Recent advances in nanotechnology-based agriculture for crop improvement: A review of promising developments.

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Abstract

Nanotechnology in agriculture involves manipulating materials at the nanoscale to enhance food productivity and address challenges facing agriculture for decades like disease resistance and environmental impact. Conventional farming practices are insufficient in ensuring nutritional quality and feeding the ever-increasing world population. This review aims to highlight recent advances in nanotechnology-based agriculture for enhancing crop productivity, plant nutrient uptake and translocation efficiency, and genetic engineering approaches for crop protection and disease management. Recent advancements in nanotechnology have shown immense potential for improving the quantity, quality, and safety of food for the increasing world population and addressing agricultural challenges. The utilization of nanomaterials including engineered nanoparticles, nano pesticides, and nano fertilizers, has enabled precise and targeted delivery of essential nutrients and growth regulators to plants. Application of nanotechnology in agriculture also involves the development of nano sensors for disease detection and plant health monitoring. Despite the promising advances in nanotechnology-based agriculture, challenges remain regarding potential environmental risks, standardization of synthesis protocols, and large-scale implementation concerns associated with the use of nanomaterials. This review presents the latest advancements in nanotechnology applications within agriculture, portraying a promising direction for crop improvement strategies. Continued research, collaboration, and policy interventions will be critical in realizing the full potential of nanotechnology in shaping the future of agriculture.

Keywords: Agriculture, Crop improvement, Nanoparticles, Nanotechnology.

Introduction

Agriculture serves as a fundamental pillar for many developing countries, with more than 60% of their population relying on it for their livelihoods [1]. However, in the 21st century, agriculture faces significant challenges in producing more food to meet the needs of the growing population. These challenges include rapid population growth, declining agricultural productivity, unpredictable climate change, a variable labor force, and increasing urbanization. A recent report by the UN predicted that the global population will reach 8.5 billion by 2030 and approximately 9.8 billion by 2050 [2]. To meet the food needs of this growing population, there is an urgent need to modernize agriculture, especially in developing countries. The first green revolution of the 1960's led to the development of high-yielding rice and wheat varieties with low land use, but these practices were ultimately unsustainable. Instead of this revolution, the second green revolution, which is emerging now, aims to meet future food demands in a sustainable way. During the last several years, scientists have developed new innovative technologies and strategies to achieve this goal. Conventional methods are available for the production of disease-resistant plants, but sometimes these methods do not meet actual needs, so countries that rely on agriculture need to adopt newer technologies, known as nanotechnology.

Nanotechnology is a rapidly developing field with the potential to revolutionize agriculture by providing more efficient and affordable ways to meet the needs of food production. It is becoming a pivotal shift and is evolving as a promising instrument to usher in a new phase of precise farming techniques and methods, and it could offer a potential solution for boosting agricultural productivity and enhancing crop improvement. It can be used to develop new agrochemicals that can help increase crop yields, reduce the use of toxic chemicals, control plant diseases, and help plants adapt to stress. Nanotechnology is gaining attention because it has unique properties that make it well-suited for agricultural applications. Nanoparticles (NPs) are very small (1 to 100 nm) and have a large surface area to volume ratio, which makes them highly reactive and efficient. They can also be used to

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deliver nutrients and other chemicals to plants in a more targeted and effective way than conventional methods.

NPs can be made of different materials and have different properties. In plant systems, NPs of metallic oxides, metalloids, carbon nanotubes, non-metals, quantum dots, liposomes, and dendrimers have been used. There are three main methods for synthesizing NPs: Physical, chemical, and biological. These methods can be further divided into two approaches: Bottom-up synthesis and top-down synthesis. Top-down methods generate small NPs from macromolecular starting materials, while bottom-up methods involve the initial creation of small nanostructures, eventually assembling them into NPs. Developing methods to synthesize NPs with specific sizes and shapes is essential for their diverse applications in catalysis, medicine, plant disease diagnosis, and the environment [3].

Nanotechnology can be used to develop new agrochemicals that can help increase crop yields, reduce the use of toxic chemicals, control plant diseases, and help plants adapt to stress. The application of nanomaterials in crop protection is primarily based on two principles: Genetic modification by the transfer of new proteins or genetic material (DNA or RNA) or the detection of plant disease using nano-sensors. Transfer devices such as nano-capsules, nanofibers, and NPs are utilized to deliver foreign DNA or gene sequences and successfully modify the target gene.

Despite recent advances in nanotechnology-based agriculture for crop improvement, several challenges persist. The environmental impact and potential risks associated with the use of NPs in agriculture remain inadequately understood, necessitating comprehensive risk assessments. Safety concerns regarding the biocompatibility of nanomaterials with plants, beneficial soil microorganisms, and human health pose critical obstacles. Moreover, the economic viability and accessibility of nanotechnology solutions, particularly for small-scale farmers in developing regions, remain significant hurdles. Additionally, bridging knowledge gaps regarding the long-term effects and interactions of nanomaterials with crops and soil is crucial, requiring ongoing research efforts to enhance our understanding and address potential risks associated with nanotechnology in agriculture. This review mainly focuses on recent advancements in nanotechnology-based crop improvement strategies in agriculture and different methods of synthesis of NPs (e.g. physical, chemical, and biological methods) and their characterization methods and support researchers from plant sciences, biotechnology, and nano-sciences, in comprehending innovative approaches to nano-based applications in agriculture.

Literature Review

Synthesis, characterization, and types of NPs used in crop improvement

NPs are small molecules that exhibit distinct properties determined by their size, shape, concentration, and stability. They are more efficient and reactive than bulk materials, and they are being studied extensively for their potential applications in plants. NPs can be classified based on their functionalization, surface morphology, chemical nature, physicochemical properties, dimension, origin, magnetic properties, and crystallinity. Nanoparticles can be synthesized using three main methods: Biological, physical, and chemical (Figure 1). These methods are further categorized into bottom-up and top-down approaches. Physical synthesis is also known as top-down synthesis, while chemical and biological synthesis is known as bottom-up synthesis [4].

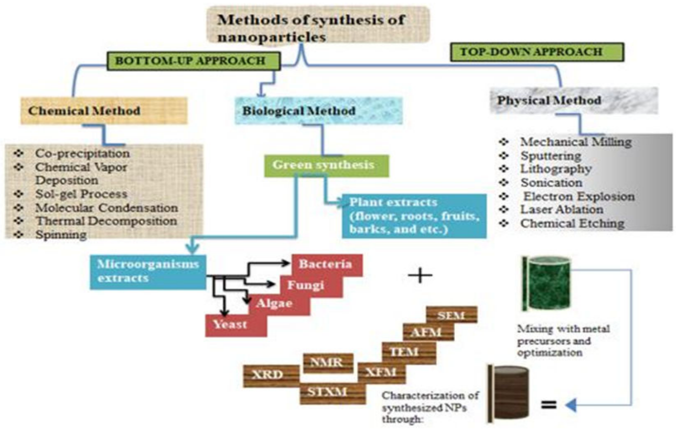

Figure 1. *General overview of methods of synthesis and characterization of nanoparticles.*

The top-down approach to NPs synthesis involves breaking down bulk materials into smaller particles. This can be done using a variety of methods, such as lithography, sputtering, mechanical grinding, chemical etching, thermal evaporation, pulsed laser ablation, and photo reduction. This approach is relatively inexpensive and can produce NPs of precise size and shape. It is also well-suited for producing structures with long-range order and for making macroscopic connections. The bottom-up approach to NPs synthesis involves building NPs from the ground up using wet-chemical processes such as chemical reduction or oxidation of metal ions, solid-gel chemistry, co-precipitation, micro-emulsion, and green synthesis. This approach is more versatile and can produce NPs with a wider range of shapes and sizes than the top-down approach. One of the main challenges of the bottom-up approach is that it often uses harmful reducing agents such as ethylene glycol, sodium borohydride, sodium citrate, and hydrazine, and additional use of capping agents such as polymers (e.g. polyethylene glycol, ascorbic acid, and polyvinylpyrrolidone and surfactants (e.g. sodium dodecyl sulfate, cetyltrimethylammonium bromide, and phospholipids) were required in steric stabilization to prevent NPs agglomeration. Additionally, the high-heat decomposition process that is often used to remove the stabilizing and reducing agents from the NPs surface can damage the NPs and reduce their reactivity [5].

Methods of synthesis of NPs for crop improvement

Biological (green) synthesis of NPs from plant extracts: Biological synthesis is a green, or environmentally friendly,

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method for making nanoparticles. Plant extracts can be used to synthesize nanoparticles in a cost-effective, environmentally friendly, and safe way. This method is advantageous over biological processes because it does not require complex cell culture or maintenance. NPs of different sizes and shapes, such as silver, gold, platinum, and titanium, can be synthesized from different parts of plants, such as leaves, fruits, bark, pericarp, and roots. Plants are a rich source of various bioactive compounds, including but not limited to phenols, flavonoids, ascorbic acids, ketones, aldehydes, carboxylic acids, terpenoids, and amides. This compound serves as a reducing and stabilizing agent in the biological synthesis of NPs.

Synthesis of NPs from plants encompasses a multi-step process (Figure 2). It commences with the collection of the required plant parts, which are washed with distilled water to rid them of impurities. Following a 10 to 15-day cleaning and drying procedure, the plant material is pulverized into a precious dried powder, and a specified gram of this powder undergoes boiling with a suitable solvent and thorough filtration to isolate particulate matter. The resulting filter is mixed with a metal particle solution, and through optimization, the NP can be synthesized and characterized through analytical techniques. The effective synthesis of NPs is often initially confirmed by the color change of the mixture due to the reduction of pure metal ions. In the optimization process, it is necessary to regularly monitor the UV-visible spectrum of the solution to identify the characteristic absorption properties of the NPs, which are indicative of the formation of NPs [6].

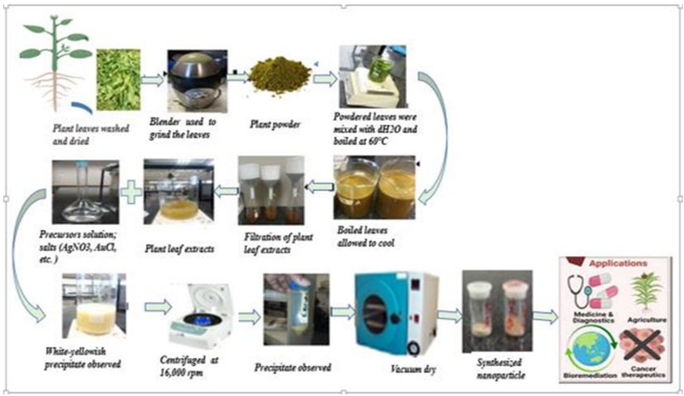

Figure 2. *Pictorial representation of the preparation process and biological synthesis of NPs using plant leaf extracts.*

Different plant extracts are currently used to produce different NPs (Table 1). A study reported that flavonoids act as reducing agents for the biological synthesis of silver NPs from *Ocimum basilicum* leaf extract. CuO NPs synthesized using *Malva sylvestris* leaf extract demonstrated potential antimicrobial activities against both gram-positive and gram-negative bacteria.

Biological (green) synthesis of NPs from microbes: The biosynthesis of NPs employing microbial agents stands as a green and eco-friendly approach. Among the commonly used microorganisms for synthesizing metallic NPs, bacteria, actinomycetes, fungi, and algae, enable the production of various NPs like silver, gold, platinum, iron, cadmium, and metal oxides such as titanium oxide and zinc oxide (Table 1 and Figure 3). These microorganisms' uptake target ions from their surroundings and subsequently convert metal ions into elemental metal through enzymatic processes arising from cellular activities. Depending on the site of NP synthesis, this method can be classified into two categories: Intracellular and extracellular. In the intracellular method, ions are transported into microbial cells, leading to NP formation in the presence of enzymes. Conversely, in the extracellular approach, metal ions are trapped on the cell surface, and enzymatic reduction leads to NP synthesis.

Table 1. *Biological synthesis of NPs using plant extracts and microorganisms.*

NPs	Microorganisms/plant extracts	Application
Pd	*Tabernaemontana divaricata*, and *Basella alba*	Effect of antibacterial and antifungal activities
Ag	*Acacia cyanophyte*	Used as antibiotic effect on *Escherichia coli*
ZnO	*Punica granatum* plant extract	Antimicrobial activities on *S. aureus*, *B. cereus*, *P. aeruginosa*, *K. pneumoniae*
Ag	*Coriolus versicolor*	Used for violating
CuO	*Eichhornia crassipes* leaf extract	Antimicrobial activities against *Staphylococcus pneumonia*, *Streptococcus aureus* and *Klebsiella pneumonia*.
TiO_2	*Aspergillus flavus*	Used in the formulation of NPs to fertilize
TiO_2	*Oryza sativa*	Photocatalyst activities

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Au	*Pseudomonas fluorescens*	Used in cancer treatment
Au	*Syzygium aromaticum*	Application in cancer treatment

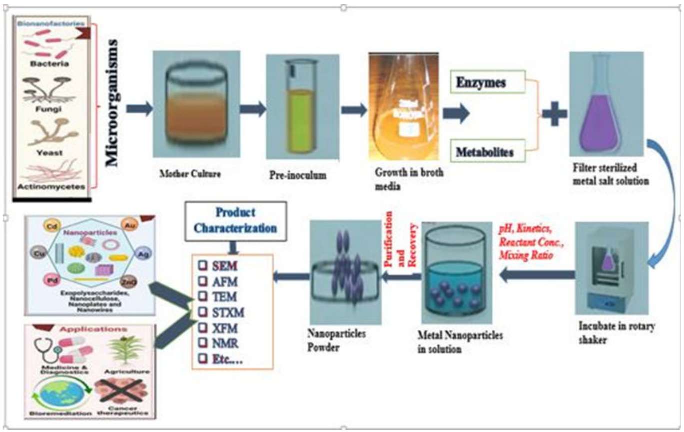

Figure 3. *A pictorial representation of the biological synthesis of different NPs, their characterization, and application using microorganisms.*

These biosynthesized metallic NPs, including silver, gold, copper, and zinc, exhibit antibacterial properties against both gram-positive and gram-negative bacteria like *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*. Notably, green synthesis of Ag NPs in *Streptomyces endophytes* showcases antimicrobial activity against four plant pathogenic fungi, namely *Alternaria*, *Streptomyces*, *Pythium*, and *Aspergillus niger*.

Physical methods for the synthesis of NPs: Physical methods are used to synthesize a variety of commercially important NPs, such as silver, copper, iron, and titanium. These methods apply different forms of energy to materials to cause abrasion, melting, evaporation, or condensation, which produces NPs. Physical methods are typically top-down, meaning that they start with large pieces of material and break them down into smaller NPs [7]. Some common physical methods for generating NPs include sputtering, electron beam lithography, sonication, electron explosion, pulsed wire discharge, laser ablation, and mechanical milling (Figure 4).

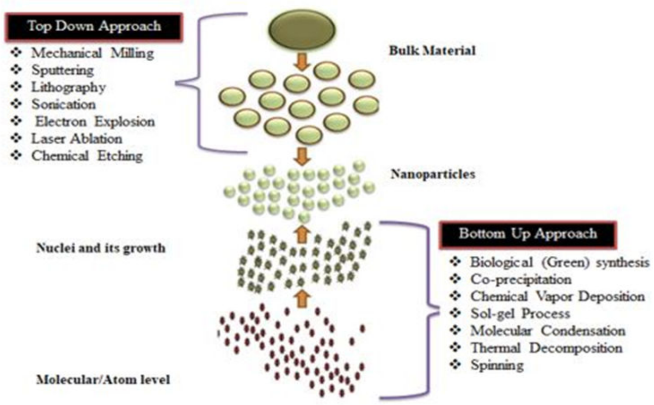

Figure 4. *The synthesis of nanoparticles by bottom-up and top-down approaches.*

Mechanical milling is a top-down approach for creating nanomaterials from bulk materials. It uses high-energy impacts to break down the bulk material into smaller and smaller pieces, eventually reaching the nanoscale and is a versatile technique to create a wide variety of nanomaterials, including carbon NPs, nanocomposites, and metallic NPs. Electrospinning is a simple and inexpensive technique that can be used to create a wide variety of nanomaterials with different properties. It is commonly used for creating nanomaterials from polymers and other hollow polymers and core-shell, organic, inorganic, and hybrid materials. Sputtering deposition is a physical vapor deposition technique that uses energetic ions to bombard a target material, ejecting atoms and clusters of atoms from the surface. The ejected atoms and clusters can then be deposited on a substrate to form a thin film. The sputtering deposition is a versatile technique that can be used to deposit a wide variety of materials, including metals, ceramics, and polymers. Lithography is another useful technique for creating NPs, typically using a focused beam of light or electrons. Additionally, it is affordable and easy to apply [8].

Chemical methods for synthesis of NPs: The chemical method is a widely used method for synthesizing NPs. It involves using various reducing agents, such as organic or inorganic chemicals, electrochemical techniques, or physicochemical processes, to convert metal ions into NPs. The shape and properties of the NPs can be controlled by the type of reducing agent and the synthesis conditions. For example, reducing silver nitrate with sodium borohydride can produce either spherical or rod-shaped silver NPs, depending on the specific parameters used. There are many different chemical mechanisms for synthesizing NPs, including co-precipitation, chemical vapor deposition, hydrothermal synthesis, sol-gel synthesis, molecular condensation, and thermal decomposition (Figure 4). Magnetic NPs are particularly important for medical imaging applications, e.g. micro-emulsions, sol-gel syntheses, sonochemical reactions, hydrothermal reactions, hydrolysis and thermolysis of precursors, flow injection syntheses, and electrospray syntheses.

Chemical vapor deposition is a gas-phase process that uses volatile precursors to deposit materials on a substrate. It is a well-known process for manufacturing two-dimensional nanomaterials and a versatile technique that can be used to produce a wide variety of nanomaterials, including carbon nanomaterials and metal oxides. It is particularly useful for producing high-quality nanomaterials with precise control over their composition, structure, and properties. Sol-gel is a wet chemical process that involves the hydrolysis and condensation of metal alkoxides to form a gel. The gel can then be dried and

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calcined to produce a nanocrystalline material. Sol-gel is a simple and cost-effective technique for producing a wide variety of metal oxide nanomaterials with controlled size, morphology, and purity.

Microwave-assisted hydrothermal synthesis is a promising new approach for engineering nanomaterials with different geometries. Hydrothermal and solvothermal methods are versatile and can operate at a wide range of temperatures, but nanomaterials produced at high temperatures may be unstable. Other methods for synthesizing copper nanoparticles include sonochemical and thermal reduction of copper hydrazine carboxylate and wet chemical synthesis using stoichiometric reactions.

Characterization of NPs used in crop improvement

Upon completing the synthesis of NPs, a crucial step entails their characterization to ensure optimal application efficacy. This includes various techniques, from exploring size, shape, atomic composition, crystal structure, and surface charge, to other essential physical, chemical, and biological properties. Employing advanced microscopy techniques like Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), Transmission Electron Microscopy (TEM), X-ray Fluorescence Microscopy (XFM), and Scanning Transmission X-ray Microscopy (STXM) allows for insightful determinations regarding NP size and morphology. Utilization of the Dynamic Light Scattering (DLS) technique facilitates the characterization of NP Brownian motion within colloidal suspensions. X-Ray Diffraction (XRD), on the other hand, emerges as an indispensable tool for NP characterization, providing valuable data regarding their crystalline structures. Meanwhile, Nuclear Magnetic Resonance (NMR) is instrumental in revealing various structural features of the NPs. Moreover, UV-visible spectroscopy sheds light on the optical properties of NPs, assessing light reflection from these nanomaterial's.

Types of NPs used in plant crop protection

The utilization of NPs in agriculture offers a promising avenue to reduce toxic agricultural practices and enhance disease control in crops. In modern times, a diverse range of NPs, including metal and metal oxide NPs, nonmetal NPs, carbon nanoparticles, chitosan NPs, liposomes, quantum dots, and dendrimers, is harnessed to combat plant pathogens.

Copper NPs (Cu NPs): It stands as an optimal choice for diagnosing plant diseases, boasting potent antimicrobial properties and disease-fighting capabilities. Impressively, fungicides formulated from Cu NPs, as revealed by Koul, et al. effectively hinder the progression of *Phytophthora infectants* in tomato plants [9]. Notably, biologically synthesized Cu NPs derived from *Streptomyces griseus* demonstrate remarkable efficacy in suppressing tea root rot disease caused by the pathogen *Poria hypolateritia*. Furthermore, Cu NPs have been found to enhance the growth performance of wheat (*Triticum aestivum*) even under heavy metal stress conditions.

Silver NPs (Ag NPs): Silver NPs are extensively researched and well-known for their antimicrobial properties. According to a study by Jagana, et al. green-synthesized nano-Ag demonstrated suppression of disease progression by nearly 70% in banana fruit against the pathogen *Colletotrichum musae* [10]. Another study by Ocsoy, et al. found that Ag NPs, which specifically targeted double-stranded DNA, effectively combated the bacterial pathogen *Xanthomonas perforans*, known to cause bacterial spot disease in tomato plants [11]. Bacterial species commonly used for the synthesis of Ag NPs include *Escherichia coli*, *Lactobacillus* sp., *Bacillus cereus*, *Acinetobacter* sp., *Pseudomonas* sp., *Corynebacterium* sp., and *Klebsiella pneumonia*.

Zinc NPs (Zn NPs): Zinc NPs possess remarkable antimicrobial properties, making them highly valuable in the control of plant diseases. They have gained significant attention due to their cost-effectiveness, large surface area, white appearance, UV-filtering capabilities, antifungal and antibacterial properties, photochemical characteristics, and high catalytic activity. Numerous studies have demonstrated the effectiveness of Zn NPs against various plant pathogenic fungi, including *Mucor plumbeus*, *Alternaria alternative*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, and *Rhizopus stolonifera*. Phytochemicals, such as polyphenols, saponins, and terpenoids, are synthesized in different parts of plants, including the roots, stems, leaves, fruits, and seeds. These phytochemicals facilitate the reduction of the metal to its zerovalent state, followed by calcination to form metal oxides. In a study conducted by Mohd Yusof, et al. ZnO NPs were synthesized using *Calotropis procera* latex [12]. The resulting NPs were spherical in shape and ranged in size from 5 to 40 nm.

Liposomes: Liposomes are commonly employed for plant irrigation due to their exceptional stability in water. Comprised of phospholipid bilayers and exhibiting a spherical shape with a hollow central core, liposomes can address plant diseases by incorporating antimicrobial agents and other compounds known for their disease-suppressing properties.

Chitosan NPs: Chitosan NPs have become increasingly popular due to their desirable traits such as being non-toxic, biodegradable, and cost-effective. Recent research has demonstrated the capability of chitosan NPs to enhance disease resistance in plants and function as carriers for drug delivery and gene transfer. Additionally, the manufacturing process of chitosan NPs is relatively simple. For example, in a tea plant-focused research, the application of chitosan NPs led to an improved defense mechanism against diseases by increasing the levels of specific enzymes involved in defense responses such as polyphenol oxidase, phenylalanine ammonia lyase, and -1,3-glucanase.

Carbon NPs: Carbon NPs possess distinct properties and serve various purposes in diverse research fields. Nevertheless, recent investigations suggest that synthetic C NPs have the potential to influence plant growth and eliminate certain plant pathogens. Disease management primarily utilizes three types of C NPs: Fullerenes, graphene oxides, and carbon nanotubes. Research has indicated that single-walled carbon nanotubes can hinder the formation of conidia in *Fusarium graminearum* and *Fusarium poae*.

Dendrimers: Dendrimers are NPs with a tree-like structure that play a crucial role as carriers for delivering chemicals or DNA to enhance disease resistance. They facilitate the transportation of disease-fighting substances into tissues, especially in areas where these substances have limited reach.

Discussion

Roles of nanotechnology in plant protection and diseases management

The introduction of nanotechnology in agriculture holds great potential for revolutionizing conventional agricultural practices by offering new tools to enhance crop disease resistance. The use of NPs in the form of nano-fertilizers can directly influence plant physiology and serve as a means to transfer genetic material or protective proteins that defend against plant pathogens by triggering the expression of pathogenesis-related genes. Various nanomaterials, such as polymer NPs, iron oxide NPs, and gold NPs, can be synthesized easily and utilized as carriers for pesticides or medicines. The use of NPs has been shown to have a regulatory effect on plant growth, seed germination, and acclimatization. A study conducted by Singh, et al. found that the application of SiO_2 and TiO_2 NPs promoted seed germination in various crops [13]. Furthermore, the application of ZnO NPs influenced root shoot growth in peanut plants and increased biomass production in chickpea plants. Recent advancements in nanotechnology offer potential solutions for promoting stress tolerance in various crop species.

NPs have shown potential in agriculture for disease and weed control (Figure 5). Inorganic NPs such as ZnO, Cu, SiO_2, TiO_2, CaO, MgO, MnO, and Ag NPs have significant roles in crop protection, particularly in microbial activity and combating bacterial diseases. For instance, recent research has demonstrated that ZnO NPs effectively inhibit the growth of various pathogens including *Fusarium graminearum*, *Penicillium expansum*, *Alternaria alternative*, *F. oxysporum*, *Rhizopus stolonifer*, *Mucor plumbeus*, and pathogenic bacteria *Pseudomonas aeruginosa*. Traditionally, farmers have used methods such as crop rotation, integrated pest management, genetic breeding, and chemical pesticides to control pathogens. However, in recent years, the use of NPs in combating plant diseases has brought about significant changes in agriculture.

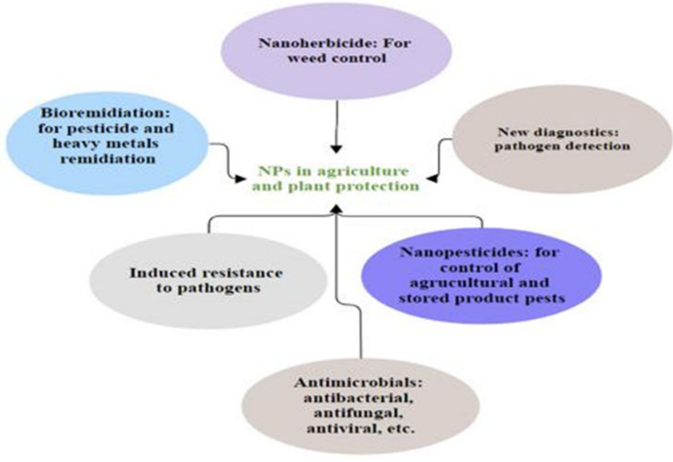

Figure 5. *Roles of nanoparticles in plant protection and disease management.*

Uptake and translocation of NPs into the plants

NPs can be taken up by different parts of plants including leaves, roots, and shoots. They can enter through natural openings such as stomata, hydathodes, stigma, and wounds. To deliver NPs to plants, various application methods like seed coating, soil drenching, and foliar spraying are commonly used (Figure 6). The process of uptake involves nutrient passage from the soil to the root surface, ion transport through the membranes of root surface cells, radial movement of ions into the root xylem vessels, transport through the xylem, and distribution of ions in the aboveground parts of the plant [14].

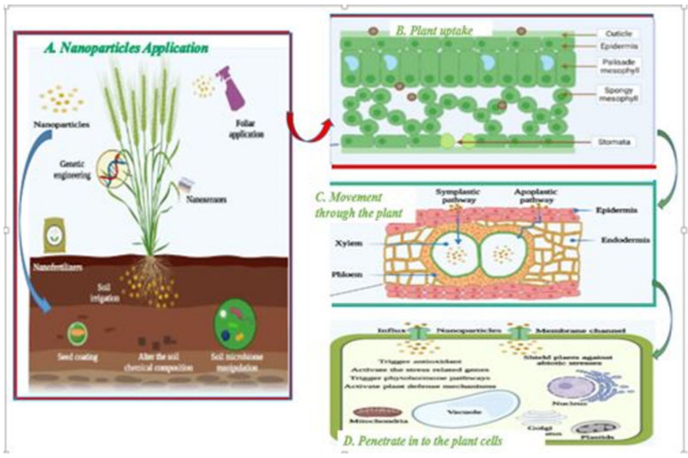

Figure 6. *Pictorial representation of a potential NPs application, uptakes, and translocation into plants.*

Carbon NPs such as fullerene C70 and fullerol ($C_6O(OH)_2O$), as well as metal NPs like TiO_2, Au, Ag, Cu, CeO_2, FeO, and ZnO, have been extensively studied. The uptake, translocation, and accumulation of NPs depend on factors such as the plant species involved, as well as the size, species, chemical composition, functionalization, and stability of the NPs. NPs can interact with membrane transporters or root exudates, which are substances released from the plant's rhizosphere and then transported into the plants. Once inside the plant, NPs need to be distributed throughout the plant tissue for their desired effects. This distribution occurs through two pathways:

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Symplastic and Apoplastic translocation. Both pathways enable NPs to move within the xylem and phloem tissues, allowing them to be transported throughout the plant. In symplastic translocation, NPs must penetrate the cell interior. However, the presence of cellulose and hemicellulose in the plant cell wall can sometimes prevent NPs from entering the cell. Nonetheless, studies have shown that NPs with a diameter of 5-50 nm, as well as carbon nanotubes, can effectively penetrate cell walls in many plant species. Apoplastic translocation, on the other hand, facilitates the radial movement of NPs into the root vessel tissue and aids in their upward movement toward the aerial parts of the plant.

Nanotechnology-mediated smart gene transfer and crop improvement: Molecular approaches

Nanotechnology is considered a crucial technology with the potential to enhance traditional agricultural practices and promote sustainable development. An essential element in achieving sustainable agricultural production is the efficient delivery of agrochemicals and organic molecules, such as DNA molecules or oligonucleotides, into plant cells. Traditional methods usually involve spraying and/or broadcasting agrochemicals onto crops. Due to factors such as chemical leaching, photolysis, hydrolysis, and microbial degradation, only a small proportion of agrochemicals reach the intended areas of crops [15]. As a result, there is a growing emphasis on utilizing nanotechnology to develop environmentally friendly agricultural practices, particularly through the synthesis of fertilizers, pesticides, and herbicides with controlled or slow-release properties. The field of nanotechnology has evolved, progressing from experimental laboratory studies to practical applications. NPs offer several advantages for effective agrochemical delivery, including their large surface area, ease of attachment, and rapid mass transfer.

The combination of nanotechnology and biotechnology not only has implications for agriculture but also presents opportunities for molecular transporters to modify genes and create new organisms (Figure 7). Researchers have successfully manipulated gene expression by utilizing nanofibers, nanocapsules, and NPs. However, the conventional method of gene transfer using viral vectors faces various challenges such as limited host range, size limitations for inserted genetic material, and difficulties in transport across cell membranes and the nucleus during genetic material transfer. Compared to viral vector-mediated genome transfer, recent breakthroughs in nano-biotechnology offer researchers greater opportunities to completely replace the genetic material of one species with another. Recent advancements in nanomaterial-based delivery systems have significantly contributed to the field of genetic engineering, particularly in the specific delivery of CRISPR/Cas9 Single-Guide RNA (sgRNA). The CRISPR/Cas9 system is a groundbreaking tool in molecular biology with the potential to revolutionize biotechnology. It consists of two components, a stretch of repeated sequences and the Cas9 protein.

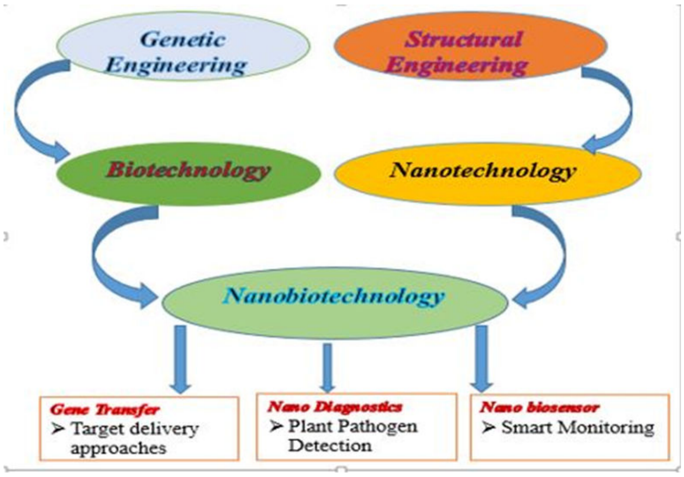

Figure 7. *A diagrammatical representation of the amalgamation of nanotechnology and biotechnology (Nano-biotechnology) for different purposes in agriculture.*

The integration of NPs in delivering the CRISPR/Cas9 system has enhanced the precision and efficiency of genome editing. By utilizing DNA-coated NPs and gene gun technology, targeted gene modifications have been accomplished in various crops. In the case of Zea May's immature embryo, genome editing was successfully performed using Mesoporous Silica NPs (MSNs). Additionally, carbon nanotubes have been employed for siRNA-mediated gene silencing in *Triticum aeativum*, *Nicotiana benthamiana*, *Gossypium hirsutm* and *Eruca sativa* [16].

Application of NPs in agriculture

Agriculture serves as the primary source of livelihood for more than 60% of the global population, particularly in emerging countries, where it plays a crucial role in economic development. The utilization of nanotechnology in agriculture aims to enhance crop production and improve the efficiency of resource utilization. NPs have a range of applications in agriculture, including their use as nanobiofertilizers, nanopesticides, nanobiosensors, nanobiofarming, and nano-insecticides (Figure 8). These various applications of nanotechnology offer promising opportunities to address agricultural challenges and promote sustainable productivity in this field.

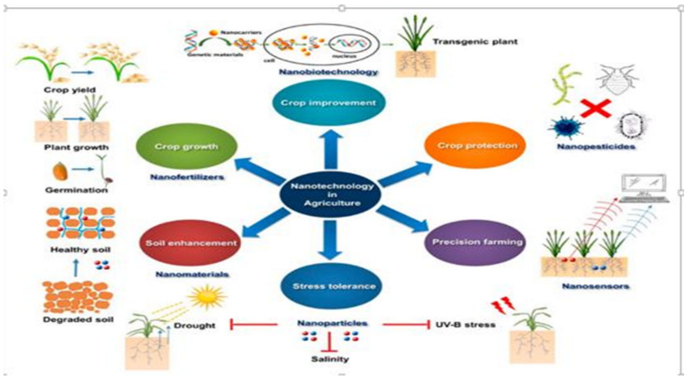

Figure 8. *Application of nanotechnology in agriculture.*

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Nano-pesticides: Nano-pesticides play a significant role in improving crop yield and efficiency in agriculture. These innovative products, some of which are already available in the market, offer promising solutions for plant protection. By utilizing nanotechnology, the transport potential of pesticides can be enhanced, leading to enhanced plant productivity. Nanomaterials such as polymeric NPs, silver ions, gold NPs, and iron oxide NPs are commonly used as pesticides in agriculture. Researchers have explored various methods for formulating, characterizing, and applying NPs in controlling plant diseases. For example, silver NPs derived from *Tinospora cordifolia* have shown insecticidal activity against larvae of *Culex quinquefasciatus* and *Anopheles subpictus*, while silver NPs have demonstrated effective anti-lice and mosquito control.

Nano-herbicides: Weed infestation poses a significant threat to crop yields if not properly managed. A solution to this problem is the use of nano-herbicides, which effectively eliminate weeds in an environmentally friendly manner, without leaving behind any toxic residues in the soil or surrounding ecosystem. When nano-herbicides are combined with soil particles, they prevent the growth of resistant weed species by targeting a specific receptor in the roots of the target weeds. Research has shown that the green synthesis of metal NPs using various natural extracts, such as *Azadirachta indica*, *Ocimum* spp., *Citrus limon* leaf extracts, *Accacia gum*, and *Punica granatum*, exhibit insecticidal activities [17].

Nano-fertilizers: Nano-fertilizers are instrumental in boosting crop yields across various types of crops. These fertilizers consist of formulations that harness the power of one or more microorganisms to improve soil productivity. They achieve this by fixing atmospheric nitrogen, synthesizing growth-promoting substances, and making phosphorus more soluble. In essence, the combination of bio-fertilizers and nanostructures is what defines nano bio-fertilizers. There are three key aspects to consider when it comes to nano bio-fertilizers: The interaction between NPs and microorganisms, the longevity of the bio-fertilizers, and how they are transported. By incorporating NPs, the stability of bio-fertilizers is enhanced. They become more resilient in terms of tolerating desiccation, heat, and UV inactivation, and overcoming shelf life limitations.

Nano-sensors: Nano-biosensors are a type of nano-sensor equipped with bio-receptor probes that specifically target certain analytic molecules. These biosensors consist of a signal receptor and a signal transducer, offering advantages such as small size, stability, reproducibility, accuracy, and low toxicity, thus promoting sustainable agriculture. Their applications range from detecting analytes like urea, glucose, and pesticides to monitoring metabolites and various pathogens. Nano-sensors can identify plant pathogens, environmental pollutants, and soil-borne diseases caused by bacteria, fungi, and viruses. Researchers have developed wireless nano-sensors that can detect insect attacks by sensing the release of volatile organic compounds from crop pathogens. Nano-biosensors have also been designed to identify Tobacco Mosaic Virus (TMV), lettuce mosaic virus, and cowpea mosaic virus [18]. In recent times, portable nano-devices have facilitated the detection of various environmental pollutants, pathogens, insects, diseases, and chemicals, leading to a decrease in the usage of pesticides, fungicides, and antibiotics.

Nanoscale carriers: Smart nanoscale devices known as nanoscale carriers have been developed to enable the targeted delivery of fertilizers, herbicides, pesticides, and plant growth regulators efficiently. These carriers are designed to anchor the roots of plants to the surrounding soil and organic matter, enhancing their stability and protecting against environmental degradation.

Precision farming: Currently, remote sensing devices, computers, and GPS systems are gaining popularity for analyzing the environment, monitoring crops, and detecting issues in crop production. By combining nano sensors with GPS-enabled smart devices, farmers can receive timely and accurate information to take corrective measures at the right stage and time. This integrated approach not only reduces the excessive use of resources like water, fertilizers, pesticides, and herbicides but also promotes a sustainable farming approach.

Negative perspectives of nanotechnology application on plant production

The agricultural sector has recognized the significant potential of nanotechnology, but its unchecked and excessive use has led to adverse effects on ecosystems. Plants, as integral components of ecosystems, play a crucial role in maintaining environmental balance. However, NPs used in plants often exhibit a certain degree of toxicity, which can negatively impact not only the plants but also the microbial life or microflora in the soil. The presence of NPs and free radicals generated by them can cause DNA damage and lipid peroxidation in plants [19].

Metal NPs, metal oxide NPs, and carbon NPs are commonly used in plant applications. However, their absorption by plants and subsequent translocation throughout the plant body can lead to the production of toxic byproducts. For instance, the application of cerium oxide NPs on soybean plants has been found to reduce pod size, inhibit growth, and impair nitrogen fixation ability. Studies have also demonstrated that excessive use of metal and metal oxide NPs in cultivated plants can trigger an oxidative burst through interaction with the electron transport chain [20].

Conclusion and Future Perspectives

Nanotechnology has great potential in agriculture, addressing challenges like population growth, plant diseases, and pollution. It can lead to sustainable farming practices and a green revolution. Advancements in nanotechnology offer opportunities for crop improvement, including enhanced growth, nutrient uptake, and disease management. Nano-fertilizers, nano-pesticides, nano-insecticides, nano-herbicides, and nano-sensors are paving the way for a more sustainable agricultural system. Collaborative research is needed to understand risks and optimize nanotechnology use in agriculture. Future studies should focus on risk assessment, soil interactions, and integrating nanotechnology with

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biotechnology for disease diagnostics. Nanotechnology has the potential to revolutionize agriculture, but further research and collaboration are necessary to unlock its full potential.

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