Jharkhand, India

Ekhlaque Ahmad
Department of Genetics and Plant Breeding, ZRS Chianki, Birsa Agricultural University, Ranchi, Jharkhand, India

Correspondence
Priyanka Kumari
Department of Genetics and Plant Breeding, Birsa Agricultural University, Ranchi, Jharkhand, India

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Agro-Nanotechnology: An innovative approach for diagnosis of plants

Priyanka Kumari, Swapnil, Jenny Priya Ekka, SK Tirkey, Ekhlaque Ahmad

Abstract
Agriculture plays a key role for economic development of the country. Food losses due to crop infection from pathogens such as bacteria, viruses and fungi are persistent issue in agriculture for centuries across the globe. For proper disease control and management to minimize crop loss early and efficient diagnosis of disease is very essential. For this nanotechnology has emerged as one of the most innovative scientific field in agriculture. The use of nanotechnology in agriculture can revolutionise the sector with new tools for rapid disease detection, targeted treatment enhancing the ability of plants to absorb nutrients, fight diseases and hold out environmental pressures and effective systems for processing etc. Smart sensors and delivery systems will facilitate the agricultural industry combat viruses and other crop pathogens. Progressively this review deals with the application of nanotechnology for rapider, cost-effective and more precise diagnostic procedures of plant diseases. In the coming years this technology will have major impact on Indian Agriculture. Controlled use of the technology will open opportunities for developing new materials and methods that will enhance our ability to develop faster, more reliable and more sensitive analytical systems. The scientists all over the world have been propagating this branch of science through their research, but very minute is done at farmers level.

Keywords: Nanoparticles, nanoemulsion, colloid, biosensors

Introduction

Nanotechnology deals with the objects calculated in a billionth of a meter. A nanometer is 1/80,000 the diameter of a human hair or roughly ten hydrogen atoms wide. Thus science of very small things is known as Nanotechnology which is not just concerned with small things but is a multi-disciplinary science which includes knowledge from biology, chemistry, physics and other disciplines. Basically Nano technology is defined as the manipulation or self-assembly of individual atoms, molecules or molecular clusters into structures to create materials devices with new or vastly different properties. This technology emphasizes the implications of individual atoms or molecules or submicron dimensions in terms of their applications to physical, chemical, and biological systems and eventually their integration into larger complex systems. The fundamental nature of Nanotechnology is the ability to make efforts at the molecular level, atom by atom, to create large structures with fundamentally new molecular organization. The plan is to exploit these properties by attaining control of structures and devices at atomic, molecular, and supramolecular levels and to learn to resourcefully manufacture and utilize these devices. This advanced technology has provided innovative solutions to problems in plants and food science (post-harvest products) and offers latest approaches to the judicious selection of raw materials, or the processing of such materials to improve the quality of plant products and make them superior.

The compassion of nanotechnology lies in the capability to compact the tools and devices to the nanometer range, and to mount up atoms and molecules in to bulkier structures while the size remains very small. The attempts are directed towards applying the disease control molecules, slow release of pesticides and developing diagnostic tools. Efforts are resting on to manufacture subsequent items in future i.e. interactive, edible nano wrappers to keep the pathogens away, targeted release of chemicals, packaging, extensive nano surveillance, interactive agrochemicals as herbicides and pesticides. Several nanomaterials such as copper, zinc, titanium, magnesium, gold, alginate and silver have been developed, but the most effective is silver nanoparticles (Nano-Ag) since they exhibit effective antimicrobial efficacy against bacteria, viruses and eukaryotic micro-organisms (Guo *et al.*, 2003) [10]. There is an eternally mounting consumption and demand for food. Thus Nanoscale science and nanotechnologies are envisioned to have the prospective to revolutionize agriculture and food systems (Norman and Hongda, 2013) [32] and has given confinement to the new era of Agronanotechnology.

The pioneering molecular and cellular biology tools are expected to provide disease prevention and treatment in plants such as disease diagnosis, screening and treatment, in farming practices which involves vector and pest detection and control, disease monitoring and smart treatment delivery systems at a Nanoscale. These smart systems deliver precise quantities of drugs or nutrients or other agrochemicals required thereby monitoring and minimizing pesticide and antibiotic use.

Why metal nanoparticles?

The crystalline nanoparticles are attractive probes of biological markers and are proved to be better diagnostic tools which is particularly due to miniature size (1-100nm), large surface to volume ratio (aspect ratio), chemically alterable physical properties, change in the chemical and physical properties with respect to size and shape, strong affinity to target particularly proteins (in case of gold nanoparticles), structural sturdiness in spite of atomic granularity, enhanced or delayed particles aggregation depending on the type of the surface modification, enhanced photoemission, high electrical and heat conductivity and improved surface catalytic activity (Garg *et al*., 2008; McNeil, 2005; Rosi and Mirkin, 2005; Shrestha *et al*., 2006) [5, 30, 39]

Nano-particles controlling the plant diseases

Some of the nano particles that have entered into the arena of controlling plant diseases are nano forms of carbon, silver, silica and alumino-silicates.

Nano Carbon

Carbon's uniqueness has been explained by many scientist in a exclusive way but many concepts are yet to be understood. At such circumstances Nanotechnology has amazed scientific community, because at Nano-level material shows diverse properties. Therefore we are exposed to a massive range of Nanosciences, wherein there are entirely new materials, new technologies and new expectation for existing problems related to agrochemicals, pesticides, herbicides regulation and smart utilization. Scientists are predominantly focussed on carbon nano tubes (CNT). Recently scientists (Khodakovsky *et al.*, 2009) [15] have reported that carbon nano tubes present in tomato seeds are not only able to penetrate into the hard coat of germinating tomato seeds but also exerted growth enhancing effect when planted in the soil. They concluded that the enhanced growth was appropriately due to increased water uptake caused by penetration of CNT. This could be a benefit for using CNT as vehicle to deliver desired molecules into the seeds during germination that can protect them from the diseases.

Nano Silver

It has long been recognized to have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities. The antifungal efficacy of colloidal nano silver (1.5 nm average diameter) solution, against rose powdery mildew caused by *Sphaerotheca pannosa Var rosae* was studied by Kim *et al.* (2008) [17]. Nano silver colloid is a well dispersed and stabilized silver nano particle solution and is more adhesive on bacteria and fungus, hence are superior fungicide. Surprisingly maximum patents are filed for 'Nano silver for preservation and treatment of diseases in agriculture field.' This popularity of nano silver has caused concern about regulating and classifying the nano silver as pesticide (Anderson 2009) [1].

Under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) nano-silver is used in products as a pesticide. Silver is therefore an accepted agrochemical replacement and eradicates unwanted microorganisms in planter soils and hydroponics systems. It is also used as foliar spray to stop fungi, moulds, rot and several other plant diseases. Furthermore, silver is an outstanding plant-growth stimulator.

Nano Silica-Silver composite

Silicon (Si) is known to be absorbed into plants to boost disease resistance and stress resistance (Brecht *et al.*, 2003; Ma *et al.*, 2001) [2, 27]. Aqueous silicate solution, used to treat plants, is reported to exhibit excellent preventive effects on pathogenic microorganisms causing powdery mildew or downy mildew in plants. Moreover, it endorses the physiological activity and growth of plants and induces disease and stress resistance in plants (Garver *et al.*., 1998; Kanto *et al.*, 2004) [6, 14]. But, since silica has no direct disinfection consequences on pathogenic microorganisms in plants, it does not exhibit any effect on established diseases. Further, the effects of silica significantly show a discrepancy with the physiological environment and as a result, they are not registered as an agricultural chemical. However silver is known as a potent disinfecting agent. It kills unicellular microorganisms by inactivating enzymes having metabolic functions in the microorganisms by oligodynamic action (Kim *et al.*, 1998) [16], and is known to exhibit superb inhibitory effects on algal growth also. Silver in an ionic state exhibits high antimicrobial activity (Kim *et al.*, 1998; O'Neill *et al.*, 2003; Thomas and McCubin, 2003) [16, 33, 47] but is unstable due to its high reactivity and consequently gets easily oxidized or reduced into a metal depending on the surrounding media. Silver in the form of a metal or oxide, is stable and constant in the environment, but due to its low antimicrobial activity it is used in relatively increased amount, which undesirable. Park *et al.* (2006) [35], developed a new composition of nano-sized Silica- Silver for the control of various plant diseases which consisted of nano-silver combined with silica molecules and water soluble polymer. It is prepared by exposing a solution together with silver salt, silicate and water soluble polymer to radioactive rays. It showed antifungal activity and controlled powdery mildews of pumpkin at 0.3 ppm in both field and greenhouse tests. The pathogens disappeared from the infected leaves 3days after spray and the plants remained healthy thereafter. Park *et al.* (2006) [35] also studied the 'effective concentration' of nanosized silicasilver on suppression of growth of many fungi; and found that, *Pythium ultimum, Magnaporthe grisea, Colletotrichum gloeosporioides, Botrytis cinere* and, *Rhyzoctonia solani*, showed 100% growth inhibition at 10 ppm of the nanosized silica-silver. Whereas, *Bacillus subtilis, Azotobacter chrococuum, Rhizobium tropici, Pseudomonas syringae* and *Xanthomonas compestris pv. Vesicatoria* showed 100% growth inhibition at 100 ppm. They have also reported chemical injuries caused by a higher concentration of nanosized silica-silver on cucumber and pansy plant, when they were sprayed with a high concentration of 3200 ppm.

Nano Alumino-Silicate
Leading chemical companies are now formulating efficient pesticides at nano scale. One of such effort is use of Alumino-Silicate nanotubes with active ingredients. The advantage is that Alumino-Silicate nanotubes sprayed on plant surfaces are easily picked up in insect hairs. Insects actively groom and consume pesticide-filled nanotubes. These are relatively environmentally-safe pesticides and are biologically more active.

Mesoporous Silica Nanoparticles
Wang *et al.* (2002) [51] have shown that mesoporous Silica nano particles can deliver DNA and chemicals into Plants thus, creating a powerful new tool for targeted delivery into plant cells. The porous, silica nanoparticles systems that are spherical in shape and the particles have arrays of independent porous channels has been developed by Lin's research group. These channels appears as a honeycomb-like structure that is facilitated to be filled with chemicals or molecules. These nanoparticles have a unique "capping" strategy that seals the chemical inside. They also displayed the caps that can be chemically activated to pop open and discharge the cargo inside the cells where it has been delivered. This exclusive aspect provides total control for timing the delivery. To penetrate rigid cell walls of the plant cell, the surface of the particle has been modified with a chemical coating and are effectively used to introduce DNA and chemicals into Arabidopsis, tobacco and corn plants.

Nano-emulsions
These are combination of two or more liquids (such as oil and water) which does not combine easily. In nano-emulsion, the diameters of the dispersed droplets are 500 nm or less. Nano-emulsions can encapsulate functional ingredients within their droplets, which can facilitate a reduction in chemical degradation (McClements and Decker, 2000) [28].

Nanotechnology for detecting plant diseases
The detection of plant disease at an early stage is required so that tons of food can be protected from the probable outbreaks which has consequentlu tempted Nanotechnologists to look for a nano solution for protecting the food and agriculture from bacteria, fungus and viral agents. A detection technique which takes less time, gives accurate and better results within a few hours without any complicated operations accordingly that even a simple farmer can use the portable system. If an autonomous nano-sensors linked into a GPS system for real-time monitoring can be distributed throughout the field to monitor soil conditions and crop, it would be of great assistance. The coming together of biotechnology and nanotechnology in sensors will construct the equipment of with augmented sensitivity thereby allocating an earlier response to environmental changes and diseases.

Nanobiosensors
NPs are capable to be used as a diagnostic and sometimes as a investigative tool for detection of plant pathogens and compounds that are indicator of disease respectively. However, this research is in preliminary phase in agriculture. Primarily the biosensor is derived from the combination of a ligand-receptor binding reaction to a signal transducer. It consists of a probe, bioreceptor and transducer. The relations of analyte with bioreceptor is premeditated to produce an effect measured by transducer, that converts the information into electrical signal. The nano-chips are known for detecting single nucleotide changes of bacteria and viruses is reported by Lopez *et al.* (2009). These nano-chips have fluorescent oligo-capture probe through which hybridization can be detected. Yao *et al.* (2009) [52] used fluorescence silica NPs in combination with antibody to detect the microorganism *Xanthomonas axonopodis* that causes bacterial spot disease in Solanaceae plant. Nano-gold based immunosensor was used to detect Karnal bunt disease in wheat by the means of surface Plasmon resonance was re [orted by Singh *et al.* (2010) [43]. The devices based on nanotechnology will automatically boost the use of sensors for real time monitoring of crops that can minimize the use of pesticides and antibiotics was analysed by Sharon *et al.* (2010) [40].

Saving post harvest plant products
The packaging of edible food films made with cinnamon or oregano oil, or nanoparticles of zinc, calcium other materials are antimicrobial that kills bacteria. The utilization of nano-fibers made from lobster shells or organic corn (both are antimicrobial and biodegradable) considered as green packaging is also a food safety effort. Therefore an advanced food packaging needs packaging materials that consists of strength, barrier properties and stability to heat and cold. These are being achieved using nanocomposite materials. In future, incorporation of silver, magnesium oxide or zinc oxide nanoparticles (which can kill harmful microorganisms) in food or beer packages will save the contamination. It is anticipated that an antimicrobial activity can also be included through addition of nano-sensors to food packages in the future. Researchers have suggested that these nanosensors could be used to detect chemicals, pathogens and toxins in foods. Radio Frequency Identification (RFID) tags could be incorporated into food packages which do not require line-of-sight for reading like bar-codes and facilitates registration of hundreds of tags in a second. Use of nanowheels, nanofibers and nanotubes are being tried to improve the qualities of food packages.

Nanolamination
Technique is another viable option for protecting the food from moisture, lipids and gases. Moreover, they can improve the texture and preserve flavor as well as color of the food. Nanolaminates consist of two or more layers of nano-sized (1 – 100) thin foodgrade films which are present on a wide variety of foods: fruits, vegetables, meats, chocolate, candies, baked goods, and French fries (Morillon, 2002; Rhim, 2004) [31, 38]. Nanolaminates are prepared from edible polysaccharides, proteins, and lipids. Park (1999) [36] has shown that polysaccharide- and protein-based nanolaminates are good barriers against oxygen and carbon dioxide, but poor in protecting against moisture. Whereas, lipid-based nanolaminates are good at protecting food from moisture. Trials are on to develop laminates that can protect against all the desired f actors. Coating foods with nanolaminates is done simply by spraying it on the food surface (Mc Clements *et al.*, 2005) [29].

Nanoparticles as Growth Promoter
Currently the researchers have reported the effects of NMs on germination and growth with the objective to promote their use of agricultural applications. Interface of NPs with plants

caused various physical and physiological changes, depending on the properties of NPs. Effectiveness of NPs depends on their concentration and it is different from plant to plant and is determined by their chemical composition, size, surface area, reactivity, and the concentration at which the response is positive was observed by Oerke (2006) [34]. NPs have both optimistic and pessimistic effects on plant growth and development. Conversely, this review deals with the positive roles played by NPs on seed germination, photosynthesis and plant growth. NPs such as carbon NMs, metal NPs and metal oxides NPs are the frequently encountered in the agricultural field.

Effect of carbon nanomaterials on plants

Amongst all the NPs, carbon NMs have acquired a momentous place due to their unique mechanical, electrical, thermal and chemical properties. Sriniwasan and Sarawathi (2010) accounted that the single walled-CNTs (SWCNTs) act as nanotransporters for delivery of dye molecules and DNA into plant cells. In a further report MWCNTs enhanced efficiency of water uptake as well as Ca and Fe nutrients uptake which increased the seed germination and plant growth (Villagarcia, (2012) [50]; Tiwari, (2014) [48]. Oxidized MWCNTs enlarged the cells in root system and promoted the activity of dehydrogenase. Moreover, CNTs persuaded the root and shoot growth of wheat plants in light and dark conditions. It has been confirmed the presence of water soluble CNTs by SEM and fluorescence microscope inside the plants by Tripathi, (2014) [49]. Lin and Xing (2007) [22] provided the information that MWCNTs enhanced 5-days root elongation in rye grass, rape and corn.

Effect of metal nanoparticles on plants

The studies by Pokhrel (2013) [37] suggests that metal NPs increase plant growth and development. AgNPs increased the root length in maize and cabbage plants in comparison with AgNO3. Au NPs have a significant role on seed germination and antioxidant system in *Arabidopsis thaliana* was determined by Kumar (2013) [20]. Biologically synthesized Ag NPs induced synthesis of protein and carbohydrate and decreased the total phenol content in *Baopa monnieri* was analysed by Krishnaraj (2012) [19]. Gruyer (2013) [9] showed that root length increased in barley exposed to AgNPs. More than two fold increase in height and fresh weight of duckweed was found when treated with Ti NPs at 0.5 gL-1 conc. was experimented by Song (2012). Biosynthesized Zn NP significantly enhanced shoot (10.8%), chlorophyll content (18.4%) and grain yield (29.5%) in pearl millet was observed by Tarafdar (2014) [46]. Almeelbi and bezbaruah (2014) reported the effect of Fe NPs on spinach in hydroponic solution. The authors reported significant enhancement in plant growth and biomass by NPs. Interestingly Fe content in spinach roots, stem and leaves increased 11-21 times.

Effect of metal oxide nanoparticles on plants

A large number of studies on the effects of metal oxide NPs on germination and growth of plants have been documented. Nano sized TiO2 promoted plant growth when seeds were soaked in NPs or sprayed with NPs was studied by Zheng (2005) [53]. Lu (2002) [25] showed that mixture of TiO2 and SiO2 NPs improved the nitrate reductase activity and stimulated the antioxidant system in soybean. Root elongation was promoted at a particular concentration of ZnO NPs in soybean was reprted by Lopez (2010) [24]. In another experiment by Sheykhbaglou (2010) [41] observed that iron oxide NPs increased soybean pod and leaf dry weight. Burman (2013) [3] reported that foliar application of ZnO NP at 1.5 mg/L concentration increased biomass as compared to ZnSO4 in chickpea. Fe_2O_3 NPs given to soybean by foliar application and soil route. Sincere field research is essential to study, promoting effects of these NPs on yields of some important crops. The mode of action of these NPs by which they take part in the growth and development of plants must also be explored.

Role of Nanoparticals in Photosynthesis

Plants converts solar energy into chemical energy by the process of photosynthesis. Only 2-4% of available energy in solar radiation is converted by plants is used in plant growth and development [127]. Gene manipulation and other techniques are being used by researchers to improve photosynthetic efficiency of plants. Nanotechnology has the potential to improve function of photosynthetic machinery. Embedded SWCNTs in the isolated chloroplast has enhanced the photosynthetic activity three folds higher than that of control. Giraldo (2014) [7] reported that it also increased the rate of electron transport to its maximum level. Ma *et al.* (2008) [26] studied and concluded that nano- anatase- induced marker gene enhanced the activities of Rubisco activase reflected in the improvement of Rubisco carboxylation and high rate of carbon reaction of photosynthetic machinery. Thus the improvement of photosynthetic mechanisms by nano-genic approach may help to design artificial light-harvesting systems.

Nanoparticles in Disease Suppression

Plant diseases are mainly caused by viruses, bacteria, fungi and nematodes are mainly resulting in decreased yield and inferior quality of plant products. Several approaches are being used to manage crop disease which includes genetics, breeding, cultural schemes with sanitation, host indexing, enhanced eradication protocols, new pesticide products, and integrated pest management. Numerous studies have reported that NPs can be used to suppress pathogens which affects crop growth and development. It was reported by Jo *et al.* (2009) [11] that Ag NPs in 200 mg/l conc. decreased 50% colony formation of pathogenic fungi that caused disease in ryegrass. Lamsal *et al.* (2011) [21] have also reported that appliance of Ag NPs augmented the disease suppression. Dimkpa (2013) [4] reported that ZnO NPs reduced growth by 26% of *Fusarium graminearum* grown in mungbean broth agar. Kanhed (2014) [13] reported that chemically synthesized Cu NPs demonstrated higher pathogenic fungal inhibition in comparison to the fungicide bavistin. Silver NPs have reduced the number of germinating fragments to a great extent relative to the control at 24 hour incubation of spores with a 2.5 ppm solution of NPs. Field tests was particularly done with silver NPs (WA-CV-WA138) at different concentrations to determine the antifungal activity. Lamsall (2010) reported that the uppermost inhibition rate for the growth of fungal pathogen on cucumber and pumpkins are 100 ppm silver NPs [96].

Conclusion

Researches are still in the very preliminary stage in the field of nanotechnology for plant disease diagnosis and its management. Several questions with incredible scientific or practical importance need to be addressed along with the genetic response of plants in the presence of NPs. Another significant aspect is to understand the penetration route of NPs into vascular tissues of the plants. Through better monitoring and targeted action, maximization of output and minimization of inputs might be possible with the help of nanotechnology. Major benefits can be brought to the farmers through nanotechnology by food production and also to the food industry in the development of novel products through food processing, preservation, and packaging. Currently the purpose of NMs in the field of agriculture includes nanosensors/nanobiosensors for detecting pathogens and soil quality along with plant health monitoring, nano enabled fertilizers for slow-release and efficient dosage of water and fertilizers, nanocapsules for agrochemical delivery, nanocomposites for plastic film coatings which is utilized in food packaging as well as antimicrobial application of NPs for preventing contamination. There has been momentous curiosity in utilizing nanotechnology to promote agriculture. Although this review displays the potential of NMs for different agricultural practices however, further investigation and research are needed to expand the application, possibilities and methodologies in agriculture.

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