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Stimulation of morphometric parameters and zinc content of native maize by priming with zinc oxide phytonanoparticles

Estimulación de parámetros morfométricos y contenido de zinc en maíz nativo cebado con fitonanopartículas de óxido de zinc

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Abstract
Nanotechnology has been a promising tool for the improvement of various crops of agricultural importance. Maize cultivation in Mexico is one of the most important activities of national interest. However, little attention has been given to the cultivation of native maize. Therefore, the objective of this work was to evaluate different treatments with ZnO-NPs on olotillo maize seeds to find out whether variations occurred on germination percentage, morphometric parameters, and zinc content. In this research, olotillo maize seeds were used, and three priming treatments were tested (nanopriming 3 h, nanopriming 3 h + hydropriming 24 h and nanopriming 24 h) with different concentrations of ZnO-NPs (0, 50, 100, 150 and 200 ppm). The parameters that were evaluated comprised germination percentage, vigor index, amylase activity, shoot and root length, and zinc content. It was observed that nanoparticles increase the percentage of germination in seeds as well as a beneficial effect on morphometric parameters, where the best treatment was nanopriming 3h + hydropriming 24 h at 50 and 100 ppm. As for zinc content in shoots and roots, the best treatment was nanopriming 24 h with 150 and 200 ppm. This indicated that ZnO-NPs in olotillo corn seeds can be an alternative for bio-fortifying this crop.
Keywords: *Zea mays*, olotillo native maize, germination, ICP, biofortification.

Resumen
La nanotecnología ha sido una herramienta prometedora para el mejoramiento de diversos cultivos de importancia agrícola. El cultivo de maíz en México es una de las actividades de interés nacional más importantes. Sin embargo, se ha prestado poca atención al cultivo de maíz nativo. Por lo tanto, el objetivo de este trabajo fue evaluar diferentes tratamientos con NPs-ZnO en semillas de maíz olotillo para conocer si se presentaron variaciones en el porcentaje de germinación, parámetros morfométricos y contenido de zinc. En esta investigación se utilizaron semillas de maíz olotillo y se probaron tres tratamientos de cebado (sin cebado, hidroceabdo y nanocebado) con diferentes concentraciones de NPs-ZnO (0, 50, 100, 150 y 200 ppm). Los parámetros evaluados fueron porcentaje de germinación, índice de vigor, actividad de amilasa, longitud de brotes y raíces y contenido de zinc. Se observó que las nanopartículas incrementaron el porcentaje de germinación en semillas, así como un efecto beneficioso en parámetros morfométricos, siendo el mejor tratamiento el hidrocebado a 50 y 100 ppm. En cuanto al contenido de zinc en brotes y raíces, el mejor tratamiento fue el nanocebado con 150 y 200 ppm. Esto indicó que las NPs-ZnO en las semillas de maíz olotillo pueden ser una alternativa para biofortificar este cultivo.
Palabras clave: *Zea mays*, maíz nativo olotillo, germinación, ICP, biofortificación.

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1 Introduction

Nanotechnology applications are a promising tool for agricultural purposes, as some nanomaterials can induce favorable effects in crops such as better development, higher yield, and an increase in their nutritional content (Del Buono *et al*., 2021). Nanoparticles (NPs) can be obtained from metals such as silver, zinc, iron, copper, gold as well as those obtained from metal oxide polymers such as titanium carbon dioxide (TiO2) and zinc oxide (ZnO) (López-Cuenca *et al*., 2019, Ying *et al*., 2020). Some of these types of NPs have been used in agriculture (Liu *et al*., 2015). ZnO has a critical function in anatomical and physiological responses in plants. Therefore, zinc oxide nanoparticles (ZnO-NPs) are mainly used as fertilizers, herbicides, fungicides, insecticides, which help to improve crop productivity (Agarwal *et al*., 2017). Also, ZnO is involved in the synthesis of tryptophan, regulating the synthesis of endogenous auxins, thus participating in the regulation of metabolism, it is also known to activate various enzymes such as oxidoreductases, lyases, isomerases, transferases, hydrolases, and ligases (Narendhran *et al*., 2016, Singh *et al*., 2018). The use of zinc oxide nanoparticles (ZnO-NPs) for mitigation of Zn deficiency and for increasing Zn biofortification in different crops has been previously reported. In tomatoes, ZnO-NPs have been applied to potentially improve seed germination and the shoot vigor index, as well as plumule and radicle length (Singh *et al*., 2016). In recent years, it has been confirmed that ZnO-NPs have a promoting action on seed germination in various plant species (Zafar *et al*., 2016). Zinc is a micronutrient with important functions in metabolic and physiological processes such as protein synthesis, chlorophyll synthesis, biomass production, cell elongation, germination, fertilization, as well as upkeep of the structural stability of cell membranes (Takahashi *et al*., 2009).

Seed priming is one of the techniques that has been applied in different crops and causes the initiation of metabolic processes to improve seed quality before establishing seed germination (Rehman *et al*., 2015). Priming is the method of pretreating seeds previously sowing them, for which conventional techniques such as imbibition and coating are used. This causes a physiological change in the seed, allowing it to germinate in less time (Bruce *et al*., 2007). Hydropriming, osmopriming, hormonal priming, nutripriming, and biopriming are models of existing priming processes that have positive effects in different crops, including improvements on germination rates, germination energy, growth, and development, as well as an increased tolerance to both abiotic and biotic stress factors, the effect of which is observed as higher crop yields and greater micronutrient concentrations (Acharya *et al*., 2020). It has been reported that germination and shoot vigor are stimulated via nanopriming in seeds such as wheat, tomatoes, peas, and onions (Paparella *et al*., 2015, Zhu *et al*., 2019, Chandrasekaran *et al*., 2020).

In different studies it has been observed that ZnO-NPs show different effects in various plant species. These studies demonstrate that ZnO-NPs can have completely different effects on plant germination and growth parameters (Lee *et al*., 2009). Furthermore, sensitivity to ZnO-NPs varies greatly from species to species. In *Black gram*, it was shown that a concentration of 600 mg/L of ZnO-NP in seeds significantly affects the germination parameters in terms of higher germination percentage, maximum root length, maximum germination length and greater vigor of germination seedlings at this concentration (Raja *et al*., 2018). On the contrary, in *Triticum aestivum* seeds at concentrations of 400 ppm it has been observed to have inhibitory effects on root growth and seedling germination (Prakash and Chung, 2016).

Maize (*Zea mays* L.) is one of the principal crops in Mexico and in the world because of its high productive value and its diverse applications. Mexico ranked fifth in the world with a production of 27,169,977 tons in 2018 (FAOSTAT, 2020), where Chiapas is one of the most important states for corn production in the country. An estimated 90% of the area where corn is grown in Chiapas contains native corn from different races and with different colors, textures, and crop cycles. The corn races described for the state of Chiapas are Olotillo, Tehua, Olotón, Tepecintle, Vandeño, Zapalote Chico, Zapalote Grande and Tuxpeño (Wellhausen *et al*., 1951). Native maize populations have received little attention within the scientific field. Hence, the application of new technologies contributing to the improvement of physiological and nutritional quality and yield in the cultivation of native maize represent an opportunity to promote this crop. The objective of the work was to evaluate the effect of nanopriming and the use of different concentrations of ZnO-NPs on morphometric parameters, α-amylase activity, and zinc content in native maize of the olotillo breed.

2 Materials and methods

2.1 Plant material

Native maize seeds of the olotillo were obtained from ranch "Capricho divino", located in Suchiapa Chiapas, Mexico at 16° 37' 30" LN and 93° 6' 0" LO, and at an altitude of 500 m. The climate is warm, sub-humid and with periodic rains, with an average annual temperature of 24.4 °C, and with precipitation averaging 956 mm per year.

2.2 *Nanoparticle preparation*

Zinc oxide nanoparticles were synthesized according to Velázquez-Gamboa *et al*., (2021), with a particle size of 13 μm and a Z potential of 22.56 mV obtained with an extract of *Moringa oleifera*. To obtain the extract, 50 g of dried and ground leaves were used in 500 mL of distilled water (pH 6.5) and subsequently subjected to ultrasound treatment at 360W (COLE-PARMER, Vernon Hills, IL, USA) for 2 hours at 25°C, finally filtered with a 0.45 μm millipore membrane. For the synthesis of zinc oxide phytonanoparticles, 15 mL of aqueous extract of *Moringa oleifera* and 1,647 g of zinc ($ZnSO_4$) dissolved in 35 mL of distilled water were used. The mixture was kept under magnetic stirring for 6 h. Subsequently, 2M NaOH was added to the solution and placed in an incubator at 60°C with constant magnetic stirring for 12 h. The colloidal solution was centrifuged (HERMLE, Germany) at 4500 rpm for 20 min, the precipitate was subjected to two consecutive washes with ethanol (96%) and distilled water; the precipitate was dried in an oven at 45-50 °C and finally a fine powder was obtained. The nanoparticles were dispersed in deionized water by using ultrasonic vibration (180 W, 40 kHz) for 30 minutes before use.

2.3 *Nanoparticle treatments*

A total of 3 sets of treatments were performed. The first set consisted of immerse the seeds with different concentrations of ZnO-NPs (0, 50, 100, 150 and 200 ppm) for three hours (Nanopriming 3 h); the second set of treatment consisted of immerse seeds for three hours with different concentrations of ZnO-NPs and subsequently placed in water for 24 hours (Nanopriming 3 h + Hydropriming 24 h). The third set of treatment consisted of treating the seeds with different concentrations of ZnO-NPs for 24 hours (Nanopriming 24 h).

2.4 *Seed germination*

Seed germination was determined by using between-paper methods. Twenty-five seeds and four repetitions were used according to experimental designs proposed by Guillen *et al*., (2018). The seeds were first disinfected with 20% chlorine for 30 min. After the time elapsed, they were rinsed with distilled water, and placed on previously moistened Anchor paper towels (Paper Company, St. Paul, Minnesota, USA). Anchor paper towels were then rolled into wads and placed in plastic bags. Bags were inserted in a container and kept in the dark in a bioclimatic chamber at 25 °C ± 2 °C for 7 days. After 7 days, the germination percentage was calculated, and the shoot length and the root were measured with a graduated millimeter ruler.

2.5 *Vigor index*

The shoot vigor index (SVI) was calculated using the formula given below:

$$\text{Vigor index} = \text{Germination}(\%) \times \text{Shoot length} \quad (\text{root length} + \text{shoot length}). \tag{1}$$

2.6 *α-amylase activity*

The extract for the determination of α-amylase activity was obtained from 3-day-old germinated seeds using the methodology detailed by Gugelminetti *et al*. (1995). Briefly described, 1 g of sample was macerated in 0.1 M TRIS-HCl buffer pH 7 containing 0.1 M NaCl and 10 mM $CaCl_2$. The resulting homogenate was centrifuged at 12,000 g at 4° C for 10 min. All supernatants were collected and stored at -20 °C until enzyme assays were performed. Starch (1% w/v) was used as the substrate for the determination of α-amylase activity. To evaluate the activity, the crude extract was kept for 15 min at 70 °C, before starting the enzymatic analyses. At the end of the reaction periods, free reducing sugars were quantified at 540 nm with the dinitrosalicylic acid (DNS) method using a Beckman Coulter Du® 73 spectrophotometer (Germany). The activity was expressed in μmol maltose $min^{-1}g^{-1}$.

2.7 *Determination of zinc (Zn) content*

The zinc content in the shoots and roots was determined following the methodology of González-Terreros *et al*., (2018). Root and shoot samples were dried in an oven at 75 °C for 48 h. After drying and pulverizing the plant material, 250 mg were subjected to acid digestion by reflux with nitric acid (4.4 mL) and 1.2 mL of 30% hydrochloric acid for 30 min. After digestion, sample volume was adjusted to 25 mL with nitric acid (1%). Zn content was determined by using an inductively coupled plasma-optical emission spectrometry (ICP-OES) spectrometer (4300 DV-PerkinElmer, USA).

2.8 *Statistical analyses*

The assessment of the effect of ZnO-NPs on the morphometric parameters and α-amylase activity was carried out with a completely randomized experimental design, whereby the least significant difference (LSD) of the treatments was determined at 95% with a confidence level of 0.05. The software used for this purpose was the Statgraphics Centurion XV statistical package.

3 Results and discussion

Nanopriming 3 h corn seeds show that, as the concentration of ZnO-NPs increases, germination percentage also increases, where a concentration of 200 ppm caused the highest values for germination percentage. The seeds with nanopriming 3 h + hydropriming 24 h had a lower germination percentage as ZnO-NPs concentrations increased. It was observed that seeds in the control and in 50 ppm treatments had a higher percentage of germination. Regarding the nanopriming 24 h, it was observed that the germination percentage increases as the concentration of ZnO-NPs increases. It has been reported that, during nanopriming, there is a rapid absorption of water molecules, which penetrate through the cell wall of the seed coat along with ZnO-NPs until they reach the embryo. This phenomenon induces the ROS generation signal, which accelerates seed germination (Mahakham *et al.*, 2017; Prerna *et al.*, 2020).

Priming with water (hydropriming) of seeds prior to sowing has been reported to improve the activation of hydrolytic enzymes, translocation, and the utilization of reserved material. Also, it has been shown that germination and shoot growth are enhanced through hydropriming (Mahakham *et al.*, 2016). The increase of germination percentage in seeds treated with NPs can be attributed to the role of Zn in stimulating a series of biochemical changes in the seed that are necessary to start the germination process. Some of these changes include imbibition, breaking dormancy, hydrolysis, and enzymatic activation (Samad *et al.*, 2014). The technique of hydropriming and the use of nanoparticles is a promising strategy for enhancements of seed germination percentage and yield (Acharya *et al.*, 2020; Anand *et al.*, 2020).

For the vigor index, the three set of treatments showed significant statistical differences regarding the control (Table 1). Among the different priming treatments, it can be observed that the vigor index with nanoprimig 3h + hydropriming 24 h at 50 ppm of ZnO-NPs registered the highest vigor index. NPs could penetrate the hard layering of the seeds, allowing the passage of water, which is the critical factor for greater growth and vigor. ZnO-NPs increase the synthesis of tryptophan, which is a precursor of IAA (Indole Acetic Acid), which causes an increase in the germination rate, greater growth and biomass production, on the other hand, it also increases the activity and expression of enzymes such as nitrate reductase and carbonic anhydrase, which play key roles in nitrogen absorption and chlorophyll synthesis, respectively, it is for this reason that ZnO-NPs increase the percentage of germination, root growth, resulting in a significant increase in the shoot vigor index (Sarkhosh *et al.*, 2022).

Table 1. Effect of the different priming treatments and of different ZnO-NPs concentrations on germination percentage, vigor index and α-amylase activity in olotillo native maize.

ZnO-NPs (ppm)	Germination (%)	Vigor index	α-amylase activity (U/g seed)
Nanopriming 3 h			
Control	76.66 ± 0.07cd	1810.64 ± 334.57g	80.23 ± 13.87cdef
50 ppm	80 ± 0.07bcd	2146 ± 350fg	127.15 ± 9.13ab
100 ppm	80 ± 0.07abc	1980 ± 463.26fg	115.98 ± 12.64abc
150 ppm	90 ± 0.05abc	2505.6 ± 514.18cdef	68. 8517 ± 13.65def
200 ppm	96.66 ± 0.03a	2383.17 ± 652.66def	115.55 ±17.41abc
Nanopriming 3 h + Hydropriming 24 h			
Control	100 ± 0a	2855 ± 460.52abcd	104.59 ± 12.90bcd
50 ppm	100 ± 0a	3290 ± 473.39a	63.85 ±10.49ef
100 ppm	95 ± 0.05ab	3205 ± 350.97ab	136.19 ± 14.43ab
150 ppm	85 ± 0.08abcd	3120 ± 252.80ab	115.12 ± 5.18abc
200 ppm	85 ± 0.08abcd	2480 ± 305cdef	145.87 ± 1.57a
Nanopriming 24 h			
50 ppm	70 ± 0.10d	2117.5 ± 375.33efg	48.74 ± 0.48cdef
100 ppm	90 ± 0.06abc	2870 ± 517.36abcd	83.74 ± 31.93fg
150 ppm	90 ± 0.06abc	2950 ± 773.32abc	10.87 ± 0.48g
200 ppm	95 ± 0.05ab	2725 ± 678.29bcde	96.93 ± 4.7bcde
LSD 1 (0.05)	0.127	506.13	39.8

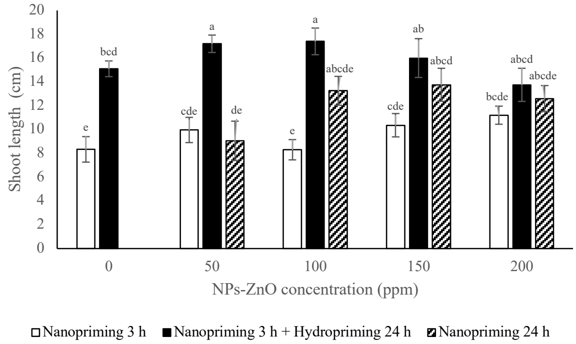

Figure 1. Effect of different priming treatments and different ZnO-NPs concentrations on shoot length of olotillo native maize.

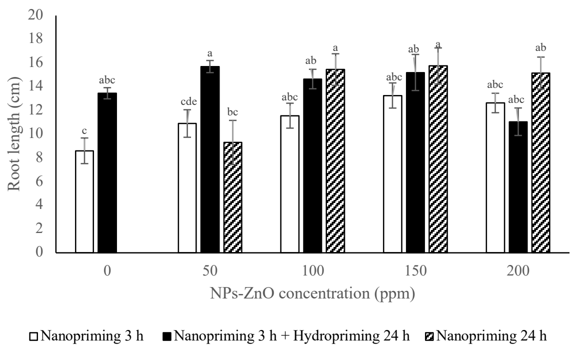

Figure 2. Effect of different priming treatments and ZnO-NP concentrations on the length of roots from olotillo native maize.

For all evaluated treatments, α-amylase activity was higher in treatment with nanopriming 3 h + hydropriming 24 h and 200 ppm ZnO-NPs (Table 1). Mahakham *et al*., (2017) and Khodakovskaya *et al*., (2011) revealed that nanoparticles enter the pores in the seed's outer layer, increasing penetration of water and inducing enzymatic activity that produces ROS-generating/starch-degrading, which physiologically improves seed germination. α-Amylase breaks down reserve carbohydrates down to soluble sugars to maintain an effective respiratory metabolism, which supports both the seed germination process and plant growth previous to photosynthesis (Konho & Nanmori, 1992). Our results show that there is no relationship between the enzymatic activity of α-amylase and germination, since in the treatment with nanopriming 3 h + hydropriming 24 h with 200 ppm of ZnO-NPs a greater activity of this enzyme is observed, however germination percentages decrease. These results contrast with what has been reported for other species such as wheat, where it has been observed that ZnO-NPs interrupt the mobilization of sugars in the endosperms due to the inhibition of α-amylase (Stałanowska *et al*., 2023).

Shoot length and root length had a positive effect with all three set of treatments and all NPs concentrations, as compared to the control. The treatment with nanopriming 3 h + hydroprimig 24 h was the treatment where greater shoot and root length was observed, compared to all the evaluated treatments (figures 1 and 2). In the treatment with nanopriming 3 h + hydroprimig 24 h an increase in the length of the shoots and roots was observed at concentrations of 50 and 100 ppm regarding the control. For rice cultivation, it has been reported that the application of ZnO-NPs on seeds caused an increase in the length of the radicle and of the plumule as the ZnO-NPs concentrations increased (0, 5, 10, 15, 20 and 50 mg/L), where it was found that a concentration of 20 mg/L was optimal for better growth (Upadhyaya *et al*., 2017). The increase in the length of roots and shoots in the presence of ZnO-NP may be related to the role that zinc plays during the biosynthesis of proteins, carbohydrates, and endogenous hormones such as gibberellins and auxins, these hormones favor the increase in root length of shoots from seeds treated with NPs (Broadley *et al*., 2007, Cakmak, 2008, Prasad *et al*., 2012). The addition of zinc to the priming solution improves root emergence as well as shoot development, probably due to the role of zinc in root development as well as coleoptile development (Ozturk *et al*., 2006). Our results show that ZnO-NPs do not generate a toxic effect on shoots at high concentrations (200 ppm), compared to other species such as *Camelina sativa* and *Brassica napus* L, where a toxic effect has been observed at concentrations over 50 ppm reduce the percentage of germination, root growth which results in a decrease in plant growth (Sarkhosh *et al*., 2022), in *Allium cepa* high concentrations of ZnO-NPs increase the production of ROS, causing abnormalities in the mitochondrial membrane and chromosome reduction or fraction (Ahmed *et al*., 2017). Thus, it can be inferred that, to stimulate germination processes and to improve physiological parameters in a crop via application of ZnO-NPs, both the species and concentration of ZnO-NPs to be applied must be considered.

The zinc content in the shoots pertaining to nanopriming 3 h treatments increased (0.39 mg/g) at a concentration of 50 ppm (figure 3), whereas nanopriming 3 h + hydroprimig 24 h did not show statistical differences at different concentrations. As for nanopriming 24 h, an increase in zinc content was observed, as the concentration of NPs was higher. As for zinc content in roots from nanopriming 24 h treatments, it was observed that this element's content increased in treatments with a higher concentration of NPs (figure 4). In the treatments with nanopriming 3 h + hydroprimig 24 h, the highest zinc content found in the root (0.39 mg/g) occurred when a concentration of 50 ppm was used. In the treatments with nanopriming 24 h, zinc content increased when using concentrations as low as 100 ppm, although a concentration of 200 ppm resulted in the highest content of this nutrient (0.97 mg/g).

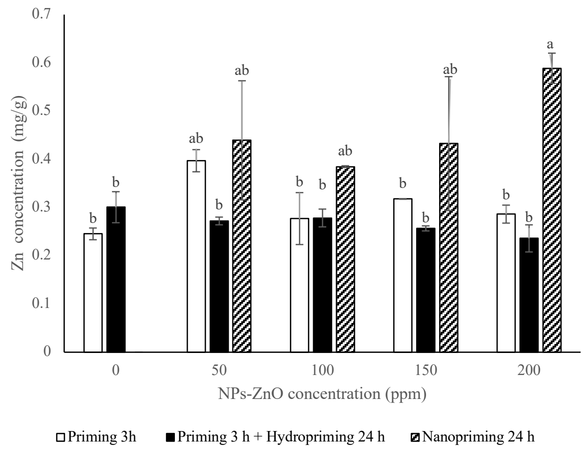

Figure 3. Effect on Zn content when using different priming treatments and ZnO-NPs concentrations in olotillo native maize shoots.

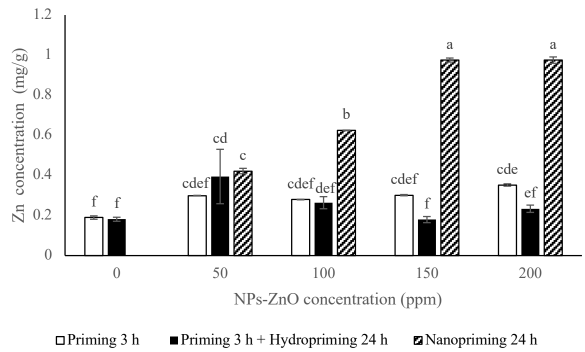

Figure 4. Effect on Zn content when using different priming treatments and different ZnO-NPs concentrations in olotillo native maize roots.

ZnO-NPs have already been used in Cherry tomatoes (*Solanum lycopersicum* L.) evaluating the effect of foliar and soil applications of a mixture of ZnO-NP and *Moringa oleifera* extract at three concentrations (25, 50 and 100 ppm), direct application to the soil showed better results at a concentration of 25 ppm, since it allowed a greater number of flowers and fruits to develop on the plant. On the other hand, it was shown that, when applied foliarly, the fruit contained greater amounts of Mg, Ca and Na, indicating that ZnO-NPs can increase some nutrients in this crop (Gutiérrez *et al*., 2021). Velázquez-Gamboa *et al*., (2021) used ZnO-NPs for the agronomic biofortification of *Stevia rebaudiana*, where no negative effects were observed on physical and morphometric parameters. In addition, the application of 75 ppm improved the nutritional quality of the crop, and it is possible that its pharmaceutical effects were also potentiated. The zinc content in olotillo native corn shoots and roots increased significantly with nanopriming treatments (150 and 200 ppm), regarding the treatments Nanopriming 3 h and hydropriming. Therefore, this pretreatment could be an alternative that allows an increase in the content of this nutrient in this crop. However, more studies are needed to verify that these NPs could be used to biofortify the corn crop. The effect of ZnO-NPs has been evaluated in various plant species, where it has been observed that the responses stimulate germination, shoot and root elongation, number of fruits and nutritional quality of the fruit. These effects have been observed in a wide range of concentrations (25 ppm to 200 ppm), indicating that the beneficial effect of zinc NPs depends on three factors: concentration of NPs, application mode, and crop.

Conclusions

The results in this study suggest that ZnO-NPs could potentially be used as stimulators of germination and vigor in native maize from the olotillo race at concentrations between 50 and 100 ppm. In addition, the potential use of ZnO-NPs at high concentrations (150-200 ppm) for biofortification of shoots could be considered.

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Nomenclature

ICP - OES	Inductively Coupled Plasma Optical Emission Spectrometry
NPs	Nanoparticles
ZnO-NPs	Zinc oxide nanoparticles
SVI	Seed vigor index
Zn	Zinc
ZnO	Zinc oxide

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