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ORIGINAL ARTICLE

Effect of zinc nanoparticles on the growth and biofortification capability of mungbean (*Vigna radiata*) seedlings

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Abstract

Zinc insufficiency is a nutritional trouble worldwide, especially in developing countries. In the current study, an experiment was conducted to evaluate the effect of supplementation of MS media culture with different concentrations of ZnO nanoparticles (NPs) (0, 10, 20, 40, 80, and 160 ppm) on growth, nutrient uptake, and some physiological parameters of 7-days-old mung bean seedlings. ZnO NPs enhanced the Zn concentration of mung bean from 106.41 in control to more than 4600 µg/g dry weight in 80 and 160 ppm ZnO NPs treated seedlings. Our results showed that ZnO NPs in the concentration range from 10 to 20 ppm had a positive influence on growth parameters and photosynthetic pigments. Higher levels of ZnO NPs negatively affected seedling's growth by triggering oxidative stress which in turn caused enhancing antioxidative response in seedlings including polyphenol oxidase and peroxidase activity as well as phenolic compounds and anthocyanine contents. Considering the positive effects of ZnO NPs treatment on mungbean seedlings growth, micronutrents, protein and shoot phenolics content, 20 ppm is recommended as the optimal concentration for biofortification. Our findings confirm the capability of ZnO NPs in the remarkable increase of Zn content of mungbean seedlings which can be an efficient way for plant biofortification and dealing with environmental stress.

Keywords Antioxidative response · Biofortification · Mung bean · Nanoparticles · Oxidative stress · Phenolic compounds · Photosynthetic pigments · Zinc oxide

Introduction

It is now apparent that a nutritional deficiency of zinc in humans is widespread, affecting nearly 2 billion individuals worldwide (Prasad 2008). Zinc has a critical role in homeostasis, immune system function, oxidative stress, apoptosis, aging, and significant disorders of great public health interest are associated with zinc deficiency. Reports confirm that dietary supplements of zinc are efficient in the control and treatment of covid 19 (reviewed by Giacalone et al. 2021). Insufficient intake of zinc from food is a major contributor to zinc deficiency in humans which somehow is related to the low Zn content of agricultural soil (Tabrez et al. 2022). Hence there is an urgent need to increase the zinc content and bioavailability in food especially in developing and poor countries (Welch and Graham 2004; Zhao and McGrath 2009).

Crops biofortification offers a sustainable solution to reduce malnutrition and deal with human diseases. Use of nanotechnology to fortify the crops in human societies has received much attention in recent years (El-Ramady et al. 2021; Khan et al. 2021a, b). Crops nano-biofortification could be achieved by seed priming (Rizwan et al. 2019), soil and foliar application (Du et al. 2019; Semida et al. 2021), and cultivation of plants in media rich of candidate nutrient using nanomaterials. In this regard, various aspects should be considered including: (i) finding the appropriate type and shape of nanomaterials, (ii) determination of optimal dose (without negative effects on plant growth and physiology), and (iii) investigation of probable negative or positive effect on the absorption and accumulation of other nutrients.

Mung bean seeds are used up by humans and its hay is used for animal feedstuff that its products can be back

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to humans through meat, milk, and dairy products. About 6 million-hectare area of the world is under mung bean cultivation (8.5% of the world's pulse area). Biofortification of crops like mung bean (*Vigna radiate* (L.) R. Wilczek) which contains carbohydrates, proteins, calcium, β-carotene, iron, and a lot of micronutrients can be a suitable strategy, especially for poor communities to cope with a nutritional deficiency. Haider et al. (2021) and Rani et al. (2022) confirmed the capability of using ZnO NPs for the biofortification of mung beans in the field and hydroponic greenhouse environments. These works have focused on zinc biofortification and failed to address the effect of ZnO NPs treatments on other nutrients uptake. Additionally, more precise studies are required to clarify the effect of ZnO NPs on plant physiology and to find the optimal dose for zinc biofortification.

Mung bean sprouts are now part of the diet of people in many countries as an appetizer and salad. One of the objectives of the current study was to evaluate the capability of the ZnO NPs in mung bean sprout biofortification, performance, and nutrient quality.

Like humans, zinc is crucial for the enzyme functioning in plants and it is very important for plant growth and development. Zn deficiency in plants is one of the major concerns globally. In recent years, the West Asia region has been severely affected by the effects of climate change, such as successive droughts, the expansion of salinized land and dust storms, which have affected agricultural production. Many reports confirm Zn supplementation could alleviate the negative effects of abiotic stresses on plants (Khan et al. 2004; Hussein and Abou-Baker 2018; Thounaojam et al. 2012; Sattar et al. 2021). Micronutrients nanoparticles because of their small size and high surface-to-volume ratio are considered a good candidate for plant uptake and biofortification. The application of appropriate concentrations of zinc oxide nanoparticles (ZnO NPs) with positive effects on plant physiology can improve the growth and protection of different plant species (Mukherjee et al. 2016; Subbaiah et al. 2016; Venkatachalam et al. 2017). A high concentration of ZnO NPs can cause phytotoxicity in plants (Salah et al. 2015; Wang et al. 2016) and produce oxidative stress, which is common in plants in response to environmental stresses. To cope with oxidative stress, plants increase their enzymatic and non-enzymatic anti-oxidants content which helps hinder reactive oxygen species. Some of those non-enzymatic antioxidants are secondary metabolites like phenolic compounds which in turn have nutritional value for humans as antioxidants.

Zinc NPs could be a potential high-performed fertilizer for enhancing plant yield and quality (Du et al. 2019). Accordingly, another aspect of the present study was to investigate the effect of ZnO NPs on the growth and physiology of mung bean seedlings. In the current study, we investigated the effects of different concentrations of ZnO NPs on seedling growth, biochemistry, and physiological response of *V. radiate*.

Materials and methods

Plant material and culture conditions

Seeds of mung bean (*V. radiata*) were surface sterilized in 1% (v/v) sodium hypochlorite solution for 15 min, followed by three washes with sterile distilled water. Ten sterilized seeds were placed into Petri dishes containing 10 ml of MS basal medium (Murashige and Skoog 1962), 7% agarose, pH = 5.7 and 0, 10, 20, 40, 80, and 160 ppm of ZnO NPs (10–30 nm, nearly spherical, US-NANO). The stock water suspension of ZnO NPs, before application in media culture, was placed for 30 min in the Elmasonic S80(H) Ultrasonic Cleaner (37 kHz of ultrasonic frequency, of 150 W of effective ultrasonic power) (Elma Schmidbauer GmbH, Singen, Germany) to achieve better dispersion of particles. Petri dishes were placed in a tissue culture room at a temperature of 25 °C, relative humidity of 55%, and a 16-h photoperiod with a light intensity of 2300 lx. Seedlings obtained after 7 days of growth were used for experiments. The biometrical data of 7-days-old seedlings were collected as mean values of the shoot and root fresh and dry weight (mg) and length (cm). For physiological and biochemical assays 7 days old seedlings were frozen in liquid nitrogen and kept at -70 °C and used for the experiments.

Multielement analysis

0.5 g of powdered freeze-dried mung bean shoot samples was placed in polyethylene tube with 8 ml of nitric acid (65%) for 12 h at room temperature. Then 2 ml perchloric acid was added to the samples and kept at 80 °C for 1 h and 150 °C for 3 h. The solution was filtered using Whatman No. 42 filter paper and diluted to 25 mL with ultrapure water and stored in the dark before analysis. ICP-MS (HP-4500, USA) equipped with an Asx-520 auto-sampler was used to define elements concentration.

Pigment contents

The total contents of carotenoids, chlorophylls a and b were measured according to the procedure described by Linchenthaler and Wellburn (1983). Briefly, fresh samples (0.2 g) were homogenized in 80% acetone (Merck, Germany) and the absorbance was recorded spectrophotometrically at 470, 646, and 663 nm. The results were calculated based on the following equations:

$$\text{Chla } (\mu g/ml) = 12.26A_{663} - 2.79A_{646}$$
$$\text{Chlb } (\mu g/ml) = 21.50A_{646} - 5.10A_{663}$$
$$\text{Chl Total } (\mu g/ml) = \text{Chla} + \text{Chlb}$$
$$\text{Carotenoids } (\mu g/ml) = (1000A_{470} - 3.27\ \text{Chla} - 104\ \text{Chlb})/229$$

Anthocyanin content was extracted using 1% HCl v/v acidified methanol. Fresh samples were homogenized in the extraction solution, centrifuged at 18 000 × g at 4 °C for 15 min, and stored in darkness for 5 h at 5 °C. The amount of anthocyanin was quantified at 550 nm spectrophotometrically (Abdel Latef et al. 2020).

Malondialdehyde and hydrogen peroxide content

Malondialdehyde (MDA) content was determined based on Heath and Packer (1968). Fresh tissues were extracted with 0.1% TCA, and 0.5 ml of the supernatant was mixed with 1 ml of thiobarbituric acid (0.5%) in TCA (20%). After heating the solution at 95 °C for 25 min, the absorbance was read spectrophotometrically at 532 and 600 nm. The amount of MDA was calculated by an extinction coefficient of 155 $mM^{-1}\ cm^{-1}$.

For H_2O_2 quantification, the 0.5 ml of extract (0.1% TCA) was mixed with 0.5 ml potassium phosphate buffer (10 mM, pH 7.0) and 1 ml KI (1 M). The absorbance was measured at 390 nm based on the Velikova et al. (2000) method.

Protein content and antioxidant enzyme activity

0.2 g of fresh samples was ground in 1 M Tris-HCl (pH 6.8) at 4 °C. The supernatant was separated at 12 000 × g centrifugation for 15 min and used for protein and enzyme assays. Protein content was quantified with bovine serum albumin as the standard (Bradford 1976).

Antioxidant enzyme assay

For measurement of peroxidase (POX) activity, 50 µl of the extract was mixed with 0.1 ml benzidine, 0.2 ml H_2O_2 (3%), and 0.2 M acetate buffer (pH 4.8), and the activity was defined at 530 nm (Abeles and Biles 1991).

Polyphenol oxidase (PPO; E.C. 1.14.18.1) activity was measured based on the method described by Raymond et al. (1993). The reaction mixture contained 0.2 ml of pyrogallol (20 mM), 2.5 ml of 200 mM sodium phosphate buffer (pH 6.8), and 50 µl of enzyme extract. The enzyme activity was recorded at 430 nm.

Total phenolic compounds content

For extraction, 0.4 g of dried powder of samples was placed in 80% (v/v) methanol for 48 h and then was put in an ultrasonic bath for 20 min. After centrifugation (10 min at 11 000 × g), the supernatant was utilized for the detection of total phenolic compounds.

For determination of total phenolic content, 100 µl of the extract was mixed with 500 µl of Folin-Ciocalteu reagent and 400 µl of sodium carbonate. Then, the reaction solution was kept for 30 min at room temperature. The absorbance was read spectrophotometrically at 630 nm (Singleton and Rossi 1965).

Statistical analyses

The experiment was set up in a completely randomized design. For experimental treatment, 10 repetitions were used with 10 seeds each (1 Petri dish). For biometrical analysis at least 20 plants were assayed in each treatment. For physiological and biochemical assays 25 seedlings were pooled and used as one replication of triplicate.

Each data point was an average of three or five replicates. Presented data were analyzed by one way ANOVA (analysis of variance) using SPSS (version 21). The significance of differences was determined according to Duncan's multiple range tests at the 0.05 level of probability. The graphs were designed with Graphpad Prism version 6 and Microsoft Excel version 2010. Principal component analysis (PCA) and Pearson correlation test were conducted using publicly available Past3.16 software.

Results

Zn content significantly increased with the increase of ZnO NPs concentration in media culture, on average, from 106.41 in control to more than 4 600 µg/g dry weight in 80 and 160 ppm ZnO NPs treated seedlings. No significant difference in Zn content was observed in the samples treated with 10 and those treated with 20 ppm ZnO NPs. Interestingly, with the exception of Zinc, the lowest concentrations of the all assayed elements were observed in 10 ppm ZnO NPs treated samples. On the other hand, the highest levels of calcium, magnesium, phosphorus, manganese, iron, cobalt, copper, and molybdenum were observed in the 40 ppm ZnO NPs treated samples (Table 1). Although none of the evaluated elements showed a positive or negative correlation with zinc, a strong correlation (≥ 90%) between K with Mg and P, Mg with P, Fe and Cu, P with K, Mn with Fe and Co, Fe with P, and Mn and Co were observed (Supplementary Fig. 1).

Seedling growth ZnO NPs treatment induced changes in the growth biomarkers of *V. radiata* seedlings (Fig. 1). The use of ZnO NPs in the concentration range from 10 to 20 ppm had a positive influence on growth. The fresh and dry weight of shoot and root increased up to 20 ppm and then decreased at 40, 80, and 160 ppm. Shoot and root length also

Table 1 Nutrient content (µg/g dry weight) in shoot of mung bean (*V. radiata*) seedlings subjected to 0 (control), 10, 20, 40, 80 and 160 ppm zinc oxide nanaoparticles

	Control	10	20	40	80	160
K	30504.01 ± 110.12e	26343.34 ± 80.39f	36993.42 ± 98.70a	36682.3 ± 160.04b	34903.01 ± 140.23c	33015.55 ± 90.04d
P	7776.04 ± 38.39e	6253.08 ± 42.45f	9463.62 ± 56.14c	10912.7 ± 105.14a	10596.2 ± 89.19b	9364.67 ± 43.12d
Ca	2899.2 ± 50.10d	2365.56 ± 25.09e	2966.73 ± 60.15c	3821.63 ± 34.56a	3645.18 ± 17.20b	3807.94 ± 80.78a
Mg	1754.26 ± 40.04d	1353.49 ± 22.15e	1840.01 ± 13.18c	2075.26 ± 35.19a	1940.8 ± 28.40b	1815.57 ± 43.89c
Na	792.88 ± 20.40d	686.38 ± 12.81e	1022.61 ± 30.08b	1014.43 ± 14.20b	926.45 ± 16.04c	1298.53 ± 22.31a
Mn	430.56 ± 14.06d	250.55 ± 8.19f	390.77 ± 20.86c	573.72 ± 19.18a	501.23 ± 31.12b	312.95 ± 25.57e
Fe	154.58 ± 10.63b	114.66 ± 19.02c	158.89 ± 21.74b	206.42 ± 13.94a	183.71 ± 8.11a	153.70 ± 9.12b
Zn	106.41 ± 15.01e	815.66 ± 24.12d	867.34 ± 37.67d	2665.91 ± 84.88c	4745.59 ± 30.15a	4644 ± 50.04b
Cu	33.91 ± 4.10a	7.52 ± 1.03c	29.91 ± 6.01b	34.49 ± 2.19a	30.84 ± 4.90a	26.66 ± 3.02b
Mo	57.83 ± 11.02c	30.24 ± 4.20e	34.88 ± 5.09e	182.45 ± 8.15a	129.75 ± 7.50b	44.36 ± 3.50d
Co	0.35 ± 0.01d	0.26 ± 0.01f	0.40 ± 0.09c	0.54 ± 0.08a	0.45 ± 0.05b	0.32 ± 0.03e

Different letters indicate means that are significantly different at ($P \leq 0.05$)

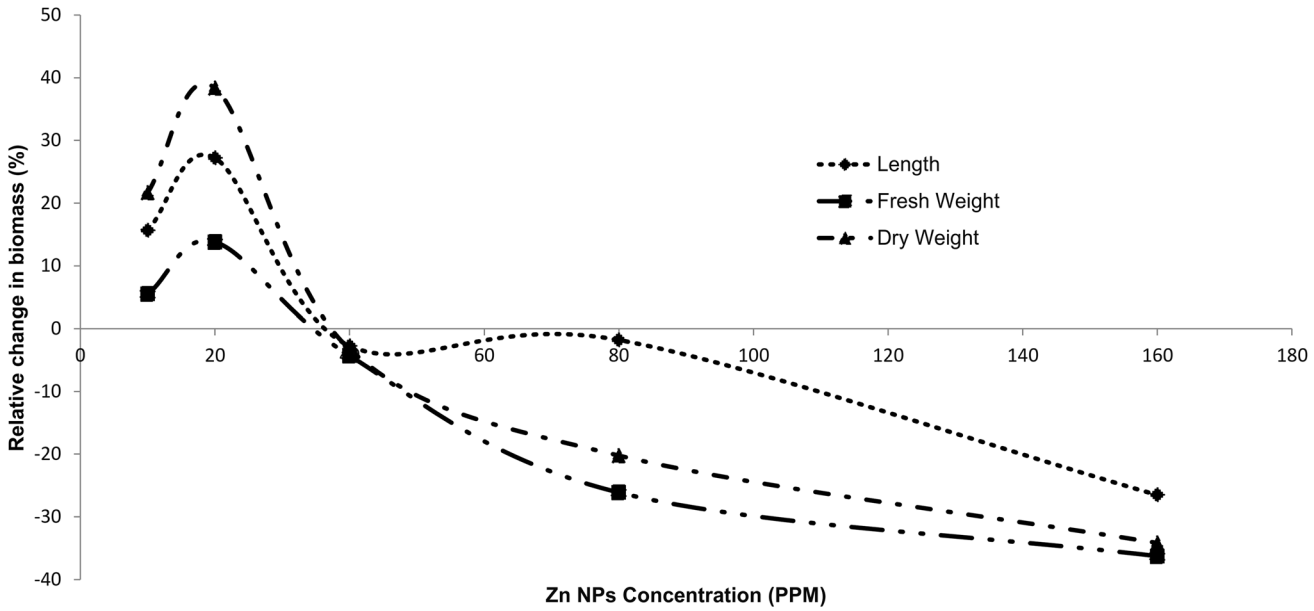

Fig. 1 Relative changes in biomass from control of the 7-day-old mung bean (*V. radiata*) seedlings in response to 10, 20, 40, 80 and 160 ppm zinc oxide nanaoparticles

increased significantly following ZnO NPs treatment up to 20 ppm, while the length of seedlings was negatively affected at higher concentrations. In summary, the highest and lowest rate of seedling biomass including plant height as well as fresh and dry weight was observed in 20 ppm and 160 ZnO NPs treated samples respectively (Supplementary Fig. 2).

Photosynthetic pigments Chl a, Chl b, Chl T and carotenoids content significantly increased ($P \leq 0.05$) up to 20 ppm ZnO NPs and then markedly reduced with the increase of ZnO concentrations in media culture (Fig. 2).

Protein content The shoot soluble protein content was observed to be the highest at 40 to160 ZnO NPs treated samples (Fig. 3). In the root, total soluble protein content significantly increased up to 40 ppm then significantly decreased under higher concentrations (80 and 160 ppm) of ZnO NPs ($P \leq 0.05$).

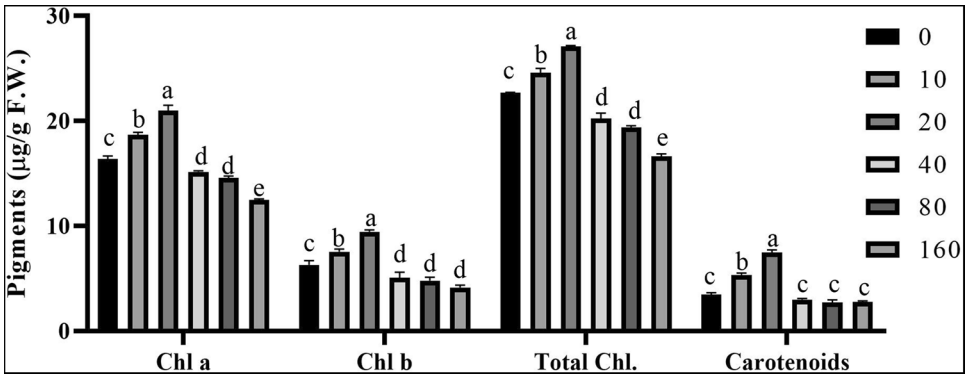

Fig. 2 The content of photosynthetic pigments: chlorophyll a, chlorophyll b, total chlorophyll and carotenoids in shoots of 7 days old mungbean (*V. radiata*) seedlings cultured on MS media containing 0, 10, 20, 40, 80 and 160 ppm zinc oxide nanaoparticles. Different letters indicate means that are significantly different at ($P \leq 0.05$)

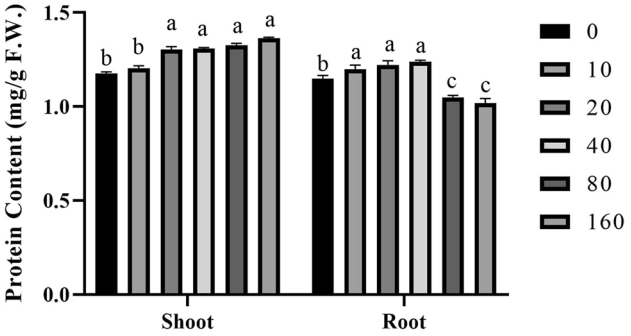

Fig. 3 The root and shoot total protein content of mungbean (*V. radiata*) seedlings grown on MS media containing 0, 10, 20, 40, 80 and 160 ppm zinc oxide nanaoparticles. Different letters indicate means that are significantly different at $P \leq 0.05$

Lipid peroxidation and H_2O_2 content The content of MDA (as a bio-indicator of lipid peroxidation) and H_2O_2 (as the most stable ROS) in the shoot samples of ZnO treated samples were significantly higher than that of controls (Fig. 4). The highest content of H_2O_2 and MDA in shoot and root samples treated with ZnO NPs were observed at 160 and 40 ppm respectively. Root H_2O_2 content in response to ZnO NPs treatment was highly increased as compared to shoot. However, lipid peroxidation in shoot was higher which probably can be justified by higher anti-oxidants in root.

Enzymatic antioxidant assay ZnO NPs treatment induced the activities of POX and PPO of *V. radiate* in both shoot and root (Fig. 5). The results showed the activity of POX in the shoot was considerably lower than in root samples. The highest activity levels of POX were observed in the shoot and root samples collected from seedlings grown on 20 ppm and 160 ppm of ZnO NPs, respectively. The highest activity level of PPO activity in both root and shoot was observed at 160 ppm ZnO NPs treated samples.

Non-enzymatic antioxidants The effect of ZnO nanoparticles on total phenolic compounds and anthocyanin was presented in Fig. 6. The treatment with ZnO NPs increased the total phenolic content of both shoot and root. The highest phenolic compounds induction was observed in shoot and root samples grown on 20 and 160 with the elevation rate of 207% and 160% percent as compared to control. Additionally, the highest level of the shoot and root anthocyanin content was observed at 160 and 80 ppm ZnNPs treated samples respectively (Fig. 6). No significant differences in anthocyanin content were observed in 20 to 160 and 40 to 160 ZnO NPs treated samples of shoot and root respectively.

The principal component analysis (PCA) was performed on Z-scored transformed data of assayed data. PCA showed that 79% variation between samples could be explained by two (PC1 = 57.60 and PC2 = 21.98%) principal components (Fig. 4). The first component (PCA1) separates the 0, 10, and

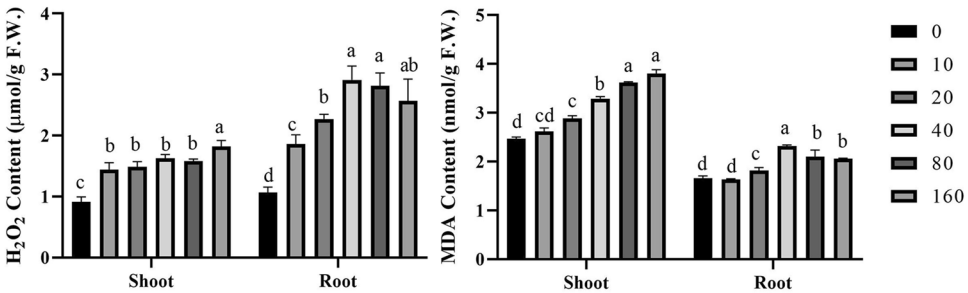

Fig. 4 The content of **A** H_2O_2 and **B** malondialdhyde(MDA) in root and shoot of mungbean (*V. radiata*) seedlings grown on MS media containing various levels of zinc oxide nanaoparticles (0, 10, 20, 40, 80 and 160 ppm). Different letters indicate means that are significantly different at $P \leq 0.05$

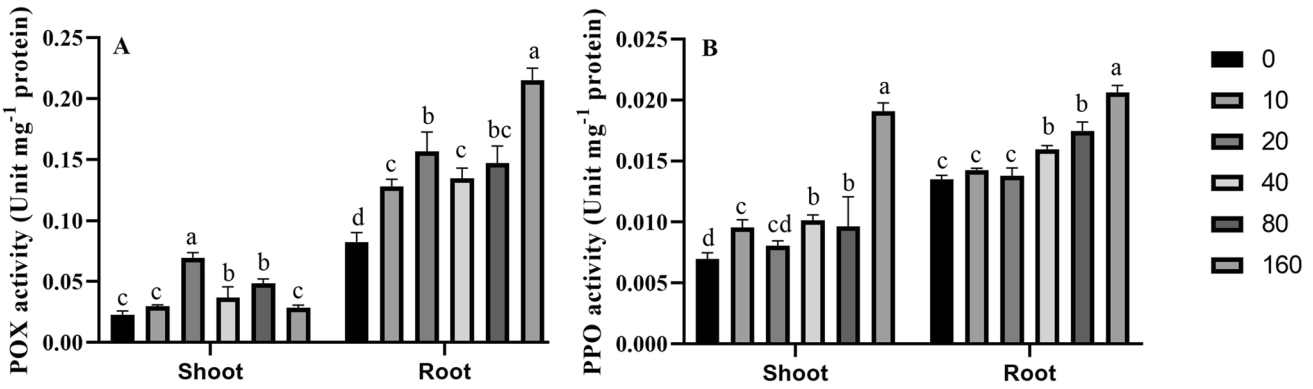

Fig. 5 The activities of **A** proxidase and **B** polyphenol oxidase enzymes in root and shoot of samples of mungbean seedlings grown on MS media containing 0, 10, 20, 40, 80 and 160 ppm zinc oxide nanaoparticles. Different letters indicate means that are significantly different at $P \leq 0.05$

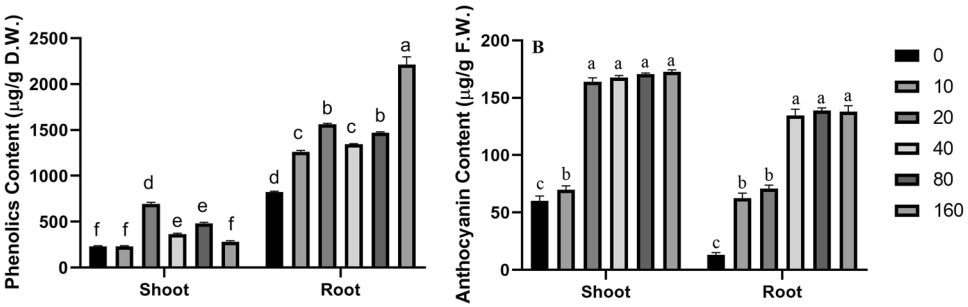

Fig. 6 The content of **A** phenolic compounds and **B** anthocyanins in root and shoot of mungbean seedlings grown on MS media containing 0, 10, 20, 40, 80 and 160 ppm zinc oxide nanaoparticles. Different letters indicate means that are significantly different at $P \leq 0.05$

20 ppm ZnO NPs treated samples from other species primarily based on and shoot zinc, calcium, H_2O_2, MDA, and chlorophyll content as well as root fresh weight (Fig. 7). The second component (PCA2) separated 40 and 60 ppm ZnO NPs treated samples from other species primarily based on cobalt, potassium, phenolic compounds contents as well as PPO and POX activities. These results confirmed that plant nutritional status is highly affected under ZnO NPs treatment followed by photosynthesis and antioxidative capability.

Discussion

The increased zinc content of shoot in response to ZnO NPs supplementation confirmed that the application of zinc nanoparticles can be an effective way to increase the zinc content of mung bean seedlings. Considering that the recommended daily intake of zinc in the diet is between 8 and 12 mg, with the consumption of 2 g of 7 days old mung bean seedlings, germinated and grown on MS medium containing 80 ppm of zinc oxide nanoparticles, this need can be met. Thus, according to the tremendous effect of ZnO NPs treatment on the zinc content of seedlings (about 45 times more than that of control), this method could lead to an augmentation that could meet the daily needs of adults.

Although the lowest concentration of ZnO NPs used in this study caused a more than 7-fold increase in zinc content of seedlings shoot, however it had a negative effect on the content of other analyzed elements. This finding, along with its positive effect on plant biomass, shows that the positive effect of zinc is related to its own nutritional value, not its effect on the absorption of other elements (at least the elements that have been studied). Because at least a part of the nutrients required for the seedling's shoot is supplied from the seed storage, it is expected that the content of elements in the shoot samples treated with ZnO NPs should not be less than that of the control samples. Therefore, it seems that the negative effect of zinc (at 10 ppm) on the content of the studied elements is related to its effect on the translocation of elements from seed to shoot not to the absorption of other elements from the medium culture.

Generally, the solubility of Zn in the media culture and soil increases with decrease in pH (Salinitro et al., 2020). It seems that the low pH of MS media culture increased the bioavailability of Zn for plant uptake. In plants, the interaction between Zn and other plant nutrients do exist and both positive and negative interactions are reported (Prasad et al. 2016).

Regarding the positive effect of zinc on plant growth, two points can be mentioned: Firstly, there are some reports that

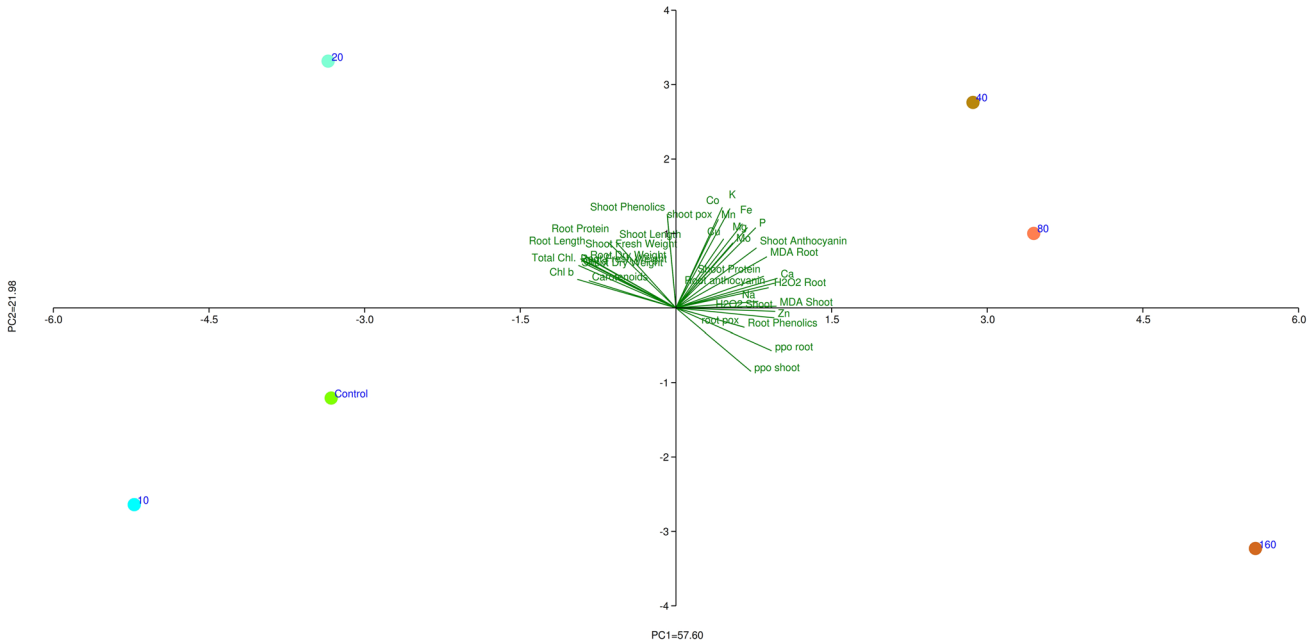

Fig. 7 Principal Component Analysis (PCA) of the z-score transformed mean of all assayed parameters of mungbean seedlings grown on MS media containing 0 (Control), 10, 20, 40, 80 and 160 ppm zinc oxide nanaoparticles. First two axes shown are 57% and 21% of the variation in composition

confirm the positive effect of zinc on tryptophan (as a precursor of auxin) and gibberellic acid content in plants which as plant growth regulators can promote plant growth (Mašev and Kutáček 1966; Wang et al. 2021). Secondly, our findings of increased leaves content of Chla, Chlb, and carotenoid (up to 20 ppm) are a good indicator of the positive impact of zinc on plant photosynthesis.

In the current study seedlings' growth (biomass) showed a strong zinc concentration-dependent trend. The positive impact of low concentration (10 and 20 ppm) and negative impact of high concentration (40 to 160 ppm) of ZnO NP media culture supplementation on seedling growth may be related to its nutritional value on one hand and toxicological effects on the other hand. Leaves photosynthetic pigments as well as root MDA and H_2O_2 content can be considered as bioindicators for the above-mentioned positive and negative effects.

Zinc is an essential nutrient for plants and it has a role in protein synthesis and activity of several enzymes, as well as membrane integrity, metabolic reactions, water uptake, transport, and gene expressions (Rudani et al. 2018). Hence, an increase in Zn level (at least up to 715% which was observed in 20 ppm ZnO NP supplemented media) was beneficial for the plants, leading to growth improvement. The positive effect of Zn application on plant chlorophyll content and photosynthesis was reported in different plants such as *Triticum aestivum* L., *Brassica juncea* (L.) Czern., and *Gossypium hirsutum* L. (Wu et al. 2015; Khan et al. 2016; Sultana et al. 2016). Similar to our findings the positive impact of low (≥ 20 ppm) concentration of ZnO NPs on plant growth and protein content was reported in *Zea mays* L. (Sabir et al. 2020), and *Lycopersicon esculentum* Mill. (Faizan et al. 2018).

The negative effect of a higher concentration of ZnO NPs on plant growth was reported in the other studies (Zhang et al. 2015; Javed et al. 2017; Tymoszuk and Wojnarowicz 2020; Rani et al. 2022). ZnO NP toxicity (at a concentration of 40 to 160 ppm) might be due to the perturbed homeostasis of Zn and its impacts on other elements' homeostasis (Srivastav et al. 2021). A strong correlation (~98%) between shoot Zn and MDA is a signal of lipid peroxidation and probable membrane damage (Supplementary Fig. 1). The finding is an indication of the creation of oxidative stress due to excessive accumulation of zinc as reported in the other plants (Remans et al. 2012; Feigl et al. 2015). Researchers have discussed about the mechanisms of nanoparticle-induced oxidative stress including light activation of electron hole pairs, active electronic configurations and functional groups generation on the surface of nanoparticles, and active redox cycling on the surface of nanoparticle (reviewed by Saliani et al. 2016). There are evidences that confirm the smaller nanoparticles have higher density of surface defects and provide greater free electrons and holes which leads to the generation of ROS (Singh et al. 2021).To eliminate excess ROS, plants activate enzymatic and non-enzymatic antioxidant defense systems. In this regard, the activation of

secondary plant metabolism and synthesis of phenolic compounds like anthocyanins is a crucial way to scavenge ROS (Sheteiwy et al. 2016; Mittler 2017; Xu and Rothstein 2018).

In the current study root PPO activity and anthocyanin content showed a significant correlation with internal Zn content (Supplementary Fig. 1). It seems that this strategy could restrain ROS accumulation in the root. A similar response of increased phenolic content to ZnO NP treatment was reported in *Brassica nigra* (L.) K.Koch, potato, and *Coriandrum sativum* L. (Marichali et al. 2014; Zafar et al. 2016; Raigond et al. 2017). However, it seems that change in the activity of PPO and POX along with the increased content of phenolic compounds has not been enough to inhibit oxidative damage in the seedling shoot (Wang et al. 2018). This observation is probably due to the fact that the photosynthesis apparatus of plant leaves are among the main site of ROS generation (Khorobrykh et al. 2020).

Comparing the content of assayed minerals in the samples, confirmed that the seedlings grown on the 40 ppm ZnO NPs, accumulated the highest amount of elements (a total of 58175.35 µg/g). Meanwhile, the highest seedling dry-weight biomass was found at 20 ppm ZnO NPs treatment. Considering the content of mineral nutrients in the total biomass produced in 7 day time period, it is determined that the highest mineral level is found in 20 ppm (2118.72) followed by 40 ppm (1602.73), 10 ppm (1324.52), 80 ppm (1308.87), control (1267.81), and 160 ppm (1021.70). These data confirm the advantage of samples grown on 20 ppm ZnO NPs over others.

Conclusion

Taken together our findings confirm the capability of ZnO NP in zinc biofrotification of mung bean seedlings. Despite the significantly increased content of many micro and macronutrients especially under 40 ppm ZnO NPs supplemented medium, its stressful effects were observed as a decrease in growth and photosynthetic pigments and an increase in the content of H_2O_2 and MDA. Considering the positive effects of ZnO NP on growth, photosynthetic pigments, protein content, activity, and content of enzymatic and non-enzymatic antioxidants in mung bean seedlings, a concentration of 20 ppm is recommended as the optimal concentration.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s11756-022-01269-3.

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Author contributions Mona Sorahinobar and Zahra Nazem Bokee designed experiments with the assistance of Ali Mehdinia, Toba Deldari carried out experiments, Mona Sorahinobar analyzed experimental results and data and wrote the manuscript.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication Not applicable.

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