Original Research Article

Assessment Various Concentrations of ZnO Nanoparticles On Micropropagation for *Chenopodium quinoa* Willd. Plant

Abstract:

Assessment influences various concentrations of ZnO-Nanoparticles (ZnO-NPs) on *Chenopodium quinoa* Willd. Micro-propagation by cotyledonary nodes explants was achieved. We used different concentrations of ZnO-NPs (0.2, 2, 10, 20 mg/l) and used the medium free from ZnO-NPs as control. The results indicated that the presence ZnO-NPs in medium was good effect on germination rate of quinoa seeds at 2 mg/l concentration, and we noticed a density in roots hair and number or length roots of seedling. In addition, the highest responding of explant to ZnO-NPs 93.33, 80.8 % were in MS that supplemented 0.0, 2.0 mg/l respectively. Maximum numbers of roots 4.0 were also observed in MS containing 0.0, 2.0 mg/l. Although, there was positive clearly effect on number leaves of shoots, but there was a sudden decline (from 8.43 to 1.0) occurred by increasing ZnO-NPs concentrations from 2.0 to 20.0 mg/L. However, the ZnO-NPs do not effect on length of shoots, where the lengthiest shoot occurred in MS without ZnO-NPs. Regarding rooting shoots, there was significant effect of ZnO-NPs on both percentage of root and root number, where percentage of root reached to 100% in 10.0 mg/l concentration and roots number was 4.80 roots in the same concentration.

Keywords: Nanotechnology, Quinoa plant, Micropropagation, ZnO Nanoparticles, Plant Tissue Culture, Germination rate.

Abbreviations: **NPs** (Nanoparticles), **ZnO-NPs** (ZnO Nanoparticles), **NaOCl** (Sodium Hypochlorite), **MS** (Murashige and Skoog medium). **GR** (Germination rates**), ROS** (reactive oxygen species)**.**

Introduction: Agriculture forms the backbone of the economy of most countries and is considered the fundamental contributor to their overall growth, industrialization, and modernization. Agriculture has made remarkable advances over the past decades, but climate change, increasing global food demand and agrochemicals demand novel and sustainable agricultural practices to improve crop yield and quality. Various strategies using nanotechnology have been explored widely to provide solutions. However, their application in the food industry is constrained by limited understanding of nanomaterial safety [1-2].

Nanotechnology, a new emerging and fascinating field of science, and in recent year's remarkable progress has been made in developing nanotechnology. This science has emerged into the limelight and become the focus of the most countries and it will enable us to get the materials which are characterized by high quality, purity and free of impurities. The growth of nanotechnology has led to the rapid development of commercial application which involves the use of a great variety of manufactured nanoparticles and which have ability to revolutionize the agriculture and food industry. Thereby, there is a crucial urgency to perform further studies on the use of nanoparticles in the agricultural field [3-7].

The term nanotechnology comes from the combination of two words: the Greek numerical prefix nano referring to a billionth and the word technology. As an outcome, Nanotechnology or Nanoscaled Technology is generally considered to be at a size below 100 nm (a nanometer is one billionth of a meter, 10^{-9} m). Nanoscale science (or nanoscience) studies the phenomena, properties, and responses of materials at atomic, molecular, and macromolecular scales, and in generalat sizes between 1 and 100 nm. In this scale, and especially below 5nm, the properties of matter different significantly from that at a larger particulate scale [8-9].

On the other hand, Nanoparticles (NPs) that ranging in size from 1 to 100 nm possess specific physico-chemical properties attributed to smaller size, large surface area and high reactivity compared to their bulk counterparts [10]. The path of nanoparticles synthesis and their relative size and structure plays pivotal role in exhibiting the biological properties of nanoparticles [11]. The interaction of nanoparticles with the biological system is of large importance, and nowadays researchers are trying to find the potential effects of various kinds of nanoparticles in plants, animals and humans [12]. Therefore, NPs can serve as “magic bullets”, containing herbicides, Nano-pesticide, fertilizers, or genes, which target specific cellular organelles in plant to release their content [13].

Zinc oxide (ZnO) one of the most used Nano-products, are used in food packaging and drugs due to their superior antimicrobial efficacy [14-15]. ZnO NPs are also used in sun-protective lotions, wall paints, ceramic manufactures, or sporting goods [16-17]. ZnO-NPs plays active role in regulating various mechanisms involved in response to abiotic stresses in plants. It has been found that zinc has an important role in the management of reactive oxygen species (ROS) and protection of plant cells against oxidative stresses [18]. According to [19], ZnO Nanoparticles considered environment-friendly and hence widely used in biological applications.

The plant tissue culture has a great impact on both agriculture and industry, through providing plants needed to meet the ever increasing world demand. It has made significant contributions to the advancement of agricultural sciences in recent times and today they constitute an indispensable tool in modern agriculture [20]. Plant tissue culture has contributed enormously to agricultural biotechnology. For example, many plants grown from seed show considerable variation in growth, flower characteristics, yield, disease resistance, resistance to environmental stress, and so forth [21-22]. It would therefore be valuable to select those that possess desirable characters for vegetative multiplication [23].

Chenopodium quinoa Willd., belonging to the C_3 group of plants and it is an annual herbaceous, dicotyledonous crop species and referred as a pseudo-cereal plant of the family Chenopodiaceae, but since 2009, phylogenetic classification (APG III) ranks quinoa in the family Amaranthaceae [24] . high-nutrition content for quinoa makes it an ideal candidate for supporting growing populations such as Africa and Asia where quinoa contains: 55.3 % carbohydrates, 12.4 % lipids, and 11.7 % proteins [25]. In 2013 the United Nations Assembly declares "International Year of Quinoa", aware Quinoa is important, where contains all the main amino acids and several important trace elements and vitamins needed for human life [26].

According to our knowledge, the effect of ZnO Nano-particles on different stages of quinoa micro propagation, such as shoot multiplication, shoot elongation, and rooting formation as well as their

phytotoxicity is not well documented. **Consequently, the main aim** of the present study is to observe the potential effects of ZnO Nano-particles on developed quinoa micro-propagation.

2. Materials and Methods:

2.1. Plant material and culture media conditions: The quinoa (***Chenopodium quinoa* Willd.)** seeds were collected from Living Now Company from **USA** and sterilized through dipped into 70% ethanol for 15 second, then washing its by sterile distilled water for many times to get rid of alcohol residue. After that, seeds were surface sterilized for 20 min. in 10% sodium hypochlorite NaOCl, then rinsing for its multiple times with sterilized distilled water, (this was done under a laminar flow hood), the culture media which used in all experiments were MS (Murashige and Skoog medium) [27]. Before media autoclaving at 121°C for 20 min, pH adjusted to 5.6 by NaOH (1N) or HCl (1N) with adding 30 g/l sucrose and solidified with 6 g/l agar.

2.2. Preparation of ZnO-NP suspension: ZnO Nanoparticles (average particle size 35-45 nm) were purchased from US Research Nano-materials, Ink Company, USA. 0.15 g of solid ZnO-NPs was dissolved in 100 ml distilled water, and a a magnetic stirrer was used to homogenize the solution at 40 kHz for 30 min. For avoid aggregation of the particles, small magnetic stirrer bar was placed in the suspensions which was automatically stirred thoroughly before use. Then, nanoparticle suspensions was filtered by (0.22 µm glass filter) prior to being added to culture media. Different concentrations of ZnO-NPs (0.2, 2, 10, 20 mg prepared and the media without ZnO nanoparticles used as control method modified by Helaly *et al*. [28].

2.3. *In vitro*: Seed Germination: For germinations, the sterilized seeds were transferred to petri dishes containing 20 ml media (10 seeds per petri dish). The cultures were incubated in growth chamber under light (12hr/ day) condition where the fluorescent light intensity was 1000 Lux and temperature was maintained at 25±2 °C. Then, Germination rates **(%) (GR %)** germination rate was calculated by the following formulae after 15 days:

Germination rates **% = (Number of Germinated Seeds/ Total Number of Seeds) x 100……...…. (1)**

2.4. Plant regeneration: After 15 days- old seedling, (which grown in ZnO-NPs medium) the shoot tip and radical were excised and cotyledonary nodes (0.5-10 mm.) which were used as explant in all experiments. After that, explants were cultured in bottles containing around 25 ml from MS sold media (free ZnO-NPs) with adding 10 g/l sucrose and solidified with 6 g/l agar. All treatments were transferred to growth chamber for 4 weeks under standard culture conditions the photoperiod light (12hr/day), the fluorescent light intensity was 1000 Lux and temperature was maintained at 25±2^{o}C.

After 4 weeks of culture, the following parameters were recorded:

Explants responding (%) = (No. Of Adventitious Buds from Explants /Total No. of Explants) × 100…...(2)
Number of Shoots (No.) = Total No. of Shoots for all Replica / No. of Replica ……... (3)
Shoot Length (cm) = Total length of shoots for all Replica / No. of Replica …… (4)
Number of leaves (No.) = Total No. of leaves for all Replica / No. of Replica ……..... (5)

2.5. Rooting and acclimatization: The shoots which obtained from the multiplication stage were transplanted to tubes contained liquid media contained half-strength medium (½ MS) for induce roots formation. Data Recorded for rooting percentage and number of roots after 4 weeks from culture. Collected Healthy plantlets from root induction medium and washed with sterile distilled water to remove

all the adherent traces of media. Then, all the healthy plantlets transferred to Vermokliet soil, watered regularly and covered with plastic bags. After two weeks around, the covers were removed gradually to harden the plantlets. Then, the acclimatized plants were shifted to a green-house for completing their developed.

2.6. Statistical Analysis: Statistical analyses were fed to the computer and analyzed using **IBM SPSS** software package version 20.0. **(Armonk, NY: IBM Corp)**. Quantitative data were described using mean and standard error. Significance of the obtained results was judged at the 5% level. The used tests were ***F-test*** (*ANOVA*) for normally distributed quantitative variables, to compare between more than two groups, and **Post Hoc test (*LSD*)** for pairwise comparisons [29].

3. Results and Discussion:

3.1. Influence of ZnO Nanoparticles on Germination Rate (%):

The results of current study showed that the role of ZnO Nanoparticles concentrations (0.2, 2.0, 10.0 & 20.0 mg./l) played an increasing in the germination rate of quinoa plant up to a certain level (2.0 mg/l ZnO nanoparticles), but with increased ZnO Nanoparticles concentration (20.0 mg/l) maybe cause toxicity as shown in **Figure (1).** The highest germination rate (22.0%) was in MS medium supplemented with 2.0 mg/l of ZnO Nanoparticles, while, the lowest germination rate (12.0%) occurred in MS medium containing 20.0 mg/l of ZnO Nanoparticles. This means that increasing concentration ZnO-NP in medium could be led to toxicity for germination of quinoa seeds, this agrees with what found by Zafar et.al. [30] they found the highest concentrations (500 to 1500 mg/l) of ZnO-NP in medium adversely affects seed germination and seedling growth of *Brassica nigra* and also lead to an increase in the antioxidative activities and non-enzymatic antioxidants. ZnO Nanoparticles improve the germination and seedling vigor of a wide of crops such as tomato [31], Cucumis sativus [32], groundnut [33]. Senthilkumar and Sridhar [31-34] they observed that the metal oxide nano-particles are efficient enough to improve the germination of aged seeds of black gram and tomato up to 30 percent. This may be due to overcome of reactive oxygen species (ROS) generated during seed storage. Both positive and negative effects was showed when some higher plants were treats with of several Nano Particles TiO2, ZnO, Mg, Al, Pd, Cu, Si, C60 fullerenes, and multiwall carbon nanotubes [7].

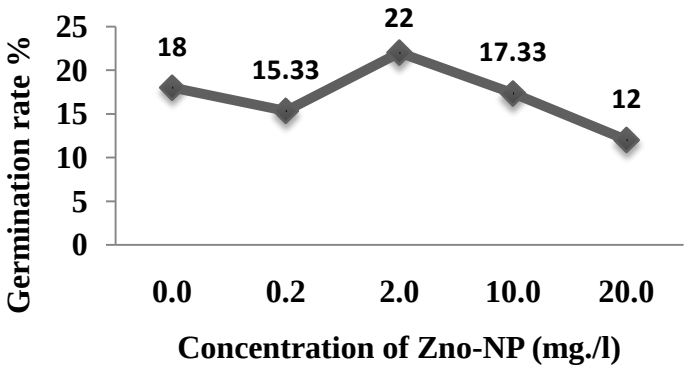

Figure (1): The Influence of ZnO-NPs (0.0, 0.2, 2.0, 10.0 & 20.0 mg/l) Concentrations on Seeds Germination Rates (%).

On the other hand, we noticed density in roots hair and number or length roots of seedling, also, increasing of shoot length in medium supplemented with 0.2, 2, 10 mg/l of Zn-NPs **Figure (2).** The our results agree with the results obtained by Raja et al. [35] they that recorded maximum germination, root length, shoot length and seedling vigor in seed that treats by Zn-NPs when compared to untreated seeds.

Figure (2): The Influence of ZnO-NPs (2.0 mg/l and 10.0 mg./l) Concentrations on Seeds Germination Rates (GR %).

3.2. Effect ZnO Nanoparticles on growth parameters:

Overall the data presented in **Table (1)** indicated that the responding explant (%) to ZnO-NPs concentrations was highly significantly ($P \leq 0.001$), maximum explant responding % 93.33, 80.8 were in 0.0 and 2.0 mg./l ZnO-NPs MS medium respectively, while the minimum responding explant (% 6.67) was obtained from explant that treated with 20.0 mg/l ZnO-NPs. Regarding shoot number, the data presented in Table (2) indicated that the deference between all ZnO-NPs treatments was highly significantly ($P \leq 0.001$), where, the highest number of shoot (4.0) occurred in MS medium containing 0.0 & 2.0 ZnO-NPs concentrations. Then, the shoot numbers began to decrease with increasing ZnO-NPs concentration until it reached the lowest number (0.20) at 20.0 mg/l of ZnO-NPs **Figure (3)**.

The results of current study could agree with [36] they found that the highest percentage of shoot regeneration (89.6%) was when nodal explants of *Stevia rebaudiana* were cultured on callus induction medium treated with 1mg/l ZnO NPs. On the other hand, [37, 38] found that low concentrations 10 mg/l, it stimulated the callus growth and indicated the nanoparticles role in decontamination, regeneration, organogenesis, callus formation and activated a protein that had a vital role in growth. However, that callus biomass decreased with increasing of ZnO NPs concentration, but with increasing concentration of ZnO-NPs in medium, the responding of explant decreased. Also, Callus proliferation was observed in all explants with all of the ZnO NPs treatments, but it reduces in high ZnO NPs concentration [39].

Table (1): Impact of different concentration of ZnO-NPs on Percentage of shoot responding %, Number of shoots, Shoots length (cm), Number of leaves (n=10)

ZNO-NPs Conc.	Shoot Responding %	Shoots Number (No.)	Shoot Length (cm)	Leaves Number (No.)
0	$93.33^{a} \pm 4.44$	$4.0^{a} \pm 0.0$	$2.33^{a} \pm 0.53$	$6.75^{a} \pm 1.28$
0.2 mg	$53.33^{c} \pm 5.44$	$2.20^{b} \pm 0.25$	$1.55^{a} \pm 0.40$	$7.93^{a} \pm 0.89$
2 mg	$80.0^{ab} \pm 5.44$	$4.0^{a} \pm 0.60$	$1.63^{a} \pm 0.17$	$8.43^{a} \pm 0.46$
10 mg	$60.0^{bc} \pm 12.96$	$2.0^{b} \pm 0.42$	$1.57^{a} \pm 0.41$	$8.33^{a} \pm 1.53$
20 mg	$6.67^{d} \pm 4.44$	$0.20^{c} \pm 0.13$	$0.10^{b} \pm 0.07$	$1.0^{b} \pm 0.67$
F	20.584^{*}	20.641^{*}	5.145^{*}	9.085^{*}
p	$<0.001^{*}$	$<0.001^{*}$	0.002^{*}	$<0.001^{*}$
LSD 5%	20.802	0.9976	1.023	2.967

Data was expressed using Mean ± SE. Means with **Common letters** are not significant (i.e. Means with **Different letters** are significant) *: Statistically *LSD* at $p \leq 0.05$

On the other hand, from the Table (1) we noticed that there are difference significantly between treatments on shoot length, but do not effect ZnO-NPs on shoot length, where the longest shoot 2.33 cm was in MS medium (0.0 mg/l ZnO-NPs), while the shortest shoot (0.10 cm) was in medium that containing 20.0 mg/l ZnO-NPs. In addition, although there was positive clearly effect on number of leaves but there was a sudden decline in numbers (8.43-1.0) occurred by increasing ZnO-NP concentration from (20.0 - 20 mg/L).

In generally, the mechanism that causes increase of growth with increasing concentration nanoparticles then a drop occurs in the growth and metabolism after reaching a certain threshold might involve the abundance of reactive oxygen species (ROS) and free radicals of Zn^{+1} ions. This ROS and free radicals produced by ZnO-NPs are internalized into the plant cell wall, cell membrane, cytoplasm, and nucleus. The translocation of ROS into the plant cells cause their destruction that occurs by the degradation of DNA and mitochondrial membranes [40-42]. Also, the denaturation of proteins and peroxidation lipids occur of cell wall leading to mutagenesis. Consequently, the inflammatory signaling cascades are activated, causing genotoxicity. Hence, the growth parameters and secondary metabolites show a fall [43].

3.3. Rooting formation and acclimatization:

Overall, the data presented in **Table (2)** showed that the highly significantly ($P \leq 0.001$) between all ZnO-NPs treatments and rooting formation and root numbers, whereas a positive effect of ZnO-NPs was most clearly on root formation (%) and number of root in both concentration (2.0 & 10.0 mg./l ZNO-NPs), while the other both concentration (0.2, 20.0 mg/l. ZnO-NPs). The best concentrations for root formation (100, 80 %) were at 10, 2 mg/l. ZnO–NPs respectively. In addition, the maximum number of root (~ 5) was in explants that treated with 10.0 mg/l of ZnO-NPs, while no rooting formation tack place in explants that treated with 20.0 mg/l of ZnO-NPs.

According to [44-47] they reported that can be explained induction of roots in two ways: (i) function of zinc in biochemical process, and (ii) role of ROS. The acidic nature of MS medium could increase dissolution of ZnO-NPs into zinc ion. Zinc ion may plays role as cofactor for several enzymes such as oxidases, dehydrogenases, a hydrases, and peroxidases, in regulating auxin synthesis, nitrogen metabolism, cell multiplication. Zinc itself regulated the synthesis of endogenous plant hormones; also presence auxin regulates local synthesis of cytokinin by controlling the expression of adenosine phosphate–isopentenyltransferase (PsIPT) gene, which encodes a key enzyme in cytokinin biosynthesis

Zinc itself regulated the synthesis of endogenous plant hormones, also presence auxin regulates local synthesis of cytokinin by controlling the expression of adenosine phosphate–isopentenyl transferase (PsIPT) gene, which encodes a key enzyme in cytokinin biosynthesis [48]. Furthermore, ROS generated in explants works as signaling molecule at non-toxic level, where it involved in induction of roots [49]. However, another aspect of ROS growth regulation involves superoxide, that affects root growth and root hair development through the regulation of calcium channels [50-51].

In contrast, the toxicity of NPs determinate by concentration of NPs and plant species, the studies that reported on phytotoxicity of ZnO-NP on plants are limited. Some plant species are sensitive toward ZnO-NP as rape, corn, lettuce, radish, ryegrass, cucumber [52], zucchini [53], garden cress, and broad bean [54], and wheat [55] are sensitive toward ZnO NP. Presence ZnO-NPs surrounding environment of plant effected on physiology and biochemistry of plant. The toxicity of ZnO-NPs due to accumulation it in root tissue and root surface and dissolution of zinc ions from NPs along with other physiochemical properties [56-57].

For acclimatization, the rooted shoots were removed from tubes and washed thoroughly to remove remnants of media and transplanted to small pots containing Vermokliet soil. Plants were covered to ensure high humidity**.** Thereafter, the plantlets were transferred to green house to follow their development as shown in **Figure (3).**

Table (2): Influence of ZnO-NPs Different Concentration on Roots Formation (%) and Number of Root (n = 10)

ZNO-NPs Conc.	**Root Formation (%)**	**Root number (No.)**
0	$60.0^{b} \pm 16.33$	$0.80^{bc} \pm 0.25$
0.2 mg	$20.0^{c} \pm 13.33$	$1.0^{bc} \pm 0.52$
2 mg	$80.0^{ab} \pm 13.33$	$1.60^{b} \pm 0.27$
10 mg	$100.0^{a} \pm 0.0$	$4.80^{a} \pm 0.74$
20 mg	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$
F	13.821^{*}	18.126^{*}
p	$<0.001^{*}$	$<0.001^{*}$
LSD 5%	31.775	1.242

Data was expressed using Mean ± SE. Means with **Common letters** are not significant (i.e. Means with **Different letters** are significant) *: Statistically *LSD* at $p \leq 0.05$

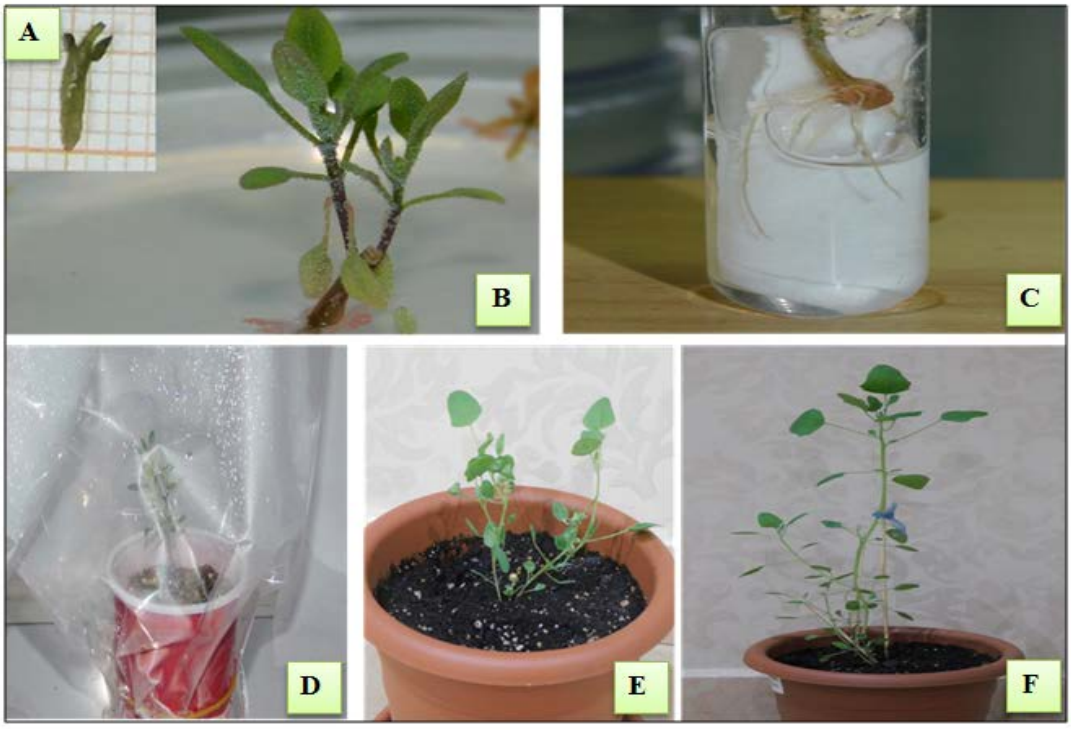

Figure (3): Micropropagation Stages of Quinoa Plant under Influence of ZnO-PNs Different Concentrations (**A)** Cotyledonary node explant **(B)** Multiplication shoots **(C)** Rooting shoots Stage **(D)** Acclimazation stage **(E) & (F)** Quinoa Plant at Green House**.**

Conclusion:

The results present form this study, concluded that the ZnO Nanoparticles could be positive effect on micrporpagation of quinoa plant through increasing germination rate, number of shoot and leaves it. When increased ZnO-NPs concentrations in medium up to 20 mg/l, the ZnO-NPs tends toward prevent plant growth. Regarding rooting formation stage, the positively effect of ZnO-NPs was clearly on both percentage and number of roots, where the percentage of roots reached to 100% at 10 mg/l ZnO-NPs concentration. Based on experimental, results we believe the concentrations of ZnO-NPs in range (2-10 mg/l) may a positive effect on micropropagtion of quinoa plant especially on rooting formation stage, but the concentrations of ZnO-NPs that higher than 10 mg/l had a negative effect on growth and developed plant.

References

[1] V. Mishra, R. K. Mishra, A. Dikshit, and A. C. Pandey, "Chapter 8 - Interactions of Nanoparticles with Plants: An Emerging Prospective in the Agriculture Industry," in *Emerging Technologies and Management of Crop Stress Tolerance*, P. Ahmad and S. Rasool, Eds. San Diego: Academic Press, 2014, pp. 159-180.

[2] S. Majumdar and A. A. Keller, "Omics to address the opportunities and challenges of nanotechnology in agriculture," *Critical Reviews in Environmental Science and Technology,* pp. 1-4, 2020.

[3] P .Ball, "Natural strategies for the molecular engineer," *Nanotechnol,* vol. 13, pp. 15-28, 2002.

[4] I. Carmen, P. Chithra, Q. Huang, P. Takhistov, S. Liu, and J. Kokini, "Nanotechnology: a new frontier in food science," *Food Technol* vol. 57, pp. 24–29 2003.

[5] R. Nair, S. H. Varghese, B. G. Nair, T. Maekawa, Y. Yoshida, and D. S. Kumar, "Nanoparticulate material delivery to plants," *Plant Science,* vol. 179, no. 3, pp. 154-163, 2010.

[6] M. Roco, "Broader societal issue of nanotechnology," *J. Nanopart. Res* ,.vol. 5, pp. 181-189, 2003.

[7] R. C. Monica and R. Cremonini, "Nanoparticles and higher plants," *Caryologia,* vol. 62, no. 2, pp. 161-165, 2009.

[8] B. Bhushan, *Handbook of Nanotechnology*. Berlin: Springer, 2004.

[9] K. D. Sattler, *Handbook of Nanophysics, Principles and Methods* New York: CRC, 2010.

[10] V. Yadav, " Nanotechnology, big things from a tiny world: a review," *AEEE* vol. 3, pp. 771-778, 2013.

[11] A. Kołodziejczak-Radzimska and T. Jesionowski, "Zinc oxidedfrom synthesis to application: a review. ," *Materials* vol. 7, pp. 2833-2881, 2014.

[12] J. Boczkowski and P. Hoet, " What's new in nanotoxicology? Implications for public health from a brief reviewof the 2008 literature," *Nanotoxicology,* vol. 4, pp. 1-14, 2009.

[13] H. S. Manzer, H. A.-W. Mohamed, F. Mohammad, and Y. A.-K. Mutahhar, "Role of Nanoparticles in Plants," in *Nanoparticles and Their Impact on Plants,* N. a. P. Sciences, Ed. Switzerland Springer 2015, pp. 19-35.

[14] R. Brayner, R. Ferrari-Iliou, N. Brivois, S. Djediat, M. Benedetti ,and F. Fievet, "Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium," *Nano Lett.,* vol. 6, pp. 866–870, 2006.

[15] N. Jones, B. Ray, K. Ranjit, and A. Manna, "Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms," *FEMS Microbiol Lett* vol. 279, pp. 71-76, 2008.

[16] Z. Fan and J. Lu, "Zinc oxide nanostructures: synthesis and properties," *J Nanosci Nanotechnol* vol. 5, pp. 1561–1573, 2005.

[17] P. Singh and A .Nanda, "Enhanced sun protection of nano-sized metal oxide particles over conventional metal oxide particles: an in vitro comparative study," *Int J Cosmet Sci,* vol. 36, pp. 273–283, 2014.

[18] N. Sheikh, G. Hassanzadeh, M. Baghestani, and B. Zabd, "Study the effect of zinc foliar application on the quantitative yield of grain maize under water stress," *Electro J Crop Prod.*, vol. 2, no. 2, pp. 59-74, 2009.

[19] A. K. Singh, V. Viswanath, and V. C. Janu, "Synthesis, effect of capping agents, structural, optical and photoluminescence properties of ZnO nanoparticles," *Journal of Luminescence*, vol. 129, no. 8, pp. 874-878, 2009.

[20] R. Garcia-Gonzales, K. Quiroz, B. Carrasco, and P. Caligari, "Plant tissue culture: Current status, opportunities and challenges ",*Cien. Inv. Agr.*, vol. 37, no. 3, pp. 5-30, 2010.

[21] A. Altman, "From plant tissue culture to biotechnology: Scientific revolutions, abiotic stress tolerance, and forestry," *In Vitro Cellular and Developmental Biology-Plant*, vol. Vol. 39, no. 2, pp. pp.75–84, 2003.

[22] T. A. Thorpe, "History of plant tissueculture," *Molecular Biotechnology*, vol. Vol. 37, pp. pp. 169-180, 2007.

[23] P. P. Kumar and C. S. Loh, "Plant tissue culture for biotechnology," in *Introduction to basic procedures in plant biotechnology* Singapore: National University of Singapore, 2012, pp. pp. 131-138.

[24] S. E. Jacobsen, A. Mujica, and C. R. Jensen, "The resistance of quinoa (Chenopodium quinoa Willd.) to adverse abiotic factors," *Food Reviews International*, vol. 19, pp. 99-10.2003 ,9

[25] D. Y. Gallego Villa, L. Russo, K. Kerbab, M. Landi, and L. Rastrelli, "Chemical and nutritional characterization of Chenopodium pallidicaule (cañihua) and Chenopodium quinoa (quinoa) seeds," *Emir. J. Food Agric.*,, vol. 26, no. 7, pp. 609-615.2014 ,

[26] FAO. (2013). *Quinoa: launch of the international year of Quinoa*. Available: http://www.fao.org/news/story/en/item/170254/icode/

[27] T. Murashige and F. Skoog, "A revised medium for rapid growth and bioassays with tobacco tissue cultures," *Physiologia Plantarum*, vol. 15, pp. 473–497, 1962.

[28] M. Helaly, M. El-Metwally, El-Hoseiny, H, S. Omar, and N. ElSheery, "Effect of nanoparticles on biological contamination of in vitro cultures and organogenic regeneration of banana," *Aust J Crop Sci* , .vol. 8, no. 4, pp. 612-624, 2014.

[29] L. A. Kirkpatrick and B. C. Feeney, *A simple guide to IBM SPSS statistics for version 20.0*. Belmont, Calif. : Wadsworth, Cengage Learning, 2013.

[30] H. Zafar, A. Ali, J. S. Ali, H. I. U., and M. Zia, "Effect of ZnO Nanoparticles on Brassica nigra Seedlings and Stem Explants: Growth Dynamics and Antioxidative Response," *Frontiers in Plant Science*, vol. 7, pp. 1-8 2016.

[31] C. Sridhar, "Effect of Nanoparticles for the Maintenance of Tomato Seed Vigour and Viability," M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore, 2012.

[32] L. Zhao *et al.*, "CeO2 and ZnO Nanoparticles Change the Nutritional Qualities of Cucumber (Cucumis sativus)," *J Agric Food Chem.*, vol. 62, no. 13, pp. 2752–2759, 2014.

[33] T. N .V. K. V. Prasad *et al.*, "Effect Of Nanoscale Zinc Oxide Particles On The Germination, Growth And Yield Of Peanut," *J. Plant Nut.* , vol. 35, pp. 905–927, 2012.

[34] S. Senthilkumar, "Customizing nanoparticles for the maintenance of seed vigour and viability in Blackgram (Vigna mungo) cv. VBN 4.," M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore,, 2011.

[35] K. Raja, R. Sowmya, R. Sudhagar, P. Sathya Moorthy, K. Govindaraju, and K. S. Subramanian, "Biogenic Zno And Cu Nanoparticles To Improve Seed Germination Quality In Blackgram (Vigna Mungo)," *Materials Letters* vol. 235, pp. 164–167, 2019.

[36] R. Javed, M. Usman, B. Yucesan, M. Zia, and E. Gurel, "Effect of zinc oxide (ZnO) nanoparticles on physiology and steviol glycosides production in micropropagated shoots of Stevia rebaudiana Bertoni," *Plant Physiol Biochem,* vol. 110, pp. 94-99, Jan 2017.

[37] S. M. Kouhi and M. Lahouti, "Application of ZnO Nanoparticles for Inducing Callus in Tissue Culture of Rapeseed. Int.," *J. Nanosci. Nanotechnol,* vol. 14, pp. 133-141, 2018.

[38] S. Kavianifar, K. Ghodrati, B. H. Naghdi, and A. Etminan, "Effects of Nano Elicitors on Callus Induction and Mucilage Production in Tissue Culture of Linum usitatissimum L.," *Journal of Medicinal Plants,* vol. 17, no. 67, pp.2018 ,54-45 .

[39] S. F. Desoukey, Z. K. Taha, H. M. El-Shabrawi, A. F. Desoukey, and A. Z. Sabh, "Biological Effects Of Zinc Oxide Nanoparticles On In Vitro Asparagus Officinalis L. Plant " *Plant Archives* vol. 19, pp. 2262-2268, 2019.

[40] O. Choi and Z .Hu, "Size Dependent and Reactive Oxygen Species Related Nanosilver Toxicity to Nitrifying Bacteria.," *Environ. Sci. Technol.*, vol. 42, pp. 4583–4588, 2008.

[41] R. Klaper, J. Crago, J. Barr, D. Arndt, K. Setyowati, and J. Chen, "Toxicity Biomarker Expression In Daphnids Exposed To Manufactured Nanoparticles: Changes In Toxicity With Functionalization," *Environ. Pollut.*, vol. 157, no. 4, pp. 1152–1156, 2009.

[42] P. Sharma, A. B. Jha, R. S. Dubey, and M. Pessarakli, "Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions," *J. Bot.* , pp. 1-26, 2012.

[43] A. Manke, L. Wang, and Y. Rojanasakul, "Mechanisms of Nanoparticle-Induced Oxidative Stress and Toxicity," *BioMed Research International,* vol ,2013 .pp. 1-15, 2013.

[44] E. J. Hewitt, "Perspective of mineral nutrition :essential and functional metals in plants, ," in *In Metals and Micronutrients: Uptake and Utilization by Plants,* D. A. Robb and W. S. Pierpoint, Eds. London, 1983, pp. 277–323.

[45] C. Tsui, "The role of zinc in auxin synthesis in tomato plants," *Am. J.Bot.* , vol. 35, pp. 172–179, 1948.

[46] J. J. Stanisławski, "Rola kwasu 3-indolilooctowego w procesach wzrostowych roslin [Role of IAA in plant growth]," *Wiad .Bot.*, vol. 21, pp.1977 ,59-43 .

[47] W. T. Shier, "Metals as toxins in plants.," *J. Toxicol. Toxin Rev.* , vol. 13, pp. 205–216, 1994.

[48] M. Tanaka, K. Takei, M. Kojima, H. Sakakibara, and H. Mori, "Auxin controls local cytokine in biosynthesis in the nodal stem in apical dominance," *Plant J.* , vol. 45, pp. 1028–1036, 2006.

[49] R. Mittler, S. Vanderauwera, M. Gollery, and F. Van Breusegem, "Reactive oxygen gene network of plants," *Trends Plant Sci.,* vol. 9, pp. 490–498, 2004.

[50] J. Foreman, V. Demidchik, and J. H. Bothwell, "Reactive oxygen species produced by NADPH oxidase regulate plant cell growth," *Nature,* vol. 422, pp. 442–446, 2003.

[51] M. A. Jones, M. J. Raymond, Z. Yang, and N. Smirnoff, "NADPH oxidase-dependent reactive oxygen species formation required for root hair growth depends on ROP GTPase," *J. Exp. Bot.,* vol. 58, pp. 1261–1270, 2007.

[52] D. Lin and B. Xing, "Phytotoxicity of nanoparticles: inhibition of seed germination and root growth," *Environmental Pollution,* vol. 150, pp. 243-250, 2007.

[53] D. Stampoulis, S. K. Sinha, and J. C. White, "Assay-dependent phytotoxicity of nanoparticles to plants," *Environ. Sci. Technol.,* vol. 43, pp. 9472–9479, 2009.

[54] S. Manzo *et al.*, "Investigation of ZnO nanoparticles' ecotoxicological effects towards different soil organisms," *Environ. Sci. Pollut. Res.* , vol. 18, pp. 756–763., 2011.

[55] W. Du, Y. Sun, R. Ji, J. Zhu, J. Wu, and H. Guo, "TiO_2 and ZnO nanoparticles negatively affect wheat growth and soil enzyme activities in agricultural soil," *J. Environ. Monit.,* vol. 18, pp. 756–763., 2011.

[56] H. Ma, P. M. Bertsch, T. C. Glenn, N. J. Kabengi, and P. L. Williams, "Toxicity of manufactured zinc oxide nanoparticles in the nematode Caenorhabditis elegans," *Environ. Toxicol. Chem.,* vol. 28, pp. 1324–1330, 2009.

[57] H. Ma, N. J. Kabengi, P. M. Bertsch, J. M. Unrine, T. C. Glenn, and P. L. Williams, "Comparative phototoxicity of nanoparticulate and bulk ZnO to a free-living nematode Caenorhabditis elegans: the importance of illumination mode and primary particle size," *Environ. Pollut.,* vol. 159, pp. 1473–1480, 2011.