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Chapter 8
Nanomaterials as new techniques in plant priming technology

Abstract: Seed priming is a presowing treatment that enhances seed performance and promotes seedling growth. The use of nanomaterials in seed priming has emerged as a promising technique in recent years due to their unique physicochemical properties. Nanomaterials, such as nanoparticles and nanotubes, have been reported to improve seed germination, increase seedling vigor, and enhance plant growth and productivity. This review summarizes recent advances in the use of nanomaterials as new techniques in plant and seed priming technology. It covers a range of topics including the mechanisms of action of nanomaterials in seed priming, their effects on seed physiology, and their potential applications in agriculture. The review highlights the potential of nanomaterials as a sustainable and eco-friendly approach to enhance plant and seed performance and to mitigate the negative effects of abiotic stresses such as drought, salinity, and temperature extremes. The review also discusses the challenges and potential risks associated with the use of nanomaterials in plant and seed priming and the need for further research to understand their long-term effects on the environment and human health. Overall, the use of nanomaterials in plant and seed priming technology represents a promising avenue for improving crop productivity and promoting sustainable agriculture.

Keywords: seed priming, nanotechnology, need germination, abiotic stress, plant priming

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8.1 Nano-priming technology (nano-prime)

8.1.1 Introduction

The continuous increase in global population and the high demand for food resource production have raised human dependency on agriculture more than ever before. Taking into account the insufficient availability and quality of land, water and labor, soil health, agrochemicals, and the inevitable climate change, agriculture sustainability has kept researchers busy (World population prospects 2019: highlights, 2019). The Intergovernmental Panel on Climate Change (IPCC) has raised many concerns about the consequences of climate change on agriculture including plant stress from temperature fluctuations, water deficiency, and flooding from heavy rainfalls [58, 59].

Rapid urbanization as well as industrialization has limited the farming land and changed the cultivation conditions, creating various stresses, such as contaminated soil from hazardous chemical wastes including heavy metals, high salinity, and high/low pH, which negatively affect the crop yield, food quality, and in many cases, food safety (World population prospects 2019: highlights, 2019; [60]). Furthermore, even though the application of pesticides and fertilizers and the use of current conventional techniques increased the agricultural production, they also deteriorate the unfavorable effects of climate change by posing other environmental and health risks [1, 61, 62].

Moreover, a high percentage of crop yield is highly affected by biotic and abiotic factors resulting in adverse effects on growth and production rate. Generally, it is common for plants to adjust fast in new environments as their position in the soil is stable, but in this case, the longer the exposure to these stresses, the higher the negative effect on the plant's growth ([63, 64]). The disturbance of plants' ideal conditions to grow automatically reduces production, the disproportion of consumption corps, and also the nutritional value of foods [65].

The economy, in many countries in the world, relies vastly on agriculture. The FAO (2021) reported a tremendous burden on the economy of many developing countries due to the negative effects that biotic and abiotic stresses have on agriculture. Between 2005 and 2015, a loss of USD 9.5 million was due to pest diseases on plants, USD 29 billion to drought, and USD 47 billion to other causes, threatening the economy and causing food security and safety warnings. Subsequently, lack of land necessities causes the depletion of natural resources and governments are called upon to face the financial damage of climate change but mostly to provide the population with sufficient sustenance.

8.1.2 Priming technology

Plants are susceptible to numerous external factors that negatively affect their development and are divided into biotic (microbial pathogens and insect herbivores) and abiotic (drought, high salinity, heavy metal toxicity, extreme temperatures, etc.) stresses. Many methods have been developed over time to enhance plants' tolerance to many abiotic and biotic stresses such as genome editing, genetic engineering, conventional breeding and mutation breeding [2–5]. Unfortunately, these techniques are expensive, require a long time to achieve the desired outcome and many are not accepted by society or even illegal in many countries.

Plant priming, hardening, or sensitization is an alternative method to the currently employed techniques that exhibits very promising outcomes to encounter both kinds of stresses. In plant physiology studies, the term priming generally means short-lasting pretreatment or preconditioning, using biological agents, physical factors, or specific compounds to enable the plant's defense mechanisms, after being exposed to an environmental stress event, resulting in enhanced plant robustness, able to respond faster and more efficiently against biotic and abiotic stress [6–8]. Plant priming can be performed on various parts of the plants and during many developmental stages of their cycle. Depending on the agricultural and economic needs, the nature of the material used, stress conditions, and plants' diversity, each application is noteworthy.

Seed priming is the most commonly used priming method, due to the many advantages from an agricultural and economical point of view. The simplest but very elegant example to understand how easy and also how efficacious seed priming can be is presoaking in plain water. Various studies have shown that hydrating and drying the seeds may increase their quality and agricultural value. Hydropriming enhances germination, seedling growth, uniform root elongation, and particularly crop yield [9]. Apart from the phenotypic improvements, the seeds also absorb biologically active and protective compounds, activating metabolic pathways, multiple enzymes, and synthesis processes, which will support the vigorous seedlings to sustain their survivability and robustness under critical environmental circumstances [10, 11]. The simplicity of seed priming with plain water allowed researchers to investigate a wide range of materials.

A similar, but still different priming method is solid matrix priming (SMP). The most common procedure for SMP is to mix seeds with solid or semisolid material (such as perlite, sand exfoliated vermiculite, moss, expanded calcined clay, and saw dust) and water or the priming agent at specific proportions. Water or priming agent is then provided to the seed slowly; therefore, a slow or controlled release occurs [12, 57].

As mentioned above, depending on the circumstances, priming application depends on many variables. Priming agents are less commonly applied on different parts of seedlings and active growth stages. This method can be used to ameliorate metallic stress tolerance with plant growth regulators, which is preferably applied on seedlings and young plants rather than on seeds [13, 14].

Also, foliar application is another technique used to apply a variety of priming agents. In some studies, comparing seed and foliar applications in terms of yield production, enzymatic, and antioxidant activity has reported similar or even better outcomes with foliar applications than seed priming [15–17].

8.1.3 Nanotechnology

The innovative approach of plant priming, in recent decades, has adopted and used nanomaterials as priming agents in many researches and studies. Some materials have the ability to break down and get smaller, reaching the nanoscale form with a size of 1–100 nm (some materials, such as polymers, are 1–1,000 nm), creating nanoparticles (NPs). NPs are introduced as chemical agents ranging from metal to polymer to carbon-based materials, exhibiting promising results in the plant's tolerances in order to face both biotic and abiotic stresses [65, 66]. These NPs possess physicochemical properties that surpass in many ways the original bulk-size material including their low cost of synthesizing and production [67], exhibiting great potential in the process of green revolution, and replacing current pesticides and fertilizers. In comparison with other priming methods, nano-priming is considerably more effective [68] (Figure 8.1).

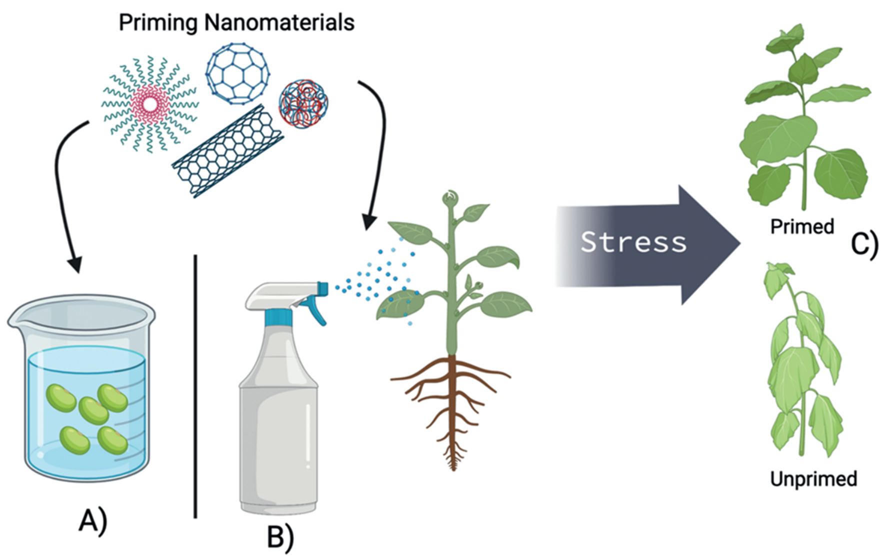

Figure 8.1: (A) Seed priming – the seeds are prepared by presoaking in nanoparticle solution; (B) foliar application the plant (usually leaves) is sprayed with the priming material; (C) plants that have undergone the priming treatment will activate a range of defence mechanisms that will prepare the plant during and after stress periods.

Functionalized NPs are used in the field of chemical priming due to their impressive properties, positively manipulating plant metabolism by preventing the rapid release of ROS and by producing secondary metabolites [18, 67, 69–72]. NPs in seed priming can alter plant cells and tissues by activating electron exchange and enhanced surface reactions [68]. NPs can also create nanopores that easily absorb water by promoting the function of aquaporines, impact the equilibrium of ROS/anxtioxidant activity in seeds, and control hydroxyl radical by loosening the cell walls. They can also behave as inducers for the rapid hydrolysis of starch driving to the stimulation of seed germination [73].

With NP application, plants gain developed capabilities in absorbing solvents and recruiting nutrients as a result of the NP’s efficiency to penetrate biological barriers [74]. NPs application display increased levels of plant growth and development as well as tolerance against various stresses [6, 18, 75, 76]. The contribution of seed nanopriming in multiple processes such as seed metabolism and signaling pathways is considered an effective way of seedling and germination improvement by inducing the plant’s cell cycle genes [18]. Many studies suggested that nanotechnology plays a vital role in promoting certain seed functions, promoting germination levels and improve plant’s resistance under challenging stress conditions [6, 65, 77].

In this chapter, nano-priming will be explored based on the utilization of different NPs in different studies that have successfully exhibited priming or growth-promoting effects on a range of plant species.

8.1.4 Nano-priming agents

8.1.4.1 Bio-polymer-based NPs

Polymer-based or polymeric NPs (PNPs) are particles with size of no more than 1,000 nm that can be loaded within or coated with active compounds. PNPs exhibit considerable potential for targeted delivery of drugs in medicine [19] and also promising results in the field of plant priming (Table 8.1).

8.1.4.1.1 Chitosan

Chitosan is attracting a lot of attention in the recent years in the world of drug delivery and plant application. Chitosan is obtained by the deacetylation of chitin. Chitin is the second most abundant organic polymer, after cellulose, and it is naturally extracted from fungi, yeast, algae, cockroaches, silkworms, honey bees, and marine aquatic animals such as crustaceans, lobsters, shrimps, mollusks, arthropods, and cephalopods [20]. Chitosan is a renewable, nontoxic, biodegradable, linear, and cationic carbohydrate polymer [21]. Due to its source material, chitosan is a renewable and cost-effective resource generated from the seafood industry waste [22].

Many researchers have studied and tested chitosan in nanoscale form as a carrier for variety of hormones and other materials. Nano-chitosan has been used for foliar application on wheat plants at salinity stress, and an increase was observed in the antioxidant activity and protection of proteins, lipid membrane, and photosynthetic apparatus [23]. At the same study, foliar application was also conducted with nano-chitosan encapsulated nano-silicon donor, which alleviated even more the adverse effects of salinity stress.

Nano-sized chitosan was also used through SMP on mung bean and then exposed to salinity stress [24]. The oxidative stress markers of H_2O_2 and malondialdehyde (MDA) have decreased and had improved growth and increased metabolism and chlorophyll content.

Another study that tested copper-chitosan NPs on wheat has induced biochemical changes through seed priming and positively affected growth and germination in wheat seedlings under salinity and hyperosmotic stress [25]. Also, a significant increase was induced in chlorophyll a, b, total chlorophyl, β-carotenoids, and total carotenoids under normal and stressed conditions. Another study with copper-loaded chitosan, also used for seed priming, had similar results on maize seedlings. The treatment increased the shoot growth and photosynthetic electron transport [26]. Also in stressed conditions the chitosan nano-encapsulated copper highly increased the antioxidant enzymes' activity.

Chitosan in its nanoform has also been studied in heavy metal stress and used as a carrier of selenium on Moldavian balm (*Dracocephalum moldavica* L.) leaves. The negative effects of cadmium toxicity stress condition were minimized and decreased MDA and H_2O_2 by amplifying photosynthetic pigments, agronomic traits, chlorophyll fluorescence parameters and antioxidant enzyme activity, proline, SPAD, and total phenols as well as a number of dominant components of essential oils [27]. Chitosan-selenium NPs also alleviated salt stress by foliar application in bitter melon [28]. The foliar application of the chitosan-selenium NPs increased the growth and yield of plants and furthermore enhanced the tolerance by decreasing MDA and H_2O_2, enhancing antioxidant enzyme activity, relative water content, K^+, and proline concentration. Another study with chitosan-functionalized selenium and anatase titanium dioxide NPs alleviated salt stress via foliar spray in *Stevia rebaudiana* Bertoni, by decreasing electrolyte leakage, MDA, and H_2O_2, and increased photosynthetic performance, plant's growth, and antioxidant enzyme activity [29].

Engineered chitosan-melatonin NPs were applied on spearmint plants under salinity stress [30]. The application, in response to the salt stress, increased the stress-related attributes including H_2O_2, MDA, and proline in addition with GP and ascorbate peroxidase (APX) enzyme activity. It is worth mentioning that solo treatment of melatonin did not combat stress as effective as the conjugated form of melatonin with chitosan.

8.1.4.1.2 Lignin

Lignin is the most abundant, available today, aromatic polymer source, and the second most abundant renewable polymer, with more than 70 million tonnes generated, from various lignocellulose processing plants, as underutilized by-product waste [31]. This waste, due to lignin's good heating value, was usually burnt for energy source [32, 33]. But, recent techno-economic studies suggested that it is not economically viable to use lignin for energy recovery alone [34, 35]. Therefore, the utilization of lignin is being explored and applied in new technologies. Amongst other ways of maximizing lignin's valorization, it has also attracted researchers' interest in plant's application.

Lignin NPs (LNPs) have the carrier properties similar as the chitosan NPs. However, plain LNPs were investigated on maize seeds as a priming agent for improved growth in different concentrations [36]. LNPs exhibited positive outcome on the first maize development stages, germination, and radicle length. LNPs, on later stages, stimulated favorable effects on seedlings concerning length of shoots and roots as well as fresh weight. In addition, LNPs also increased chlorophyll (a and b), anthocyanin, and carotenoid. In response to specific dosages, a positive trent has been shown on soluble protein content. These findings indicate that LNPs do not only act as a carrier for other beneficial materials but also stimulate physiological, chemical, and biochemical traits.

A recent study synthesized lignin, zinc oxide hybrid NPs, and for the first time used the particular hybrid system to prime maize seeds [37]. Compared to the controlled samples, higher contents of carotenoids, chlorophyll, total phenols, and anthocyanins were found and increased antioxidant activity.

8.1.4.2 Metal-based NPs

Metal-based NPs are usually smaller than 100 nm with fascinating and quiet different properties from same bulk materials, individual atoms, or surfaces [38]. These particles in nanoscale form may unlock a variety of communications with biomolecules inside the cell and on the cell surface, in a way that allows the application to have an effect on different biochemical and physiochemical properties of the cells [39]. Many of these metals used for plant priming are present and have important roles in plant's metabolism and biofortification [1, 40] (Table 8.1).

8.1.4.2.1 Iron oxide NPs

One of the most studied metal-based NPs is iron. As one of the main vital elements for plants development, iron plays an important role in photosynthesis, respiration, and cell metabolism. Due to the need of iron in the synthesis of chlorophyll protein in the chloroplasts, any deficiency will cause the yellowing of the leaves and reduced photosynthetic capacity. Iron NPs (FeNPs) have the ability to act as an essential element, with the activating reactive oxygen species, oxidation defense system, and the promotion of iron film formation on the root surface [41].

Green synthesized iron oxide NPs (FeONPs) have been used for seed priming for watermelons [42]. The green synthesis was done by simply adding drops of onion extract, at pH 5 to 0.1 M $FeCl_3$ (pH 2) at room temperature. The FeNPs were used on watermelon seeds, and at studied concentrations (20, 40, 80, and 160 mg L^{-1}), the treatment did not have any toxic effect on germination, seedling development, and biosynthesis of chlorophyll. Metabolome of diploid and triploid was distinctly altered in watermelon seedlings and significantly modulated 12-oxo phytodienoic acid level.

Another eco-friendly synthesis of FeONPS was utilized for priming rice seeds [43]. This synthesis used *Cassia occidentalis* L. flower extract for the preparation of FeONPs. Compared to the controls (hydro-primed and $FeSO_4$), 20 and 40 mg/L of FeONPs treated rice seeds had efficiently improved germination and seedling vigor. Root length and dry weight of 20 mg/L FeONPs had been found to be 50% superior than the rest of the treatments as well as sugar and amylase content. Antioxidant enzyme activity was remarkably stimulated at FeONPs 20 mg/L in superoxide dismutase (SOD) activity at 29%, APX activity at 50%, and catalase (CAT) activity at 60% compared with controls.

The synergistic effects of zinc oxide and FeNPs on seeds have been investigated on the growth and accumulation of cadmium (Cd) by wheat (*Triticum aestivum*) [44]. Compared to the controls, NPs phenotypically had higher rates in roots, shoots, spikes, grains, plant height, and dry weights and also enhanced photosynthesis of wheat. Electrolyte leakage, SOD, and peroxidase activities in stressed leaves have been reduced, and the concentration of Cd in roots shoots and grains was efficiently decreased. Additionally, Zn and Fe concentrations were increased in plants' elemental contents.

Iron acquisition and biofortification were enhanced as well with FeNPs by seed priming on wheat (*Triticum aestivum L.*) grains [45]. Priming was done at the range of 25–600 ppm with the outcome of distinctive accumulation of iron contents. In high concentrations of 200 and 400 ppm the two contrasting wheat genotypes for determination of accumulation of grain iron contents, WL711 (low-iron genotype) and IITR26 (high-iron genotype), have been increased as well as germination percentage and shoot length. Treatment with 25 ppm Fe_2O_3 had a significant increase in iron accumulation to 26.8% in WL711 and 45.7% in IITR26.

8.1.4.2.2 Zinc oxide NPs

Zinc belongs to the micronutrients, an essential element to all plants. Being present inside the associated enzymes with energy and proteosynthesis processes, zinc is necessary at maintaining the sustainability of biomembranes. It also has an important role in the formation of seeds and generative organs [46]. Due to their substantial antimicrobial efficacy, zinc oxide NPs (ZnONPs) are one of the most commonly used nanomaterials, used in drugs and food packaging [47, 48]. Zinc deficiency in plants is currently being ameliorated by using zinc fertilizers, usually zinc oxides and zinc sulfates. But, due to the lack of solubility in soil, zinc is unavailable in plants, and these fertilizing applications are limited [49]. Zinc in nanoscale form has the ability to overcome this issue by providing highly reactive zinc in more soluble form. In a range of studies, seed priming with ZnONPs has increased zinc content in seeds, aiding in germination, seedling growth, and yield [50–52].

Zinc NPs (ZnNPs) have also been studied as a priming agent under several types of stress with very promising outcomes. An investigation of ZnONPs was done on lupine (*Lupinus termis*) plants under salt stress [49]. Lupine seeds have been soaked in ZnNPs 20, 40, and 60 mg/L for 12 h. Priming has stimulated the growth of stressed plants and has increased the levels of organic solutes, photosynthetic pigments, ascorbic acid (ASA), total phenols, and Zn content. Enzyme activity was also triggered in CAT, SOD, APX, and peroxidase (POD) in comparison with unprimed salinized plants. A reduction of MDA and sodium in stressed plants was also observed in ZnNPs primed plants compared with unprimed stressed plants.

Another study, investigating the behavior of ZnONPs on primed rice seeds, was conducted under water deficit environment [53]. Seed priming with ZnONPs has resulted in an increase in the plants' fresh and dry weight, height, and total chlorophyll contents as well as straw yield under drought stress. A 53% decrease in MDA content was also the result of seed priming with ZnONPs in water shortage environment as well as increased antioxidant enzyme activity and reduction in the levels of oxidative stress indicators.

Seed priming with ZnONPs was also investigated on rice plants in cadmium stress conditions. ZnONPs, other than the phenotypic improvements and the increased enzymatic activity in roots and shoot under low concentrations, have significantly improved the α-amylase and total amylase activity, when exposed to Cd stress conditions [51].

8.1.4.2.3 Silver NPs

Silver NPs (AgNPs) have appeared in a range of publications, aiding in seed germination and seedling vigor and ameliorating stress-induced effects and activating several antioxidant and enzymatic activities. Photosynthesized AgNPs, in concentrations as low as 5 and 10 ppm, enhanced germination of aged rice seeds and starch metabolism as well as higher soluble sugar content, a major component in assisting in seedling growth, from enhanced α-amylase activity [54].

Salt-induced toxicity was drastically reduced in pearl millet by seed priming with AgNPs [55]. AgNPs at 20 mM with or without salinity stress relatively improved plant height, water content, fresh and dry weight, and proline contents. Antioxidant enzyme activities, under salt stress, have been reduced in pearl millet leaves by AgNPs and additionally decreased sodium and increased potassium contents.

Priming, using turmeric oil nano-emulsions and AgNPs synthesized with agro-industrial byproducts from turmeric and onions, was done on watermelon (*Citrullus* *lanatus*) seeds [diploid (Riverside) and triploid (Maxima)] and then seedlings have been transplanted in four different locations in Texas [56]. At first, seed germination was significantly enhanced by AgNP and glucose and fructose contents were increased during germination. After transplantation higher yield was observed in AgNPs primed Riverside (31.6%) and Maxima (35.6%) compared to the control.

8.1.4.2.4 Titanium dioxide NPs

TiO_2 is a component found in high quantity in nature and it is the ninth most abundant element on Earth, with remarkable properties in plant growth and development [78]. More specifically, plants' morphological, biochemical, and physiological features have been affected by the application of TiO_2 [79].

The application of titanium oxide NPs (TiO_2NPs) in low concentrations on Moldavian balm plants (*Dracocephalum moldavica* L.) improved plant performance and agronomic traits under both normal and saline conditions. This was due to the reduction of oxidative stress damage and increased antioxidant capacity of enzymes as well as increased essential oil content [80].

A study by Shah et al. [78] also found that seed priming with TiO_2NPs improved maize (*Zea mays* L.) seedling growth by increasing root and shoot length, fresh, and dry weight as well as boosting antioxidant activity.

Furthermore, the application of TiO_2NPs and bulk material to marjoram seedlings under salinity stress improved seedling growth, reduced the negative effects of free radicals, and increased essential oil content levels and yield. This was achieved by enhancing free radical scavenging activity and positively affecting seedling emergence, plant height, chlorophyll content, and total shoot dry weight of stressed plants compared to nonprimed plants [81].

Cadmium-stressed coriander plants *Coriandrum sativum* L., after being treated with TiO_2NPs, had lower levels of chlorophyll content, photosynthetic rate, growth, and seed yield. Nevertheless, seedling biomass, number of seeds, and yield improved as well as cadmium levels. The study showed that TiO_2NPs are effective in reducing the toxic effects of cadmium by reducing oxidative stress and Cd uptake and improving the antioxidant system and stress markers [82].

8.1.4.3 Carbon-based NPs

Carbon nanomaterials (CNMs) are derived from carbon, the most abundant element on the Earth's crust, forming different allotropes. CNMs consist of a broad spectrum of structures from polyatomic planar to rod-shaped to spherical or voided. Those structures are designed to form fullerenes, graphene oxide, and carbon nanotubes [93].

Table 8.1: Synopsis of studies on plant priming using nanoparticles, their applications, and mode of action under different stress condition.

Nanomaterial	Plant species	Application	Stress	Mode of action	Reference
Polymer-based nanoparticles					
Nano-chitosan-encapsulated nano-silicon	Wheat (*Triticum aestivum* L.)	Foliar application	Salinity stress	Increased antioxidant activity and osmoregulatory	[23]
Nano-sized chitosan	Mung bean (*Vigna radiata* L.)	Solid matrix priming	Salinity stress	Decreased H_2O_2 and MDA; increased metabolism and chlorophyll content	[24]
Copper-chitosan nanoparticles	Wheat (*Triticum aestivum* L.)	Seed priming	Salinity and hyperosmotic stress	Increased seed germination, biochemical changes, chlorophyll A & B content total chlorophyl, β-carotenoids, and total carotenoids	[25]
Copper-loaded chitosan nanoparticles	Maize (*Zea mays* L.)	seed priming	High relative humidity and temperature	Increased the shoot growth, photosynthetic electron transport, and antioxidant enzyme activity	[26]
Selenium-loaded chitosan nanoparticles	Moldavian balm (*Dracocephalum moldavica* L.)	Foliar application	Heavy metal stress (Cd)	Decreased H_2O_2 and MDA; amplification of photosynthetic pigments, agronomic traits, chlorophyll fluorescence parameters, and antioxidant enzyme activity, proline, SPAD, total phenols, and essential oils	[27]
Chitosan-selenium nanoparticles	Bitter melon (*Momordica charantia*)	Foliar application	Salinity stress	Increased the growth and yield of plants; decreased H_2O_2 and MDA; enhanced antioxidant enzyme activity, relative water content, K^+, and proline concentration	[28]

(continued)

Table 8.1 (continued)

Nanomaterial	Plant species	Application	Stress	Mode of action	Reference
Chitosan-functionalized selenium and anatase titanium dioxide nanoparticles	Candyleaf (*Stevia rebaudiana*)	Foliar application	Salinity stress	Decreased electrolyte leakage, MDA, H_2O_2; increased photosynthetic performance, plant's growth, and antioxidant enzyme activity	[29]
Chitosan-melatonin nanoparticles	Spearmint (*Mentha spicata* L.)	Foliar application	Salinity stress	Enhanced morphological traits, proline, and antioxidant enzymatic activities	[30]
Lignin nanoparticles	Maize (*Zea mays* L.)	seed priming	No stress	Increased seed germination, radical length, shoot and root length, fresh weight, chlorophyll (a and b), anthocyanin, and carotenoid and soluble protein content	[36]
Lignin-zinc oxide hybrid nanoparticles	Maize (*Zea mays* L.)	Seed priming	No stress	Increased antioxidant activity; higher contents of carotenoids, chlorophyll, total phenols, and anthocyanins	[37]
Metal-based nanoparticles					
Iron oxide nanoparticles	Watermelon (*Citrullus lanatus*)	Seed priming		No toxicity effect on germination, seedling development, and chlorophyll biosynthesis. Altered diploid and triploid metabolome and modulated 12-oxo phytodienoic acid level	[42]
Iron oxide nanoparticles	Rice (*Oryza sativa* L.)	Seed priming		Improved germination and seedling vigor. Increase in root length and dry weight as well as sugar and amylase content. Enhanced antioxidant activity of SOD (29%), APX (50%), and CAT (60%)	[43]

Synergistic effect of iron oxide and zinc oxide nanoparticles	Wheat (*Triticum aestivum*)	Seed priming	Heavy metal stress (Cd)	Higher rates in roots, shoots, spikes, grains, plant height, dry weight, and photosynthesis. Reduced electrolyte leakage, SOD, and peroxidase activities. Cd concentration in roots, shoots, and grains has been decreased. Increased Zn and Fe concentrations in elemental contents.	[44]
Iron oxide nanoparticles	Wheat (*Triticum aestivum*)	Seed priming		Increased germination and shoot length. Accumulation of grain iron contents, WL711 (low-iron genotype), and IITR26 (high-iron genotype) has been increased.	[45]
Zinc oxide nanoparticles	Lupine (*Lupinus termis*)	Seed priming	Salinity stress	Enhanced growth; increased levels of organic solutes, photosynthetic pigments, ascorbic acid, total phenols, and Zn content. Triggered enzyme activity in CAT, SOD, APX, and POD. Reduction of MDA and sodium.	[49]
Zinc oxide nanoparticles	Rice (*Oryza sativa* L.)	Seed priming	Drought stress	Increase of fresh and dry weight, height, total chlorophyll contents, and straw yield. Increased in antioxidant enzyme activity. Decrease in MDA content; reduction in the oxidative stress indicators.	[53]
Zinc oxide nanoparticles	Rice (*Oryza sativa* L.)	Seed priming	Heavy metal stress (Cd)	Phenotypic improvements; enhanced enzymatic activity in roots and shoots; improved α-amylase and total amylase activity.	[51]
Photosynthesized silver nanoparticles	Jasmine rice (*Oryza sativa* L. cv. KDML 105)	Seed priming	Aged seeds	Enhanced germination, α-amalyase, and starch metabolism; higher soluble sugar content; improved seedling growth.	[54]
Silver nanoparticles	Pearl millet (*Pennisetum glaucum* L.)	Seed priming	Salinity stress	Enhanced antioxidant enzyme activities; decreased sodium and increased potassium contents.	[55]

(continued)

Table 8.1 (continued)

Nanomaterial	Plant species	Application	Stress	Mode of action	Reference
Silver nanoparticles	Watermelon (*Citrullus lanatus*)	Seed priming	Low germination rate of triploid seeds	Enhanced seed germination by AgNP; increased glucose and fructose contents during germination; Higher yields.	[56]
Titanium dioxide nanoparticles	Moldavian balm plants (*Dracocephalum moldavica L.*)	Foliar application	Salinity stress	Improved agronomic parameters and antioxidant activity; increased levels of secondary metabolite	[80]
Titanium dioxide nanoparticles	Maize (*Zea mays* L.)	Seed priming	Salinity stress	Improved plant growth; increased seedling root-shoot; increased fresh and dry weights; enhanced antioxidant activity and relative electrolyte leakage response.	[78]
Titanium dioxide nanoparticles	Coriander (*Coriandrum sativum* L.)	Seed priming	Heavy metal stress (Cd)	Improved agronomic traits; enhanced biosynthesis of osmatic regulators; diminished oxidative injuries	[82]
Titanium dioxide nanoparticles	Marjoram (*Origanum majorana* L.)	Seed priming	Salinity stress	Improved seedling growth and growth performance with increased essential oils yield and content levels; enhanced free radical scavenging activity (FRSA)	[81]
Carbon nanoparticles					
Multiwalled carbon nanotubes	Wheat (*Triticum aestivum* L.)	Seed priming	Toxicity audit	Root and shoot growth; elevated levels of biomass; cell enlargement; increased length of xylem and phloem	[83]
Single-walled carbon nanotubes	Soybean (*Glycine max* L. *Merrill*)	Seed priming	Drought stress	Enhanced germination; increased fresh weight, root, and shoot length; higher H_2O_2 and MDA contents; increased ASA, CAT, SOD, and POD activities.	[24]

Single-walled carbon nanohorns	Medicinal plant (*Sophora alopecuroides* L.)	Foliar application	Salinity stress	Enhanced seedling development with better metabolite accumulation rate	[84]
Carboxylated multiwalled carbon nanotubes	Green alder (*Alnus viridis* L.); buffalo berry (*Shepherdia canadensis* L.)	Seed priming		Cell membrane lipid metabolism control	[85]
Water soluble carbon nanoparticles	Little Gem, Parris Island, Breen, Butter Crunch, Muir, and Jericho varieties (*Lactuca sativa)*	Seed priming	Salinity stress	Increased chlorophyll content; improved root development and germination	[86]

Pourkhaloee et al. [87] and Joshi, Kaur, Singh, et al. [88] found that carbon nanotubes (CNTs) increased germination levels and enhanced seedling growth and physiological activities. CNMs may have different forms including single-wall and multiwalled carbon nanotubes. Their functions can be altered when modified with surfactants, amine, carboxylic acid, or polymer groups and can further be used for different purposes [89]. In a study conducted by Husen and Salahuddin Siddiqi in 2014, it was indicated that with this functionalization, water retention in plants appeared better as well as biomass and fruit yield [90].

Additionally, CNPs water solubility was exploited for seed priming under multiple salt concentrations on lettuce *Lactuca sativa* and on salt sensitive Little Gem, Parris Island, Breen, Butter Crunch, Muir, and Jericho varieties. The results suggested that seed priming with soluble CNPs was beneficial as an increased germination was observed under 150 mM NaCl and high temperature conditions. Furthermore, the amount of chlorophyll concentration increased in lettuce seedlings undergoing salinity stress as well as lateral root development. The study pointed out that although different varieties behave differently to CNPs due to genetic factors so mandatory tests run prior to application, salt stress consequences can be eliminated [86].

Another study investigated the effects of multiple forms of CNMs on antimicrobial activity and their contribution to preventing the induction of oxidative stress on plants and the metabolic procedures of bacteria. CNMs application caused physical and mechanical damage by collapsing the bacterial outer membranes and discharging the inner cell substances [91].

8.1.4.3.1 Multiwall carbon nanotubes

Multiwall carbon nanotubes (MWCNTs) are a subcategory of CNTs made of rolled-up multiple graphene layers and have unique properties. The unique mechanical, electrical, and thermal properties of MWCNTs make them ideal for plant priming applications in agriculture, with the ability to enhance plant performance [88].

Seed priming with MWCNTs in low doses has been found to impact the time of germination and growth development of wheat *Triticum aestivum* L. Also, root and shoot lengths were better at multiple growth periods, enlarged cells, xylem and phloem size, and increased levels of biomass. Toxicological analysis of MWCNTs on plants and soil presented no indications of harmful effects in the specific concentrations used in the study, demonstrating the likelihood of a green approach to raising crop productivity [83].

A 2019 study on Boreal Upland Forest species tested MWCNTs functionalized with carboxylic acid against seed dormancy and positively affected propagation on green alder (*Alnus viridis* L.) and buffalo berry (*Shepherdia canadensis* L.). Seeds were primed in low concentrations of MWCNT-COOH and gibberellic acid (hormonal priming), resulting in inhibited seed dormancy and improved germination to 90% in both species. The study supports that nano-priming with MWCNT-COOH has a critical role in cell membrane lipid metabolism control, preventing seed dormancy [85].

8.1.4.3.2 Single-wall carbon nanoparticles

Single-wall carbon nanotubes (SWCNTs) are also a subcategory of CNTs, made of rolled-up single graphene layers. SWCNTs were known to penetrate the mammal cell membranes and for the first time Liu et al. [92] demonstrated the ability to penetrate also plant cells, suggesting the potential to deliver a range of contents into plant cell organelles.

SWCNTs have been used under drought stress on soybean seeds. Under osmotic stress, with PEG 6,000, the germination percentage of SWCNTs-treated seeds was higher than the untreated (control) as well as in fresh weight and root and shoot length was longer. H_2O_2 and MDA contents were lower, but in the ASA, CAT, SOD, and POD activities were higher. The overall results of the study suggested that SWCNTs improved soybean seeds tolerance to drought stress and antioxidant capacity [24]

Single-walled carbon nanohorns (SWCNHs), horn-shaped sheath aggregate of graphene sheets, have been studied under salinity stress on *Sophora alopecuroides.* Compared with untreated plants, foliar spraying with SWCNHs increased leaf soluble sugar content along with root length and fresh weight, indicating the growth-promoting effect of the NPs on *S. alopecuroides*. Foliar spraying, while under salt stress, also increased the Photosystem II activity, leaves and roots' total protein contents and leaf copper (Cu), and soluble sugar contents. Interestingly, the metabolomic analyses showed that salinity-stressed seedlings had a higher metabolite accumulation rate. Also, increased contents of pyruvic acid, glucose-6-phosphate, fructose-6-phosphate, and L-malic acid were observed, which have the ability to benefit plants in terms of energy by participating in glycolysis and tricarboxylic acid cycle [84].

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