Zinc Oxide Nanoparticles Seed Priming against Environmental Stresses

Subjects: Materials Science, Coatings & Films

Contributor: Domenica Tommasa Donia, Marilena Carbone

Drastic climate changes over the years have triggered environmental challenges for wild plants and crops due to fluctuating weather patterns worldwide. This has caused different types of stressors, responsible for a decrease in plant life and biological productivity, with consequent food shortages, especially in areas under threat of desertification. Nanotechnology-based approaches have great potential in mitigating environmental stressors, thus fostering a sustainable agriculture. Zinc oxide nanoparticles (ZnO NPs) have demonstrated to be biostimulants as well as remedies to both environmental and biotic stresses. Their administration in the early sowing stages, i.e., seed priming, proved to be effective in improving germination rate, seedling and plant growth and in ameliorating the indicators of plants' well-being. Seed nano-priming acts through several mechanisms such as enhanced nutrients uptake, improved antioxidant properties, ROS accumulation and lipid peroxidation. The target for seed priming by ZnO NPs is mostly crops of large consumption or staple food, in order to meet the increased needs of a growing population and the net drop of global crop frequency, due to climate changes and soil contaminations.

Keywords: seed priming ; ZnO NPS ; crops ; stress alleviation

1. Introduction

The 2030 Agenda of sustainable development goals of the United Nations foresees the "Zero hunger" pursuit as its second point. It is estimated that in 2022, approximately 735 million people—or 9.2% of the world's population—found themselves in a state of chronic hunger. Food insecurity and hunger are global issues, exacerbated by climate changes triggering different types of stressors [1]. The worsening of climate conditions constantly affects crops yields and quality, causing food shortages worldwide, especially in areas under threat of desertification. A combination of climate stressors, such as drought and heat, significantly impacts crops yield by decreasing harvest index, shortening crop life cycle, and altering seed number, size and composition [2]. A robust negative association is projected among warming, caloric yield and crop frequency. By the 2050s, it is foreseen that crop frequency augmentation in cold regions will be offset by larger decreases in warm regions, resulting in a net global crop frequency reduction (−4.2 ± 2.5% in high-emission scenario), suggesting that climate-driven decline will exacerbate crop production loss [3]. Soil degradation and poor water retention lead to an imbalance between food and feed production as well as carbon storage in the ecosystem. On a larger scale, micronutrient deficiency (or hidden hunger) lead to soil erosions and nutrient runoffs, causing soil infertility, affecting human beings through malnutrition and other related diseases [4][5]. On the other hand, heavy metals from various anthropogenic sources are dumped into the environment, causing accumulation in soil and posing a severe threat for soil, plants, food security and human health. Heavy metals are persistent and non-degradable, and above threshold levels, they alter the soil–plant systems by disrupting physiological and biochemical metabolisms [6]. Therefore, contamination of soil by heavy metals, especially those used for the cultivation of food/feed crops, is a serious environmental and agronomical issue [7].

A major role in holding the pledge against the environmental stressors induced by climate changes is played by nanomaterials, since they can be employed with several functions. They are more efficient than traditional fertilizers, with a site-specific and controlled release of nutrients that increases the efficacy of plant uptake and reduces negative environmental effects related to nutrients loss into the environmental matrices. In addition, they offer a great contribution to enhancing agricultural productivity via gradual release and proper pesticide dosage to aid pest control [8]. The administration of nanomaterials to food crops may help to readily supply nanoscale nutrients, strengthening crops, contributing to food crops biofortification and improving the nutritional quantity and quality of foods [9]. In addition, it may enhance the defense potential against exposure to toxic concentrations of heavy metals resulting in a higher capability for plants to survive and develop under stress conditions [10].

The application of nanomaterials at various phases of plant growth is labelled as priming and is considered a form external stimulus or short-lasting preconditioning that may be applied to several compartments with the purpose of enhancing physiological and biochemical mechanisms of defense, thus promoting earlier, faster and stronger responses to stresses [11][12]. Priming may be applied to seedlings or their parts, as well as young plants in active growth phases, allowing the amelioration/evaluation of defense responses in specific plant regions or organs (for instance, the root system [13][14] or the foliar district [15]). However, the most frequent application is seed priming, a pre-sowing treatment, before the planting phase, aimed at physiological changes in the seeds that serve as a germination synchronizer with numerous advantages from an agricultural viewpoint [16][17]. Seed priming may reduce dormancy by the induction of physiological changes before planting and enhance the growth and yield of treated plants. In addition, it may mitigate the negative effects of long seed storage by counteracting the alterations of biochemical properties responsible for low seed vigor and diminished capability of enacting protection mechanisms against pathogens [18]. Different seed priming solutions were proposed to boost plant germination, including phyto- and biopriming [19], and hydropriming, halopriming, osmopriming, hormopriming and nano-priming [20]. Seed nano-priming, i.e., priming with solutions containing nanoparticles (NPs) [21], is an innovative, promising, simple and cost-effective strategy to improve plant growth and development, tolerance to biotic and abiotic stresses, and the production capacity of crops [19][22][23]. Nano-priming is a considerably more effective method compared to all other types of seed priming, its efficacy being based on the enhanced capability of seeds to rapidly absorb nutrients and renovate seed metabolism [20]. As nanoscale materials have versatile physicochemical properties and a large surface/volume ratio, they are expected to be better absorbed by seeds as compared to bulk chemicals, thus triggering enhanced molecular interactions at the cellular level. The flexibility in altering the surface chemical properties of nanomaterials can facilitate better interaction with the seeds/seedlings while inhibiting the wastage of priming agents. Their action is displayed by promoting the plants' production of phytohormones, of antioxidant enzymic and non-enzymic molecules, and by inducing the overexpression of new water channels [9]. In addition, several studies have demonstrated their bacteriostatic effect towards a wide panel of pathogenic and non-pathogenic bacteria and fungi [24]. The striking features of nanoparticles are connected to the possibility of developing electron exchange and enhancing the surface reaction of various components of plant cells and tissues. Nano-priming is responsible for the formation of nanopores in shoot, fostering water uptake. It activates reactive oxygen species (ROS), as well as antioxidant mechanisms in seeds, thus stimulating fast hydrolysis of starch. It also induces the expression of aquaporin genes that are involved in the intake of water and mediates H_2O_2, or ROS, dispersed over biological membranes.

Nano-priming by metal oxide nanoparticles (MO NPs), and especially by ZnO NPs, has largely increased in recent years, also on account of several methods implemented for their achievement and detection [25][26][27][28][29][30][31][32][33][34][35][36][37][38].

In general, ZnO NPs are widely applied in agriculture and food industries, and can be used as fertilizers and micronutrients to improve yield and food quality, especially when crops are grown in zinc-poor soils. Initially, ZnO NPs priming was put in place for biofortification in response to Zn deficiency in soil and consequent poor dietary intake [39]. Comparison with routine supplements indicated better plant development with nano-priming [40]. ZnO NPs were applied through several pathways, including the foliar district of seedling and plants, though seed priming became the primary choice due to its greater efficacy. Since high seed Zn content has a starter fertilizer effect, it is responsible for achieving good crop yield and improvements in rice grain. The concentration of Zn in plants is a result of enhanced Zn uptake by roots after flowering. Therefore, supplementary Zn by ZnO NPs application became important for improving both grain yield and grain Zn content [41][42]. Further developments foresaw the investigation of the beneficial effects of stress alleviation [43].

It must be added that the benefits of ZnO NPs priming are very much dose dependent and need to be offset against the shortcomings of too high a dose, in terms of inhibition of seed germination and root growth as well as retarded growth of seedlings. In particular, a dose of 1000 mgL^{-1} is considered the threshold level, beyond which, significant changes occur in the activity of soil enzymes, such as the inhibition of protease, catalase and peroxidase, which are bioindicators of soil quality and health [44][45][46][47].

2. Seed Nano-Priming Mechanism NPs Entry

Seed priming by ZnO NPs may alleviate plants' abiotic and biotic stress, act as a biostimulant causing an increase in germination rate, seedling and plant growth and overall fresh weight, and improve the biomass and photosynthetic machinery.

The effects of seed priming are implemented through several mechanisms. NPs affect the germination and vigor of plants by the stimulation and improvement of seed metabolic rate, vigor index and seedling characteristics [48], especially in the case of ZnO NPs priming [49]. Furthermore, seed nano-priming plays a role in nutrient uptake. This is particularly important, since poor nutrient uptake negatively affects the whole growth process, including root formation, seedling growth, flowering, and fruit formation [50][51]. Implementing nutrient delivery by conventional management systems may not be efficient, thus opening the way to nano-priming technology [52][53]. In fact, NPs may improve nutrient uptake by altering the metabolism of seeds, for instance, by a steep rise in α-amylase activity via gibberellic acid [54], the breakdown of stored starch, and stimulation of the release of growth regulators, ultimately fostering the growth and productivity of plants. This can also be monitored by the activity of enzymes like α-amylase, which has a steep increase upon seeding.

Management of the antioxidant system is crucial to a plant's well-being, since it prevents potential cellular molecule damage by maintaining ROS homeostasis [55]. Enzymatic and non-enzymatic antioxidant enzymes participate in the ROS management system: catalase (CAT), peroxidase (POX), ascorbate-peroxidase (APX), superoxide-dismutase (SOD), phenylalanine ammonia lyase (PAL), glutathione, and glutathione reductase. Seed priming affects the antioxidant enzyme system of plants at several levels.

It was observed that the activity of antioxidant enzymes increased at various degrees, depending on the type of plant and on the specific priming, though the exact action pathway was not quite fully disclosed. Some correlations were hypothesized, though. For instance, H_2O_2 radicals were significantly reduced in tomato, cucumber, and pea nano-primed seeds due to increased SOD and CAT activity [56][57][58]. On the other hand, H_2O_2 is responsible for carbonylating proteins, initiating and changing kinase transduction pathways; therefore, it can interfere with the expression of multiple genes in the germination process [59]. Seed nano-priming plays a role in ROS and lipid peroxide regulation. Seeds accumulated in the seed coat induce ROS accumulation and activate a number of downstream processes [60]. ROS are produced in plants' chloroplasts, mitochondria, and peroxisomes as a by-product during aerobic metabolism [61][62]. They may irreversibly damage DNA, but they also act as signaling molecules that allow plants to grow normally and mitigate abiotic stresses [63][64]. Increased ROS accumulation in plant cells upon seed nano-priming helps to break the bonds among the polysaccharides in the cell wall of seed endosperm, thus facilitating quick and healthy seed germination [65]. It triggers processes of breaking seed dormancy and stimulating seed germination [66] through the activation of the synthesis of gibberellic and abscisic acid [67][68]. The lowering of ROS by NPs may result in a large rise in antioxidant enzyme levels. This may help increasing tolerance towards stress factors, such as salinity and drought [69][70]. Heavy metals affect crop growth development and production, hampering plant growth progressions. However, in general, NPs regulate plant physiological and biochemical parameters to reduce their detrimental effects [71]. Several studies indicate a connection between NPs priming and decreases in toxic metal accumulations by stimulating antioxidant enzyme activities and decreasing ROS and lipid peroxidation [72]. A scheme of many nano-priming mechanisms is summarized in **Figure 1**.

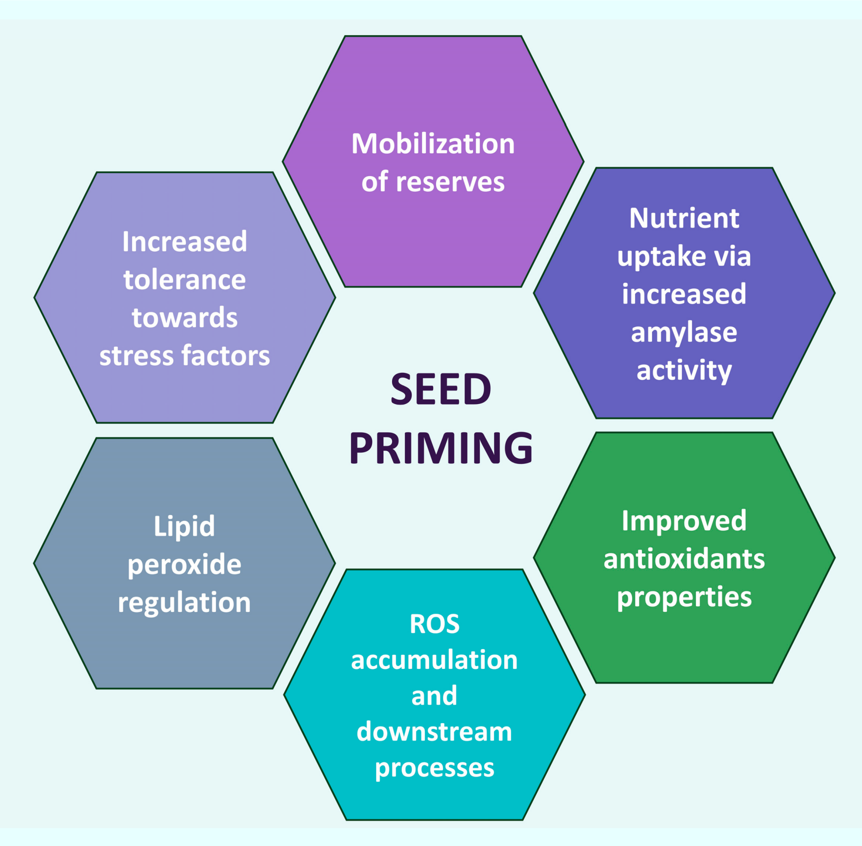

Figure 1. Main nano-priming mechanisms.

Upon seed priming, the entry of the NPs is proposed by three mechanisms [73]. In the first one, NPs are considered as molecules crossing the plasma membrane by a direct diffusion process, the efficacy depending on the NPs' properties, i.e., the size, hydrophobicity, constitution, charge, and shape of the particles [74]. In the second mechanism, NPs are actively transported into the cell by endocytosis [75]. The third mechanism occurs through transmembrane proteins or through channels that regulate the movement of NPs into cells [76]. However, the actual limiting factors for NP entrance are mostly related to their high degree of specificity, the capability of generating a "least open" pathway, and the presence of small pores [77].

3. Application to Different Crops

Nano-priming techniques are deemed beneficial for improving crop yields, frequencies and seedling and plant well-being. In this framework, great effort has been devoted to promote and support nanotechnologies in agriculture under different flagships such as the Nano Mission [78] of the Indian government to encourage private-sector investment and support the growth and commercial application of nanotechnology. Broad, systematic and low-cost applications of NPs on fields are general requirements for sustainable agricultural stimulation. The success of seed nano-priming as a form of biostimulation and an agent of contrast against abiotic and stresses varies based on the species of plants and the characteristics of treating agents. However, ZnO NPs appear to be very efficient and versatile among nanoparticles, for seed nano-priming purposes, since they are suitable for targeting different types of crops worldwide. Besides this, their production is low cost and can be achieved with environmentally friendly methods [79]. Crops which were successfully tested for ZnO NPs nano-priming include produce grown in localized areas, as well as global harvests. Large consumption cereals are a primary target for improved production, in dry, salty and contaminated soils, especially since cereal crops play an important role in satisfying daily calorie intake in the developing world. However, the Zn concentration in grain is inherently very low, particularly when grown on Zn-deficient soils. Rice alone (*Oryza sativa* L.) is one of the major staples, feeding more than half of the world's population. It is grown in more than 100 countries, predominantly in Asia [80]. Hence, rice, wheat, maize, sorghum, chickpeas and several varieties of faba beans (lupine, *vigna mungo*, *vigna radiata* and *vicia faba*) were recently probed for seed priming with ZnO NPs. However, tests are also being performed on different types of edibles, such as spinach (*basella alba*) and tomatoes. Finally, textile fibers (cotton) and vegetables for animal feed and industrial oil production (rapeseed, *Brassica napus* L.) were also investigated for ZnO NPs seed priming.

4. ZnO NPs Seed Priming against Abiotic Stresses

The efficacy of seed priming is dose dependent, and investigations may be carried out in vitro, in pot or in field. In the following sections, a summary of the latest research is reported on the effects of ZnO NPs priming on the most widespread crops worldwide. Drought, soil salinity and heavy metal and arsenic accumulation are listed among abiotic stresses as well as improper seed storage. In addition, action against biotic stresses is considered in association with biotic remedies. The sheer biostimulating effect of ZnO NPs is also reported. Details are mentioned for the major effects, doses and mechanisms when specified in the original papers. The ZnO NPs used in the experiments were of two types, commercial or purposely synthetized. Information on ZnO NPs preparation and characterization methods was reported where available. **Figure 2** summarizes the main actions of ZnO NPs seed priming and the consequent effects on crops. The type of stress, ZnO NPs doses, target plants and major benefits of seed priming are reported in **Table 1**, whereas the type of ZnO NPs and the synthesis and characterization methods (if available) are reported in **Table 2**.

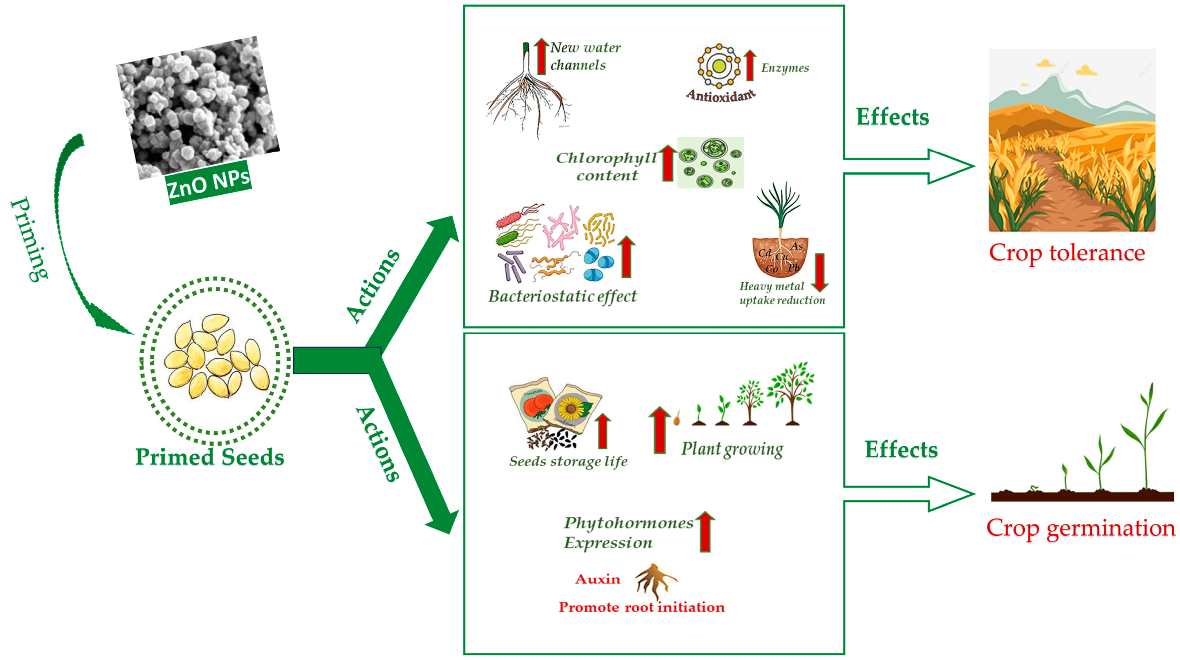

Figure 2. Scheme of the main actions of ZnO NPs seed priming and consequent effects on crops.

Table 1. Summary of the latest advances on applications of ZnO NPs seed priming, their doses and effects.

Application	Type of Seed	Doses	Effects	Ref.
Drought stress alleviation	Rice	0; 5; 10; 15; 25; 50 ppm	Agronomic profile yield	[81]
	Wheat	40; 80; 120; 160 ppm	Chlorophyll content and plant nutrients	[82]
	Wheat	10 mg/L	Reduced DNA damage	[83]
Salt stress alleviation	*Sorghum bicolor*	5; 10 mg/L	Increased Fresh weights	[84]
	Wheat	50 mg/L	Increased plant growth, grain yield and macronutrients	[85]
	Wheat	50 mg/L	Improved germination	[86]
	Rapeseed	25; 50; 100 mg/L	Increased vigor indexes	[87]
Cu stress alleviation	Wheat	20 ppm	Improved growth	[86]
Co stress alleviation	Maize	500 mg/L	Improved plant growth, biomass and photosynthetic machinery	[88]
Pb stress alleviation	*Basella alba*	200 mg/L	Increased seed germination, seedling and roots growth, seed vigor; reduced Pb uptake	[89]

Application	Type of Seed	Doses	Effects	Ref.
Cd stress alleviation	Rice	0; 25; 50; 100 mg/L	Improved early growth and related physio-biochemical attributes	[90]
Arsenic stress alleviation	*V. mungo* (bean)	50; 100; 150; 200 mg/L	Increased germination by modulation of metabolic pathways	[91]
Biotic stress counteraction	Chickpea	0.25; 0.50; 0.75; 1.0 µg/mL	Antifungal Activity	[92]
		1000 mg/kg	Reduction in disease indices	[93]
Seed storage	*V. radiata* (bean)	1000 ppm	Increased germination percentage	[94]
	Chickpea	100 ppm	increased seed storage life; decreased disease incidence	[95]
Crop improvement	Wheat	5; 10; 15; 20 ppm	Increased plant growth	[96]
	Oryza sativa (rice)	10 µmol	Seedling vigor; increased plant chlorophyll content; nutrients uptake	[97]
	Tomato	100 ppm	Improved germination process	[98]
	Cotton	400 ppm	Increased germination and seed parameters	[99]
	Wheat	10 mgL^{-1}	Increased germination, vigor index and chlorophyll content	[100]
	V. faba (bean)	50, 100 mgL^{-1}	Increased biometric parameters, chlorophyll content	[101]

Table 2. Summary of the ZnO NPs preparation conditions and characterization, where reported.

ZnO NPs Source	Reagents and Conditions	Characterization	Size and Purity	Refs.
Purchased		Not Reported	20–30 nm 98% purity level	[81]
Purchased		UV–Vis, TEM, XRD *	Not reported—99,99%purity level	[82]
Purchased		Not Reported	Not Reported	[83]
Phyto-Synthesis	$Zn(NO_3)_2 \cdot 6H_2O$, *Agathosma betulina*, 80 °C, calcination 600 °C	UV–Vis, TEM, XRD, FTIR, SEM	20–30 nm	[84]

ZnO NPs Source	Reagents and Conditions	Characterization	Size and Purity	Refs.
Purchased		Not reported	<50 nm	[85]
Purchased		Not reported	<100 nm	[86]
Precipitation	$ZnSO_4$·$7H_2O$, NaOH, drying 100 °C	XRD, IR, SEM, TEM	~20 nm	[87]
Purchased		XRD, EDX, TEM, SEM	~20 nm-99% purity	[88]
“Chemical”	$ZnSO_4$, citrate, 70 °C	DLS, TEM	spherical shape ~ 193 nm High purity	[89] **
Purchased		SEM, TEM *	36 nm	[90]
Phyto-Synthesis	$Zn(ac)_2$ ·$2H_2O$ *V. mungo* extract, 70 °C	UV–Vis, SEM, XRD	30–80 nm	[91]
Biogenic synthesis	$Zn(ac)_2$ ·$2H_2O$, *T. Harzanium*, 40 °C, calcination 500 °C	UV–Vis, FTIR, TEM, SEM, EDX, XRD	5–27 nm, agglomerated	[92]
Purchased		XRD	15–25 nm	[93]
Precipitation	$Zn(NO_3)_2$ ·$6H_2O$, NaOH, 55 °C, vacuum 60 °C	SEM, EDAX, TEM, Raman	Narrow rods— 80–150 nm	[94]
Wet chemical synthesis	*Trichoderm asperellum*	UV–Vis, SEM, TEM, FTIR	spherical shape, 33.4 nm	[95] **
Precipitation	$Zn(ac)_2$ ·$2H_2O$, KOH, 60 °C, dried at 60 °C	UV-Vis, XRD, SEM, FTIR	Hexagonal shape 20 nm	[96]
Biogenic synthesis	$Zn(NO_3)_2$, $ZnSO_4$, $ZnCl_2$ fungi, from arid field	DLS, TEM-EDX, zeta potential	10 nm	[97] **
Phyto-Synthesis	$Zn(ac)_2$ ·$2H_2O$, *Coriandrum sativum*, 70 °C, calcination 500 °C	UV–Vis, FTIR, TEM, SEM, XRD	spherical shape 30 nm	[98]
Precipitation	$Zn(NO_3)_2$ ·$6H_2O$, NaOH, 55 °C, Dried at 60 °C	UV –Vis, FTIR, TEM, SEM	spherical shape 36 nm	[99]
Purchased		UV –Vis, XRD, TEM	spherical shape 20–30 nm	[100]

ZnO NPs Source	Reagents and Conditions	Characterization	Size and Purity	Refs.
Precipitation	$Zn(NO_3)_2 \cdot 6H_2O$, NaOH 40 °C–60 °C	XRD, FTIR, SEM, TGA	58–60 nm	[101]

* data provided by the manufacturer; ** and references therein.

5. ZnO NPs Seed Priming against Biotic Stresses

Nanoparticles have been revolutionary for pest management in agriculture, since they facilitate a substantial decrease in pesticide use. At the same time, biopriming techniques, i.e., priming by microorganisms, provide alternative remedies to managing soil- and seed-borne pathogens. A proper coupling of the two approaches, such as the simultaneous application of ZnO NPs and endophytic bacteria, may ensure a sustainable crop production, improving yields and quality [102]. This was successfully achieved when facing a variety of biotic stresses that affect the yield of the most valuable pulse crops worldwide, i.e., the destructive fungus *Fusarium oxysporum*, which causes the main seedborne and soil fungus diseases in legume-growing areas [103]. In this regard, ZnO NPs were used in association with *Trichoderma harzianum,* a fungus widely employed as a biopesticide and biofertilizer, for nano-priming chickpea seeds (*Cicer arietinum* L.) [92]. Several concentrations of ZnO NPs were initially probed on the in vitro inhibition of the mycelial growth of the fungus. Based on the optimal outcome of this first phase of investigation, a concentration of 0.50 µg/mL of ZnO NPs was selected for further seed priming experiments, which caused a significant increase in the antioxidant activity of germinated plants and a decrease by 90% in the incidence of *F. oxysporum* disease. An overall increase in the defensive enzymatic activities was revealed, as well as a positive effect on plant growth, recognizing ZnO NPs as a very effective promoter agent in host tolerance under fungal stress. It must be added that *Trichoderma harzianum* was also used in the mycological synthesis of ZnO NPs. In the procedure, $Zn(ac)_2$ and a filtrate of the fungus were mixed and shaken in an incubator for 24 to 48 h. Centrifugation, drying and calcination at 500 °C yielded the ZnO NPs in power form, which could easily be transported to the field for large-scale application. The NPs were characterized by XRD, FTIR, SEM, EDX and UV-Vis techniques, revealing a size in the range of 5–27 nm (TEM), and a high level of agglomeration. Furthermore, residual carbon and Al were present (determined by EDX), as well as a few functional groups, such as -NH, C-I, C-Br, C-Cl and -OH (determined by FTIR).

In addition to fungi, bacteria and parasites are major threats to legume crops. Among this sort of pathogen, *Pseudomonas syringae pv*, *pisi* and *Meloidogyne incognita* are responsible for the “bacterial blight disease complex” of pea, causing constantly increasing losses in yields [93]. The pathogens’ damage to plants may be reduced by the symbiotic action of soil bacteria with legume roots, enhanced by the associated action of the NPs. This was achieved, for instance, by exploiting the combined antimicrobial properties of *Rhizobium Frank* and ZnO NPs to pursue disease management. In vitro studies and in-pot experiments indicated a larger plant growth, chlorophyll and carotenoid content when NPs-primed seeds were grown with *R. leguminosarum* and exposed to pathogens, as compared to plants subjected to only one of the treatments. In addition, plants inoculated with *R. leguminosarum* showed higher root nodulation, whereas only a few nodules were observed in untreated plants. Both tested pathogens had adverse effects on nodulation, while the use of ZnO NPs in combination with *R. leguminosarum* reduced nodulation, blight disease indices, galling and nematode population. Finally, the segregation of various treatments in the biplot of principal component analysis demonstrated the suppressive role of ZnO NPs on the blight disease complex of pea [93]. ZnO NPs in this investigation were purchased and the average size was assessed by XRD and TEM (though the data are not shown). NPs were claimed to have a diameter in the range of 15–25 nm, and 79.1–81.5% NPs are smaller than 100 nm.

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