Chapter 9
Nanobiotechnology and Its Applications in Plant System Biology

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9.1 Background

Materials with a dimension less than 100 nm and a high surface-to-volume ratio are called nanomaterials due to their small size. They have unique physicochemical properties like increased reactivity and a small surface area with a typical surface structure. They are highly reactive because of their small size, accumulation of nanoparticles, stability, surface structure, shape, and chemical composition (Wang et al. 2016). Nanomaterials, in addition to their unique physicochemical features, are highly receptive to surface conjugation, allowing them to be produced as adaptable platforms with a wide range of applications in plant science (Machado et al. 2020; Hu et al. 2020). Nanotechnology has showed great promise in agriculture: it can increase plant stress tolerance by scavenging reactive oxygen species (ROS) with nanozymes (nanomaterials that imitate antioxidant enzyme functions) (Gao et al. 2007; Pirmohamed et al. 2010). Cerium oxide enhances tolerance against abiotic stresses (heat, cold, salinity, and drought) in plants (Liu et al. 2021b; Wu et al. 2018; Djanaguiraman et al. 2018; Wu et al. 2017b). Nanobiotechnology also enables the targeted and controlled release of agrochemicals (Zhang et al. 2020b; Santana et al. 2020); stress detection at early stages (Giraldo et al. 2019; Kwak et al. 2017) by using carbon nanotubes for sensing Ca^{2+}, H_2O_2, and NO (Wu et al. 2020); successful delivery of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) in non-model plant species (Demirer et al. 2019; Kwak et al. 2019); as well as stress tolerance by seed priming (nanopriming) with nanoparticles (Rizwan et al. 2019; Mahakham et al. 2017).

By 2050, the world's population is expected to reach 9 billion, and feeding such a large number is a great challenge. Scientists have estimated that Agriculture production must grow by 60% from 2005–2007 levels to feed a population of nearly 9 billion people by 2050 (Van Ittersum et al. 2016). Many efforts have been made through plant breeding, cultivation practices, and farm management to reduce the gap between demand and supply of food, but it is still an emerging problem that can only be solved by modern techniques, like nano-enabled agriculture, to mitigate food shortage. An emerging field called nano-enabled agriculture has the potential to increase plant tolerance to biotic and abiotic challenges, as well as plant breeding and agriculture. By 2050, it is expected that plant nanobiotechnology will address food shortage and have great importance in sustainable agriculture.

Previously, plant biotechnology was not much focused on by researchers; rather, they emphasized nanosensors, nanotoxicity, and nanoparticles in agricultural production (Zhao et al. 2020; Xin et al. 2020; Zhang et al. 2020a; Acharya et al. 2019). Nanomaterials' biosafety concern might be substantially handled with adequate management and design (Gilbertson et al. 2020; Adisa et al. 2019). The use of nanomaterials in sustainable agriculture is being focused on in the current chapter. The

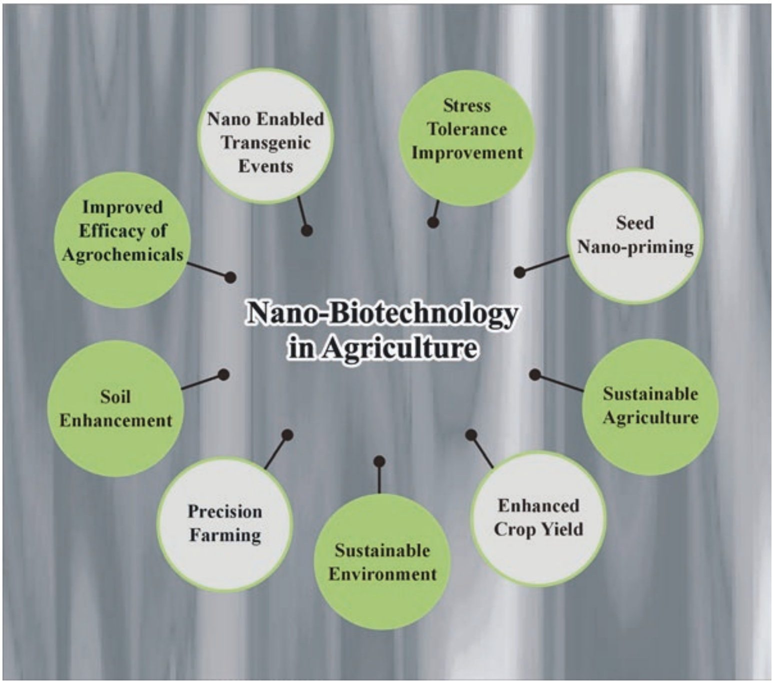

Fig. 9.1 Role of nanobiotechnology in various aspects of agriculture

major objective of this chapter is to encourage researchers specializing in plant biotechnology to further work on nano-enabled agriculture. Figure 9.1 illustrates the dynamic role of nanobiotechnology in various aspects of agriculture to improve the current agricultural production system.

9.2 Plant Stress Tolerance by Nanoparticles

9.2.1 Nano vs Bulk

Many researchers have recently worked on nano- and bulk materials in plants for agricultural production. By comparing the technique of using nanofertilizers and nanopesticides with conventional approaches, it was proved that nanomaterials are 20–30% more efficient (Dietz and Herth 2011). Therefore, we emphasize the higher efficacy of using nanomaterials as compared to conventional methods. Nanomaterials offer features like catalytic ROS (reactive oxygen species) scavenging ability and self-fluorescence that bulk or commercial equivalents lack due to changes in physical and chemical properties at the nanoscale level. It was reported that CeO_2 nanoparticles are ROS scavengers, and they have a wide application in plant sciences, industries, and medical research (Kah et al. 2018). CeO_2 nanoparticles feature a significant number of surface oxygen vacancies that alternate between two oxidation states Ce^{3+} and Ce^{4+} to offer substantial ROS scavenging capacity, in contrast to bulk cerium oxide (Walkey et al. 2015). ROS is catalyzed; the dangling bonds in Ca^{3+} efficiently scavenge ROS, while lattice strain increases surface oxygen vacancies by redox reactions (Boghossian et al. 2013). Another newly developed nanoparticle is Mn_3O_4, which has a greater in vivo ROS scavenging ability because it exists in two oxidation states, Mn^{2+} and Mn^{3+}, at a ratio of 1:2 (Yao et al. 2018). Salinity stress tolerance in crop plants (*Brassica napus*) was observed after the application of cerium oxide. The plants treated with cerium oxide had 48% more fresh biomass than the control plants (Rossi et al. 2016).

In two other studies, polyacrylic acid coated cerium oxide nanoparticles improved salt tolerance in Arabidopsis (50 mg L^1, 10.0 nm, –17.0 mV, foliar spray) and cotton (100 mg L^1, 8.0 nm, –15.3 mV, foliar spray), with plants under salinity stress showing 18% and 40% increases in biomass, respectively, compared to no-nanoparticle controls (Wu et al. 2018; Liu et al. 2021b). The mechanism was described as nanoceria scavenging of ROS. Thus, it enabled the modulation of channels and transporters for K^+ retention in the mesophyll (KOR gene expression with downregulation) and the exclusion ability of N^+ from the shoot (HKT1 gene expression with upregulation) (Liu et al. 2021b; Wu et al. 2018). Salt tolerance in cucumber was induced by foliar application (1 mg $plant^{-1}$, 226.4 nm, –7.7 mV) of Mn_3O_4. A 19% increase in the fresh biomass of cucumber was observed as compared to the control (Lu et al. 2020). Sorghum plants were treated with cerium oxide nanoparticles and were recorded to have drought stress tolerance. CeO_2 nanoparticle application also enhanced the temperature and UV and high light tolerance in *Arabidopsis thaliana*.

Carbon-based nanoparticles are another example of nanomaterials being used in the improvement of crop production. Carbon quantum dots (CD) have a wide application in agriculture due to their various physical and chemical properties, *i.e.*, superficial synthesis, lower toxicity, higher stability, adjustable surface functions, higher water stability, strong photoluminescence, and biocompatibility. Drought resistance has been induced in peanut plants by applying carbon nanodots through leaf infiltration (Su et al. 2018); however, its mechanism is still unclear. The application of multiwalled carbon nanotubes increased salinity stress tolerance in *Brassica napus*. The nanotubes were added into Skoog medium and Murashige (Zhao et al. 2019). Hydroponically applying carbon nanotubes also induced salinity stress tolerance in broccoli (Martínez-Ballesta et al. 2016). Carbon nanotubes promoted nitric oxide (a gas-signaling molecule) and the transduction of aquaporins. Silicon nanoparticle is another nanomaterial used to increase stress tolerance in crop plants. The soil application of silicon nanoparticles increased fresh biomass (up to 27%) and chlorophyll content (up to 17%) in barely grown plants under drought stress, so recovery from drought stress was observed in these plants (Martínez-Ballesta et al. 2016). ZnO, Fe nanoparticles, and TiO_2 nanoparticles have also played a crucial role in improving plant tolerance against abiotic stresses (Sun et al. 2020; Abdel Latef et al. 2018). Drought tolerance was also observed in maize through the application of ZnO nanoparticles in soil. Plants treated with ZnO (100 mg L^{-1}, 37.7 nm, 14 mV) had a 75% increase in proline and an 18% decrease in H_2O_2 (Sun et al. 2020). A list of nanoparticles, their concentration, and their effective performance against certain abiotic stress are provided in Table 9.1.

Table 9.1 Nanoparticles, their concentrations, plant species, application, and the type of abiotic stress

Sr #.	Nanoparticle	Concentration	Species	Treatment	Stress	Reference
1	Ag	25, 50, 75, and 100 mg/L	*Triticum aestivum*	Potting soil	Heat stress	Iqbal et al. (2019)
2	Ag	0, 2, 5, and 10 mM	*Triticum aestivum*	Seed priming	Salinity stress	Mohamed et al. (2017)
3	Ag	0, 10, 20, 30, and 40 µg mL−1	*Trigonella foenum-graecum*	Petri dish exposure	Salinity stress	Hojjat and Kamyab (2017)
4	Ag	1 mg/L	*Triticum aestivum*	Seed priming	Salinity stress	Abou-Zeid and Ismail (2018)
5	Al2O3	50 ppm	*Glycine max L. cv.*	Petri dish exposure	Flooding stress	Mustafa and Komatsu (2016)
6	CeO	500 mg/L	*Gossypium hirsutum L.*	Seed priming	Salinity stress	An et al. (2020)
7	Chitosan NPs	0, 30, 60, and 90 ppm	*Hordeum vulgare L.*	Foliar application	Drought stress	Behboudi et al. (2018)
8	Chitosan-PVA and CuNPs	50, 100, and 150 mg/L	*Solanum lycopersicum L.*	Nutrient solution	Saline stress	Hernández-Hernández et al. (2018)
9	CNTs and graphene	50 and 200µg/ml	*Catharanthus roseus*	Murashige and Skoog medium	Salinity stress	McGehee et al. (2019)
10	CNTs and graphene	50 and 200µg/ml	*Gossypium hirsutum*	Seed priming	Drought stress	Pandey et al. (2019)
11	Cu	3.333, 4.444, and 5.556 mg/L	*Zea mays*	Plants priming	Drought stress	Van Nguyen et al. (2021)
12	Fe	0, 25, 50, and 100 mg/kg	*Triticum aestivum*	Potting soil	Cadmium and drought stress	Adrees et al. (2020)
13	Fe2O3	0, 10, 20, and 30 $_{\mu}$M	*Mentha piperita L.*	Hoagland solution	Salinity	Askary et al. (2017)
14	Fe3O3	0, 30, 60, and 90 ppm	*Dracocephalum moldavica L.*	Foliar application	Salinity stress	Moradbeygi et al. (2020)
15	Fe3O4	0.8 ppm	*Fragaria × ananassa*	Murashige and Skoog	Drought stress	Steinfeld et al. (2015)
16	$FeSO_4$	2 g/L	*Helianthus annuus*	Foliar spray	Salinity stress	Torabian et al. (2017)
17	Mn	0.1, 0.5, and 1 mg/L	*Capsicum annuum L.*	Nanopriming	Salinity stress	Ye et al. (2020)
18	MWCNT	10, 30, 50, 100, and 200 mg/L	*Dodonaeaviscosa L.*	Nanopriming	Drought stress	Yusefi-Tanha et al. (2020)

(continued)

Table 9.1 (continued)

Sr #.	Nanoparticle	Concentration	Species	Treatment	Stress	Reference
19	Poly(acrylic)-CeO	~50 mg/L	*Arabidopsis thaliana*	Leaf infiltration	Multiple stress	Wu et al. (2017a)
20	Se	10 mg/L	*Sorghum bicolor (L.) Moench*	Foliar and water spray	Heat stress	Djanaguiraman et al. (2018)
21	Se	0, 1, 4, 8, and 12 μM	*Lycopersicum esculentum*	Hydroponic solution	High and low temperature stress	Haghighi and Pessarakli (2013)
22	Si	10 μM	*Triticum aestivum*	Nutrient solution	UV-B stress	Sedghi et al. (2013)
23	SiO_2	0.5, 1, 2, and 3 mM	*Solanum lycopersicum L.*	Exposure *in vitro*	Salinity stress	Almutairi (2016)
24	SiO_2	50 and 100 mg/L	*Strawberry*	Exposure in nutrient sol	Salinity stress	Avestan et al. (2019)
25	SiO_2	0, 200, 400, and 600 mg/L	*Musa acuminata "Grand Nain"*	*In vitro*	Salinity and water deficit	Mahmoud et al. (2020)
26	TiO_2	0, 10, 100, and 500 mg/L	*Linum usitatissimum*	Leaf treatment	Drought	Aghdam et al. (2016)
27	TiO_2	0.01, 0.02, and 0.03%	*Triticum aestivum L. cv. "Pishtaz"*	Spraying by backpack sprayer	Drought	Jaberzadeh et al. (2013)
28	TiO_2	0, 2, 5, and 10 ppm	*Cicer arietinum L.*	Amended soil	Cold stress	Mohammadi et al. (2013)
29	TiO_2	500, 1,000, and 2,000 mg/kg	*Triticum aestivum*	Amended soil	Drought stress	Faraji and Sepehri (2020)
30	Yttrium doped	100, 160, 200, and 400 mg per plant	*Brassica napus*	Nutrient solution	Drought	Palmqvist et al. (2017)
31	ZnO	0, 0.5, and 1 g/L	*Glycine max*	Petri dish exposure	Drought	Sedghi et al. (2013)
32	ZnO	10 mg/L	*Abelmoschus esculentus L.*	Foliar application	Salinity stress	Alabdallah and Alzahrani (2020)
33	ZnO and Si	50, 100, 150 mg/L; ZnO NPs and 150, 300 mg/L; SiNPs	*Mangifera indica L.*	Foliar spray	Salinity stress	Elsheery et al. (2020)

Additionally, diseases and insects can impact the quality and harvest of crops. There are concerns about the health and environmental impacts of pesticides and an increase in fungal and insect resistance (Damalas and Eleftherohorinos 2011). Despite this, novel tactics for biotic stress defense that are both environmentally benign and effective are still required. The use of nanobiotechnology in the production of insecticides (Jameel et al. 2020), fungicides (Ma et al. 2020), and herbicides (Cao et al. 2017) has the potential to improve their effectiveness. For example, *Spodoptera litura* larvae fed with a mixture of thiamethoxam (10–90 mg L^{-1}) and ZnO nanoparticles (TEM size 30 nm) died at a 27% greater rate than the larvae fed in the control. Plants have been exposed to a wide range of nanoparticles to determine whether they can improve their biotic stress tolerance, including Ag- and Cu-based nanomaterials. Ag-based nanoparticles are effective in inhibiting diseases as well as nematodes (Mishra et al. 2014; Ali et al. 2015). Disease control and pest activity inhibition are two applications for copper-based nanoparticles that are commonly applied (Cumplido-Nájera et al. 2019; Borgatta et al. 2018; Ayoub et al. 2018). A 58% reduction in the progression of *Fusarium oxysporum* infection in watermelon was demonstrated by $Cu_3(PO_4)23H_2O$ nanoparticles (10 mg L^{-1}, 151 nm) because of their smaller size, distinct structure, and faster initial release of copper ions (Borgatta et al. 2018). Carbon nanotubes (Wang et al. 2014), carbon dots (Li et al. 2020), Si-based nanoparticles (Buchman et al. 2019), MgO nanoparticles (Huang et al. 2018; Cai et al. 2018), TiO_2 nanoparticles (Paret et al. 2013), and CeO_2 nanoparticles (Adisa et al. 2018) are examples of nanomaterials that have shown the capacity to prevent plant diseases. Many researchers have applied nanoparticles to reduce biotic stress on plants (Servin et al. 2015).

Crop losses resulting from diseases, insects, and weeds amount to more than $2000 billion every year globally (Popp et al. 2013). Applied fungicides for disease management cost more than $600 million per year in the United States (González-Fernández et al. 2010). *Pyricularia oryzae* is a pathogen that causes rice blast, reducing rice production by as much as 80% in Thailand (Kongcharoen et al. 2020; Srivastava et al. 2017). The fungi can quickly evolve and become resistant to present fungicides if they are applied to resistant rice varieties. Therefore, the use of nanoparticles to combat rice blasts is worth studying in the future.

Drought stress is a substantial hindrance to the production of agricultural crops in semi-arid regions. Crop yields are increased by using environmentally safe nanoparticles. Farmers in water-stressed areas may benefit from drought resilience, which can help them maintain or enhance their revenue. The use of nanotechnology to strengthen drought tolerance has been demonstrated in several plant species (Sun et al. 2021; Taran et al. 2017). Drought tolerance in sorghum increased with the use of cerium oxide nanoparticles (nanoceria). Salt tolerance in cotton was improved by seed priming using polyacrylic-acid-coated nanoceria (An et al. 2020). Making polyacrylic acid-coated cerium oxide nanoparticles for nano-priming cotton seed for sowing a one-hectare area costs less than $30, while foliar spraying a one-hectare area costs more than $100. If output were to grow, chemical expenses would be reduced as a result. Personnel, equipment, and utilities (such as water, gas, and electricity) are all factors that contribute to the cost of manufacturing a commercial product.

9.2.2 Dispersion of Nanomaterials

Heavy metal nanoparticles, such as Cd^{2+} quantum dots (Li et al. 2018), cerium oxide nanoparticles, and Ag nanoparticles, may constitute a threat to human health and the environment. The use of cerium oxide nanoparticles to improve plant stress tolerance has raised concerns about their biosafety, despite cerium being the most abundant rare-earth element in the soil (Tan and Chi-Lung 1970). Nanoparticles applied to plants can also reduce their ability to respond to stress (Tan et al. 2017). Therefore, aggregated nanoparticles have a smaller surface area and less cellular absorption than scattered ones (Spicer et al. 2018). As a result, the use of appropriate nanomaterials will be required for the development of nano-enabled agriculture. One technique for increasing the longevity of nanomaterials is surface conjugation, which is shown to limit heavy metal leakage (Sharifi et al. 2012). Gold nanoparticles with diameters ranging from 4 to 22 nm displayed the least intracellular degradation (Balfourier et al. 2020), with the smallest particles degrading the most rapidly. It would be good to increase the dispersion quality of nanoparticles to prevent agglomeration after application to support consistent biological activity and the application of nanomaterials in agriculture (Kobayashi et al. 2014). The dispersion of Cu—the combination of copper and chitosan—improves chitosan nanomaterial compared to their bulk equivalents (Saharan et al. 2015). Cu–chitosan nanomaterials boost tomato fresh weight (16%) and seedling length (18%) while simultaneously decreasing harmful fungus mycelial development and spore germination (Saharan et al. 2015).

Nanomaterials that are free of heavy metals and are highly dispersible in water should be considered. These molecules should be used in sustainable agriculture, which can be accomplished by using nanotechnology. It seems that employing nanoparticles derived from plant nutrients would be beneficial in preliminary studies. For example, manganese is vital for plant health and is typically found in agricultural fertilizers due to its high concentration. Mn_3O_4 nanoparticles, a novel type of nanoenzyme, can scavenge ROS. Utilizing Mn_3O_4 nanoparticles in cucumber cultivation enhanced salt resistance in cucumber (Lu et al. 2020).

9.3 Stress Detection and Early Exposure by Nanoparticles

9.3.1 Nanoparticle Sensors

Sessile crop plants have evolved erudite stress-tolerance systems. Stress sensing and signaling are two very important systems. The oxidant H_2O_2 has been shown to operate as a signaling molecule in plants (Mittler 2017; Gilroy et al. 2016). Other signaling molecules involved in plant stress responses include sugars and gaseous molecules like acetic acid esters, acetic acid, ethylene, methyl salicylate, jasmonic acid, abscisic acid, hydrogen sulfide, carbon monoxide, and nitric oxide. The signals were transported from the root to the shoot in plants subjected to salt stress mediated by Ca^{2+} signaling events (Choi et al. 2014). In response to various environmental stresses, the patterns of these signaling molecules can change. Plants produce different Ca^{2+} signals when exposed to salinity and drought stress (Shabala et al. 2015). It is still possible to detect several signaling chemicals, including Ca^{2+} (Toyota et al. 2018), H_2O_2 (Nietzel et al. 2019), glucose (Zhu et al. 2017), and sucrose (Chaudhuri et al. 2011) in nonmodel plant species without becoming invasive plants. Several technologies, including ratiometric quantum dot sensors, DNA aptamers coated on single-walled carbon nanotubes (SWCNTs), AT15-coated carbon nanotubes for nitrogen oxide detection (Giraldo et al. 2014), and nanoneedle transistor-based Ca^{2+} sensors are effective for monitoring signaling molecules in nonmodel plant species. Glucose concentration in the leaf of *Arabidopsis* plants was evaluated using the TGA–QD method after 60 minutes of incubation. QD and boronic acid are used to determine whether the glucose level in the leaf has changed. In addition, they created QD nanosensors (11.3-nm leaf infiltration) to use in environmental monitoring. These nanosensors have demonstrated excellent performance in conjunction with other sensors that monitor temperature, humidity, and even stomatal activity (Di Giacomo et al. 2015; Oren et al. 2017; Koman et al. 2017). Researchers developed an electrical conductometric sensor that measures the delay between single stomata opening and closing in real time.

Nanosensors can be useful tools in the plant research field since they can fix some fundamental complications. Still, there is not much information about how plants detect the presence of Na^+ in their surroundings (Jiang et al. 2019; Wu 2018). Through Na^+-specific nanosensors, the researchers were able to track Na^+ transit in plants at both granular and temporal scales. A similar problem exists with hydroxyl radicals (Mittler 2017), the most destructive ROS. This is owing to a lack of a reliable tool for researching their biological activities in stressed plants. Because fluorescent dyes such as hydroxyphenyl fluorescein do not detect hydroxyl radicals only (Setsukinai et al. 2003), also present visualization approaches rely on them to detect changes in plant development and defense. Their inclusion hampers this research in these luminous dyes. Developing hydroxyl radical-specific nanosensors will allow scientists to better understand how hydroxyl radicals function within plants.

9.3.2 Plants Sensors for Initial Stress Recognition

Early detection of stress may aid in the reduction of agricultural losses. Modern approaches for detecting chlorophyll fluorescence in leaves, evaluating morphological changes in plants, and monitoring water status employ remote sensing or hyperspectral imaging. However, these features only represent plant performance after the stress has been established (Giraldo et al. 2019). Therefore, the early detection of stress signals should be carefully observed and investigated. It is possible to improve remote sensing by using high-resolution nanosensors that monitor stress-signaling molecules to identify crop stress. It was also proposed in the case of transforming plants into smart plants using nanosensors (Giraldo et al. 2019). Nanosensors can detect and indicate the presence of analytes by quenching or changing the fluorescence of the light emitted by the sensor (Rong et al. 2019; Lew et al. 2020; Kwak et al. 2017). The detection of chemical signals by agricultural equipment is made possible by nanosensors, which can detect and respond to stress-signaling molecules such as H_2O_2, glucose, and NO, and convert them to optical or radio waves. Agricultural management and the early identification of plant stress could be made more efficient and effective in the future using this type of technology. Recent advancements in the noninvasive real-time in vivo detection of glucose H_2O_2 in stressed plants by a QD system (Lew et al. 2020) illustrate the agricultural potential of nano-enabled smart plants. Using nanosensors is possible to detect stress in plants at an earlier stage and develop smart plant sensors.

Making the smart plant sensor a reality will necessitate further effort, particularly in the field of agriculture. A common occurrence in the field is a combination of high temperatures and drought (Suzuki et al. 2014). It is possible for chemical signaling to become more complex when many stresses are present at the same time. In this case, the employment of nanosensors in response to specific signaling molecules can decode signals with a higher resolution. It is possible to improve nanosensor sensitivity, selectivity, and accuracy to get a higher-decoded signal resolution in field conditions. Therefore, an important study objective should be to create a database of chemical signaling molecule alterations that respond to stress, such as Ca^{2+} and ROS scavengers. An organic field-effect transistor for detecting carbon monoxide, a signaling molecule in plants, was constructed using zinc oxide nanoparticles as the active medium (Narayana et al. 2020).

9.4 Agrochemicals Based on Nanoparticles

9.4.1 Nanofertilizers and Nanopesticides

Commercial fertilizers are expected to account for 30% and 50% of total crop yields. However, 40–90% of agrochemicals are lost to the environment each year. Even though plants have high nutrient utilization efficiency for essential nitrogen (N), phosphorus (P), and potassium (K) components, there is still room for improvement in terms of agrochemical efficacy in plants (Adisa et al. 2019). This is because plant nutrient utilization efficiency for essential N, P, and K components is between 30–35%, 18–20%, and 35–40%, respectively (Adisa et al. 2019). Plant performance can be improved by using engineered nanomaterials. There are two types of engineered nanomaterials (ENMs) that deliver one or more nutrients directly to plants and boost plant performance. “Engineered nanomaterials” are “any pesticide formulation or product that comprises designed nanoparticles as active components and demonstrates biocidal action.” Nanoagrochemicals are expected to perform better in agricultural production compared to conventional agrochemicals. Nanoagrochemicals are projected to boost efficacy by 20–30% as compared to conventional ones (Kah et al. 2018). Foliar Mn nanoparticle treatment increased rootlet number, root and shoot length, and biomass by 40–70% (Pradhan et al. 2013). Nanoparticles encapsulating common agrochemicals like dsRNA, siRNA, ascorbic acid, and abscisic acid might be used to release the nanoparticles in a controlled and targeted manner. Several recent studies have added to the body of knowledge about nanopesticides and nanofertilizers (Mikula et al. 2020; Dimkpa and Bindraban 2017).

It is possible to control the release and dispersion of nanoagrochemicals by altering their surface properties. In experiments involving the controlled release of nanoformulations (37% of the studies), the most frequently used trigger is pH (Camara et al. 2019). Using the GA3-HMSN/Fe_3O_4 combination, the release of the growth promoter GA3 (gibberellic acid 3) at pH greater than 5 or less than 4 resulted in a 44% increase in cabbage growth. Temperature- and pH-responsive nanopolymers were developed to administer and release agrochemicals into tomato plants (Zhang et al. 2020b). Three days after foliar spraying tomato with the star polymer PAA50-b-PNIPAm450, up to 43% of the star polymer was translocated to other plant compartments, including the roots (Zhang et al. 2020b). Stimuli including light, temperature, enzymes, and the redox state are all used to produce a response. To improve the surface functionalization of nanoagrochemicals, aptamers, which are oligonucleotide or peptide molecules that bind precisely to specific targets, have been proposed for use. It was discovered that beta-cyclodextrin-conjugated quantum dots coupled to a shorter chloroplast transit peptide were significantly more effective than random peptides or controls that did not contain any peptides. A better understanding of the uptake and fate of customized nanofertilizers or nanopesticides and their impacts on plants and the surrounding environment may pave the way for their application in sustainable agriculture.

Until now, nanofertilizers and nanopesticides are still not commonly used in agriculture due to concerns about biosafety, ambiguities about their long-term environmental impact, and the likelihood of interspecies transfer. However, these concerns have been addressed. A lack of information about the long-term effects of nanoproduct accumulation in the environment on ecological systems and a lag in the development of legislation and regulations governing their usage are also contributing to the current situation. Still, more research work is needed to understand the long-term environmental destiny and accumulation of nanomaterials. It is necessary to develop policies and laws governing the use of nanoparticles in agricultural production. Farmers and the general public may be more accepting of nanotechnology-enabled farming if they are taught about the benefits of the technology and the most effective ways to use it.

9.4.2 Targeting the Chloroplast to Synchronize Plant Performance

Nanomaterials can be used to aid the regulated release and transport of agrochemicals to specific organelles, organs, or tissues in plants and the transport of agrochemicals to specific organelles, organs, or tissues in animals, and this can help reduce agrochemical waste by increasing the efficiency of the process. Nanoparticles are transported in a regulated manner in animal cells (Anselmo and Mitragotri 2016), suggesting that the distribution of nanoproducts to plants might be tailored. Methyl viologen and ascorbic acid have been successfully delivered into chloroplasts to manage their redox state by employing quantum dots coated with beta-cyclodextrin and truncated guidance peptides (Santana et al. 2020) to regulate the redox status of the chloroplasts. This work indicated fine-tuning in the activities of cell organelles while using fewer agrochemicals in their experiments. To obtain superior chloroplast colocalization, the results imply that QDs alone are insufficient and that an optimal nanoplatform design is required.

However, it has taken an extremely long period and high cost to get to the high efficiency of targeted distribution in plants. It has been achieved principally through a combination of nanomaterials and peptide-guiding molecules (Zhu et al. 2012; Santana et al. 2020). The discovery of novel technologies for the targeted administration of nanoparticles will enable a wider range of agricultural applications for this methodology. Changing the size and charge of nanoparticles allow for the infiltration of cell compartments such as the apoplast in plants and the epidermis and guard cells in leaves (Hu et al. 2020). This method has certain advantages and also limitations. Future nanoparticle delivery systems may be capable of efficiently delivering nanoparticles to plant tissues or organs and specific cell types and compartments through the engineering of nanomaterials with controllable features, such as size, charge, shape, and hydrophobicity/hydrophilicity, among other characteristics.

It is necessary to develop methods for dispersing nanomaterials into certain cell organelles, along with developing ways for dispersing nanomaterials to specific plant tissues such as the apical meristem of a shoot or organs such as flowers. Neutral and negatively charged nanoparticles are more effective at migrating from the roots to the shoot compared to positively charged CeO_2 nanoparticles, which are demonstrated to attach to and primarily reside on roots (Spielman-Sun et al. 2019). Therefore, an in-depth examination of the nanomaterials transported into plant tissues and organs, cells, and organelles is required. Furthermore, the development and management of nanoparticle mobility in plants should be carried out within the intended application of the studied materials.

Abiotic stress causes a decline in photosynthetic activity in all plants, regardless of the type of stress experienced (Foyer and Shigeoka 2011). This mechanism is related to an increase in ROS in plants when subjected to abiotic stress. An excess of ROS can induce oxidative damage to proteins, lipids, and cell structures and programmed cell death (Liu et al. 2021a; Das and Roychoudhury 2014). Plant biologists and breeders are working on producing more efficient plants against scavenging reactive oxygen species to increase their abiotic stress tolerance. Genetic transformation is restricted to model plants or species that are easily altered. Thus, plants must be protected from abiotic stress, and their photosynthetic efficiency must be increased. A scalable and universal approach is needed to achieve these goals. This technique may use ROS-scavenging nanoparticles, such as cerium oxide nanoparticles and manganese oxide nanoparticles, to reduce the formation of reactive oxygen species. When plants are treated with nanoceria, the improved ROS scavenging capacity of leaf mesophyll cells increased the carbon assimilation rate. Plants treated with SWCNT had higher ROS scavenging capacities and higher electron transport rates than control plants (Giraldo et al. 2014). Other ROS-scavenging nanomaterials may aid in protecting chloroplasts from oxidative stress and increasing photosynthesis in plants. By utilizing a newly developed targeted delivery method, it may be possible to preserve chloroplasts from stress and convert them into a "chloroplast factory," allowing for a wider range of applications. This newly developed technique may have applications in the pharmaceutical and bioenergy industries and the field of plant photosynthesis research.

9.5 Transgenic Events Assisted by Nanoparticles

9.5.1 Nanomaterials as Delivery Platform

Agrobacterium tumefaciens or gene gun bombardment are the two most prevalent methods of transformation to produce transgenic plants. These two approaches target a small number of genetically sensitive plant species or cause harm to the plants (Landry and Mitter 2019; Yu et al. 2017). Other than model species, callus cultivation (Altpeter et al. 2016) is inefficient and labor-intensive. Nanobiotechnology has recently demonstrated a considerable promise in creating transgenic wild-type plants, and it has the potential to be used in a much wider range of plant species than model plants. Single-walled carbon nanotubes have the potential to transport functional genetic elements into chloroplasts and nuclei (Demirer et al. 2019; Kwak et al. 2019). Wide properties have been shown to allow carbon nanotubes to enter plant cells (Wong et al. 2016; Chaudhuri et al. 2011). Variations in pH across cell organelles should trigger the release of plasmid loads. These studies advocate employing nanoparticles rather than *Agrobacterium* or gene cannon bombardment to provide functional genetic resources to plants (Kwak et al. 2019).

The use of positively charged carbon dots (2.0–10.0 nm in diameter) to transport siRNA to plants resulted in a reduction in GFP (green fluorescent protein) expression in plants. For the first time, carbon dots were used to deliver siRNA to plants to silence genes. GFP was precisely delivered to tobacco chloroplasts by using peptide/pDNA complexes (Thagun et al. 2019). They employed nanomaterials to make transgenic plants, which included nonmodel and model species. Nanomaterials can also be used as scaffolding to transfer unstable molecules such as RNA and RNA polymerase (e.g., siRNA or dsRNA). After loading dsRNA onto a clay nanosheet, which had a mean diameter of 45 nm and side dimensions ranging from 20–80 nm (*d*-value = 0.82), the stability of the loaded dsRNA significantly improved for 20 days (Mitter et al. 2017). A siRNA delivery system based on DNA nanostructures was used to enhance the growth of tobacco (Zhang et al. 2019).

Researchers and farmers may favor carbon nanotubes over other nanomaterials because they are less expensive or more biocompatible (Mohanta et al. 2019). It is possible that increasing the number of nanoparticles for supplying functional genetic resources may improve adoption. A study of *Arabidopsis* plants found that polyethylenimine (PEI)-coated gold nanoparticles effectively delivered siRNA to the NPR1 gene (Lei et al. 2020). Carbon dots built on PEI can be used to carry DNA or RNA molecules. It is feasible under theoretical conditions to use positively charged nanomaterials such as carbon dots or silica nanoparticles to transport negatively charged functional genetic components into plant cells. The fact that nanoparticles are associated with biosafety issues suggests that this could be a cost-effective method to conserve costs. The availability of nanomaterials will impact the frequency by which this nano-enabled transgenic approach will be employed.

9.6 Nano-Enabled-CRISPR-Cas Complex

As an added benefit to the construction of transgenic plants, nanomaterials may be employed to provide a platform for organelle-specific CRISPR-Cas genome editing, which would otherwise be impossible. Although tissue culture is now widely employed in plant breeding, it is still crucial in the process. It is still restricted to a few numbers of plant species, genotypes, and organs. It was demonstrated that nanoparticles might be used to deliver the CRISPR-Cas9 system (Wei et al. 2020). However, there have been no reports of plant-based nano-enabled CRISPR-Cas genome editing that have been published. One of the most likely causes is barriers within the plants' cell wall (Albersheim et al. 2011). Recently, it was discovered that a virus was carrying the ultra-compact genome editor CRISPR Cas, which was previously thought to be inactive. A minimally functional CRISPR-Cas system comprises the Cas protein (70 kDa, about 3 nm Rmin (Erickson 2009) and a CRISPR array (Pausch et al. 2020)). Due to the ability to manipulate the size of the complex, it may be possible to deliver nanoparticle CRISPR-Cas complexes to plants more efficiently and precisely using this technology. The complex's size can be adjusted to pass more easily through the plant's cell membrane. As a result, nanoparticles may prove to be a useful vector for the CRISPR-Cas system, targeting certain organelles or plant regions. The CRISPR-Cas system may be supplied to the chloroplast using nanoparticles directed by a chloroplast transit peptide, transforming it into a plant factory (Santana et al. 2020). Nanotechnology can be utilized to enable CRISPR-Cas gene editing in plants (Demirer et al. 2021). Details on the application of nanomaterials for CRISPR genome editing in transportation, species independence, germline transformation, and gene editing efficiency have been discussed.

9.7 Seed Nanopriming

Drought, salt, and heat are all factors that impact seedling growth, with the most noticeable influence occurring during the germination stage of most crops. The germination, establishment, and adaptation of plants to a range of conditions are all assisted by seed vigor. The rate of seed germination and the uniformity of seed germination can be improved by utilizing a range of approaches and strategies. A range of techniques is available for priming seeds, including the use of a salt solution, an osmotic solution with a low-water potential, bioactive chemical combinations, solid matrix priming, and chemo-priming. As the stressed green gram (*Vigna radiata* (L.) Wilczek) variety Pusa Ratna was halo primed with 35 mmol L^{-1} NaCl, the fresh weight increased by 47% (under salt stress) and 28% (under drought stress) compared to unprimed controls under stress (Jisha and Puthur 2014).

It is a novel method of seed priming that, compared to conventional priming strategies, can significantly improve crop development and performance, particularly in adverse conditions such as drought, salty environment, and heat. Priming seeds with nanoparticles has the potential to improve crop development and performance significantly. When cotton seeds were primed with cerium oxide nanoparticles (2nm, 51.7 mV, 500 mg L^{-1}, nanopriming) and grown under salt stress (200 mmol L^{-1} NaCl), fresh seedling weight increased by 41% (An et al. 2020), compared to a water-primed control. Many crop species have been shown to benefit from nanopriming, including wheat, Fe_2O_3 nanoparticles (Sundaria et al. 2019), ZnO nanoparticles (Rizwan et al. 2019), rice, silver nanoparticles (Mahakham et al. 2017), sorghum (Maswada et al. 2018), broad bean (Younis et al. 2019), cotton, cerium oxide nanoparticles, onion (An et al. 2020), and gold nanoparticles (Acharya et al. 2019). In comparison to an untreated control, onion seed nanopriming with gold nanoparticles (93.6 nm, –8.5 mV, 5.4 mg L^1, nanopriming) resulted in a 69 percent increase in emergence percentage and a 24 percent increase in mean yield. Gilbertson et al. stated that nanoZn/ZnO is one of the most promising seed-coating options based on the increase in seed germination and the environmental effect of the embodied energy (Gilbertson et al. 2020).

Despite the promising findings obtained by seed nanopriming, additional research is required to comprehend the fundamental principles fully. Nanoceria priming can improve crop salinity stress resistance, which modulates the plant's reactive oxygen species (ROS) and ion homeostasis signaling pathways (An et al. 2020). Many additional seed nanopriming methods have been identified or proposed. There are four techniques for reducing electrolyte leakage: using ZnO nanoparticles (Rizwan et al. 2019), Fe_2O_3 nanoparticles (Maswada et al. 2018), or silver nanoparticles (Younis et al. 2019) or a combination of the three methods. The first goal is to reduce lipid peroxidation, and the second is to improve the amount of water in plants and the effectiveness of photosynthesis and respiration.

What methods of seed nanopriming persist when a single nanomaterial is applied to a range of plant species, and how does this affect the experiment's outcome? What processes, such as changed redox state or seed dormancy, can be used to explain the phenomenon of seed nanopriming? The uptake, distribution, and fate of nanoparticles and their interactions with seeds should all be investigated further by looking at the mechanisms that cause these events to occur in the first place. When it comes to nanomaterial uptake, the size of the hole pores in the seed coat matters. A concern has been expressed regarding differences across plant species in the spread of nanomaterials. Is there a relationship between the distribution pattern of nanomaterials in seeds and their biological effects? What is the relationship between this and the scavenging of reactive oxygen species (ROS) mediated by nanomaterials or epigenetic changes? Is there a critical stage or position in the seed nanopriming process critical for nanomaterials' biological impacts? Combining seed nanopriming with seed coating technologies may be worth investigating to ensure that efficacy and performance are not degraded over time. The nano-enabled seed coating technique used in the agrochemical business is only vaguely known by the general public (Acharya et al. 2019).

9.8 Light Harvesting by Nanoparticles

Photosynthesis is largely dependent on the availability of visible light. Plants do not make good use of most natural light sources. nIR light is absorbed mostly by chlorophylls in plants, whereas UV light causes chlorophylls to deteriorate (Antonaru et al. 2020). Even though the ozone layer prevents UV-C (100–280 nm), UV-A (315–400 nm) and UV-B (280–315 nm) are still able to reach the Earth (Stapleton 1992). It is difficult to see through the bottom leaves of plants in high-density cropping systems because of limited visible light. Visible light is diminished during overcast or rainy days. Sustainable agriculture may benefit from developing new photosynthesis technologies that allow plants to utilize a greater proportion of lost light resources.

Although cyanobacteria contain chlorophyll d and f, which absorb near-infrared light (Airs et al. 2014), cyanobacteria could only utilize light with wavelengths up to approximately 750 nm. Chlorophyll d and f are pigments that can be introduced into higher plants to escape the photochemical red limit of the light spectrum. nIR light has a wavelength limit of 800 nm and cannot be used. The use of nanomaterials to transform ultraviolet and near-infrared radiation into visible light for plant photosynthesis may pique the interest of scientists, farmers, and even the industrial sector. By fine-tuning and complementing plant photosynthesis, this revolutionary technology can potentially enhance food production by as much as 50% considerably.

The upconversion and downconversion of nanoparticles are two types of nanoparticles commonly employed in biophotonics and nanomedicine to convert near-infrared and ultraviolet light to visible light (Loo et al. 2019). It has been demonstrated that upconversion nanoparticles (UCNPs) doped with Yb, Nd, and Er can convert light stimulated between 808 and 980 nm to visible light between 510 and 570 nm (Wiesholler et al. 2019). When downconversion nanoparticles (DNCP), such as bNaYF$_4$:Gd^{3+} and Tb^{3+}, were encapsulated in PEI, they converted ultraviolet light with a wavelength of 273 nm into visible light (between 480 and 630 nm in wavelength) (Malik et al. 2019). Shoot length and dry weight were increased in rice by 19% and 64%, respectively, when CD 1:0.2 was used as a converter for converting UV radiation to PAR (photosynthetically active radiation). These UCNP and DCNP nanoparticles can be sprayed on the surface of leaves or injected directly into cells to assist plants in maintaining photosynthesis during periods of low light, such as shadow or continuous cloudy days. Because of advances in nanotechnology, this approach to photosynthetic light amplification may be helpful for agriculture and allied industries, such as biofuel manufacturing. Nanomaterials can be used to transform near-infrared and ultraviolet energy into visible light in plants.

9.9 Capturing More Electrons

Photosynthesis eliminates more than 120 billion tons of CO$_2$ from the atmosphere each year in terrestrial ecosystems. Photosynthesis in green plants is based on the harvesting of light and the passage of captured electrons into the electron transport chain, both critical processes. Reactive oxygen species can form when an excessive number of electrons are removed (Foyer 2018). On overcast and rainy days, especially when plants are shaded, they might not capture enough electrons to complete the light response. New tactics that allow plants to gather more electrons in low-light conditions may considerably boost plant photosynthesis and output. Plants may be able to collect more electrons with the assistance of nanomaterials. A structure is formed by a combination of organic and metallic components.

Nanomaterials are capable of both absorbing and transmitting electromagnetic radiation. The excited state can be transmitted to gold nanoparticles that have been triggered by light (Robatjazi et al. 2015). Thus, photosynthesis could be enhanced in low-light conditions by incorporating light-capturing nanomaterials into the chloroplasts. Research on innovative light-harvesting nanomaterials and their tailored distribution to chloroplasts should be carried out in the future. Scientists have proposed the use of nanomaterials to fine-tune plant photosynthesis under low-light conditions. These materials have the potential to trap more electrons.

9.10 Future Perspectives

We discussed the potential applications of plant nanobiotechnology in modern and sustainable farming practices. Plant nanobiotechnology could improve stress tolerance, sensing and early detection, pesticide targeted delivery and controlled release, nonmodel crop species transgenic events, and seed nanopriming. Heavy metals should be avoided in agricultural nanomaterials, and their dispersibility should be as high as possible. As previously asserted, more research into the biological effects of nanoenzymes, such as Mn_3O_4 nanoparticles, on stressed plants is required. It is critical to keep looking into the mechanisms that influence nanoparticle absorption, dispersion, and fate, as well as their interactions with seeds. With the help of nanomaterials, plants can be converted into chloroplast factories, enhancing their functionality. In addition, nanomaterials can transform ultraviolet and near-infrared light into visible light, enabling more electrons to be retrieved for photosynthesis when employed in low-light settings. According to the researchers, understanding how nanoparticles help plants cope with stress should make it easier to develop nanomaterials specifically useful in agricultural applications. Legislation and regulatory restrictions may help reduce the biosafety risks associated with the use of nanoparticles in agriculture and assuage public worries about nanomaterials. Nanotechnology has the potential to have a substantial impact on agriculture.

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