

EFFECTS OF ZINC OXIDE NANOPARTICLES ON GERMINATION AND SEEDLING ESTABLISHMENT OF PEA (*PISUM SATIVUM*) AND BEANS (*PHASEOLUS VULGARIS*)

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Abstract. The number of studies on nanomaterials, the ecological significance and the possible ecotoxicity have been increased over the last decade. Nanoparticles have been considered a potential health risk and a massive menace to the environment. The aim of the study was to explore the effect of zinc oxide nanoparticles on the growth, metabolism, stress and defense systems during seed germination and seedling development. Pea (*Pisum sativum* L. var. Alicia), white beans [*Phaseolus vulgaris* L. ‘Snowdon’ (Pv1)] and red beans [*Vigna angularis* ‘adzuki bean’ (Pv2)]. Seeds were germinated for 5 days. Seedlings were then transplanted into several trays filled with nutrient solution for 15 days. At harvest, plants were divided into leaves, stems and roots. Application of ZnO nanoparticles (150 mg/L and 300 mg/L) improved vegetable production. The antioxidant activities in cytosolic, chloroplast, and mitochondrial compartments varied according to ZnO doses, organs and plant species. Significant changes were found caused by genetic variability. A gradation of quantitative differences in ZnO-NPs resistance is more typically observed between plant genotypes.

Keywords: cellular responses, environmental risk, genotypes, harmful effects, nanomaterials, resistance, tolerance, yield losses

Introduction

Agricultural practice has shown that certain substances promote plant growth and development. These substances, of natural or anthropogenic origin, allow for better absorption of nutrients (Missaoui et al., 2020). Among these substances are nanoparticles with dimensions smaller than 100 nm (Missaoui et al., 2017, 2018; Chemingui et al., 2019a, b; 2021; Guey et al., 2020). Publications often present discordant results (Dietz et al., 2011; Missaoui et al., 2021). Indeed, studies on plants show both beneficial and harmful effects (Larue, 2011). Nanoparticles (NPs) stimulate plant growth and represent an interesting potential in agriculture (Missaoui et al., 2021). The results obtained depend on the type of nanoparticles and their properties (Ghafariyan et al., 2013; Missaoui et al., 2017), but also on the plant species and their stage of development, time and doses (Ma et al., 2013). They are expressed indirectly via mineral nutrition, by improving the bioavailability of mineral elements, or directly, by the assimilation of organic molecules that modify biochemical processes and plant cell metabolism. These effects are either positive or negative depending on the experimental conditions. Several levels of variability are involved in the study of the biological impact of NPs. Reducing the application of fertilizers and biocides on crops limits the risks of soil, groundwater and watercourse pollution, and would reduce the energy and environmental costs associated with the production and distribution of agrochemicals (Koller, 2004). However, minimizing environmental risk often implies yield losses. Modern agriculture uses techniques that would allow a reduction in the use of chemical inputs without affecting crop yields or farmers’ incomes. For these different reasons, we present in this work the results of the treatment of pea and bean seedlings

with the aim of analyzing the biological, physiological and cellular responses. We asked the following questions: is it a general response for both plants? Could it be a kind of plant resistance, or is it a genetically controlled tolerance?

‘Resistance’ and ‘tolerance’ are the terms used to denote the ability of the plant manage the stress, be it biotic or abiotic. A plant is considered resistant when it has the ability to exclude, hinder or overcome the effects of a given pathogen or another damaging factor. A plant may be resistant to one pathogen or condition but not others. Tolerance is the ability of a plant to be colonized by a pathogen or exposed to an abiotic factor without dying or demonstrating disease symptoms (Cooper, 2007; Catiempo et al., 2021). According to the definition of Koch et al. (2016), plant resistance can be categorized into three categories: antibiosis, antixenosis or non-preference, and tolerance. Antibiotic plant traits negatively impact a pest’s biology through increases in mortality, reduced growth, longevity, and fecundity. Antixenosis, often referred to as non-preference, is a host-expressed trait that has adverse effects on insect behavior. In essence, insects have a non-preference for antixenotic hosts, and a preference for susceptible ones. Tolerance traits reduce the negative effects of herbivory on plant fitness after herbivory has occurred, all the while maintaining insect populations similar to those seen on susceptible plants. In an evolutionary context, tolerance is defined as the slope of the line describing the association between fitness and level of damage for a set of genetically related plants (Strauss and Agrawal, 1999). In agronomic situations, tolerant crop varieties are able to withstand injury and produce acceptable yields (Qiu et al., 2011). From an ecological perspective, tolerant plants can maintain fitness in response to pest injury. Both antibiosis and antixenosis involve a plant response and a pest response (Peterson et al., 2017). However, in the case of tolerance only a plant response is involved. Therefore, there is a nonreciprocal process associated with tolerance (Smith, 2005).

The two major mechanisms of plant defense against Zinc oxide nanoparticles are resistance (the ability to limit ZnO-NPs-plant interactions) and tolerance (the ability to reduce the effect of ZnO-NPs on plant metabolism regardless of the dose of ZnO-NPs). Qualitative differences in stress resistance can be observed when multiple specimens are compared after treated by the ZnO-NPs at similar levels in similar environments. A gradation of quantitative differences in ZnO-NPs resistance is more typically observed between plant genotypes.

The purpose of this research is to understand and explain the variable effects of zinc oxide nanoparticles on *Pisum sativum* and *Phaseolus vulgaris* seedlings.

Materials and methods

Genotypes tested comprised three commercial cultivars in Saudi Arabia. Seeds of pea (*Pisum sativum* L. var. Alicia), white beans [*Phaseolus vulgaris* L. ‘Snowdon’ (Pv1)] and red beans [*Vigna angularis* ‘adzuki bean’ (Pv2)] were disinfected with sodium hypochlorite (2%) for 10 min, washed thoroughly with distilled water and then germinated in Petri dishes (10 seeds/Petri dish) on 2 sheets of moist filter paper for 5 days at 25 °C (Basahi, 2018).

The seedlings were transplanted into several trays filled with nutrient solution (Hoagland and Arnon, 1950). pH 6.5 was evaluated as optimum for hydroponic culture. Aeration was provided by a modular aerator. The temperature was about 25 °C. The luminosity was 150 lx. Relative humidity was 50%. The photoperiod was

8 h. Seedlings were imbibed with distilled water (control) or treated with 150 mg/L and 300 mg/L ZnO nanoparticles (<50 nm particle size (BET), Purity = 99.9%, Sigma-Aldrich, St. Louis, MO, USA; *Table 1*) for 15 days. Nutrient solutions were changed every four days during the experiment. The dissolution of zinc oxide (ZnO) nanoparticles (NPs) is a key step controlling their environmental fate, bioavailability, and toxicity. ZnO nanoparticles were not soluble at pH 6.5 (Copur, 2010). The different treatments used were presented in *Table 2*. At harvest (20-days old; phenophase: plant growth during spring), plants were divided into leaves, stems and roots, dried for 8 days at 70 °C for dry weight determination or kept in ultra-deep freezer at -80 °C for biochemical studies.

As for morphological parameters axis length, (2) length of internodes, (3) root length, (4) number of secondary roots, (5) leaf area, and (6) number of leaves were determined. Leaf area had been measured using CI-202 portable laser leaf area meter.

For the determination of chlorophylls, they were extracted by grinding 100 mg fresh leaves in 5 mL ethanol (80%) using a mortar and after 72 h in the dark and at 4 °C, the absorbance was measured at 663 and 645 nm (Lichtenthaler and Welburn, 1983). Pigment contents (chlorophyll a (Cha) and chlorophyll b (Chb)) (mg g⁻¹ FW) were determined using a spectrophotometer (Lamba 2, PerkinElmer, Waltham, MA, USA).

Cytosol, chloroplasts, and mitochondria were isolated according to method described by Smiri et al. (2009). Enzyme activities expressed as units per gram of fresh weight Ug⁻¹ FW and determined using spectrophotometer (Lamba 2, PerkinElmer, Waltham, MA, USA).

Table 1. Size, surface area and form of ZnO-NPs obtained from Sigma-Aldrich

Properties of ZnO-NPs (677450 - Zinc oxide; Sigma-Aldrich)	
Quality level	For non-regulated applications with no change notification requirements
Purity	99.9% Based on trace metals analysis
Form	Nanopowder
Contains	6% Al as dopant
Reaction suitability	Reagent type: catalyst
Particle size	Core: zinc
Surface area	< 50 nm (BET)
	> 10.8 m ² /g
Safety information	
Symbol	GHS09
Signal word	Warning
Hazard statements	H410
Precautionary statements	P273 - P391 - P501
Personal protective equipment	Dust mask type N95 (US), Gloves

Table 2. The different treatments

Treatments	<i>Pisum sativum</i>	<i>Phaseolus vulgaris</i>	<i>Vigna angularis</i>
Controls (ZnO (0 g/L))	CPs	CPv1	CPv2
ZnO (150 mg/L)	ZnO-1Ps	ZnO-1Pv1	ZnO-1Pv2
ZnO (300 mg/L)	ZnO-2Ps	ZnO-2Pv1	ZnO-2Pv2

Guaiacol peroxidase (GPOX) activity was measured according to Fielding and Hall (1978). The enzyme extract was added to the reaction mixture containing 50 mM of potassium phosphate (pH 7.0), 10 mM of H₂O₂, and 9 mM of guaiacol. Enzyme activity was estimated by the increase in absorbance at 470 nm. GPOX activity was determined using the extinction coefficient of 26.6 mM⁻¹ cm⁻¹. One unit of GPOX activity was defined as the amount of enzyme that caused the formation of 1 μM of tetraguaiacol per minute under the assay conditions. Catalase (CAT) was measured according to Aebi (1984). The enzyme extract was added to the reaction mixture containing 50 mM of potassium phosphate buffer (pH 7.0), 10 mM of H₂O₂, and 1 mM of dithiothreitol (DTT). The enzyme activity was quantified by recording the decrease in absorbance at 240 nm. CAT activity was determined using the extinction coefficient of 39.4 mM⁻¹ cm⁻¹. One unit of CAT activity was defined as the amount of enzyme required to decay 1 μM of hydrogen peroxide/min/mg protein under the assay conditions.

Seedling data were arcsine transformed before statistical analysis to ensure homogeneity of variance (Ahmed and Khan, 2010). For statistical analysis, the factors were the species and the ZnO concentrations. The effects of the tested factors on growth, productivity, chlorophyll metabolism, oxidative stress and antioxidant systems were analysed. Post hoc tests were performed to compare treatment means.

Results

ZnO-NPs treatments have significant effects on stem growth and the number of internodes of two tested plants (*Fig. 1A, B*). The variability of the results could be explained by the tolerance of the ZnO-NPs, which depends on the genetic variability of the plants (interspecific variability). These results could be explained by a Zn-dependent control of stem growth (intraspecific variability). Results in *Figure 1C* showed the variation in root lengths. The ZnO (150 mg/L) and ZnO (300 mg/L) treatments give the longest roots in pea, which reach 30 cm and 35 cm, respectively, and significantly different from the control (15 cm). In white beans, which are more sensitive to contamination of the nutrient medium by ZnO-NPs, root development regressed by more than 20% compared to the control. These results proved that the effectiveness of treatment with ZnO-NPs depended on the applied dose in both varieties of beans (inter- and/or intra-specific variability). No significant effect of ZnO-NPs on the number of roots was recorded for the three species (*Fig. 1D*). The impact of nanoparticle on plant varied in relation to its size, concentration, and exposure methodology. Based on the available reports, we proposed the possible implication of the same mechanism against ZnO-NPs stress for Fabaceae plants. It is a general response for both plants.

Results in *Table 3* show significant effects of ZnO-NPs varying within the same species. This was a kind of specific resistance. Results presented in *Table 4* showed a significant variation in responses between species. We suggested a genetic control of ZnO-NPs induced growth. The results presented in *Figure 1E* and *F* show that the addition of ZnO-NPs induced leaf area and number of plant leaves after 20 days of development in hydroponic environments.

Water content, fresh weight and dry weight varied significantly when plants were exposed to ZnO-NPs (*Fig. 2*). The application of zinc oxide nanoparticles induced an

excessive accumulation of water in plant organs. The highest levels were highlighted for the ZnO- treatment (150 mg/L). The treatment of beans with ZnO-NPs (300 mg) kept organ water contents of control levels. pea dry weight showed no significant effect when changed ZnO-NPs dose, in contrary for beans (Table 5). The responses varied significantly among the three species (Table 6).

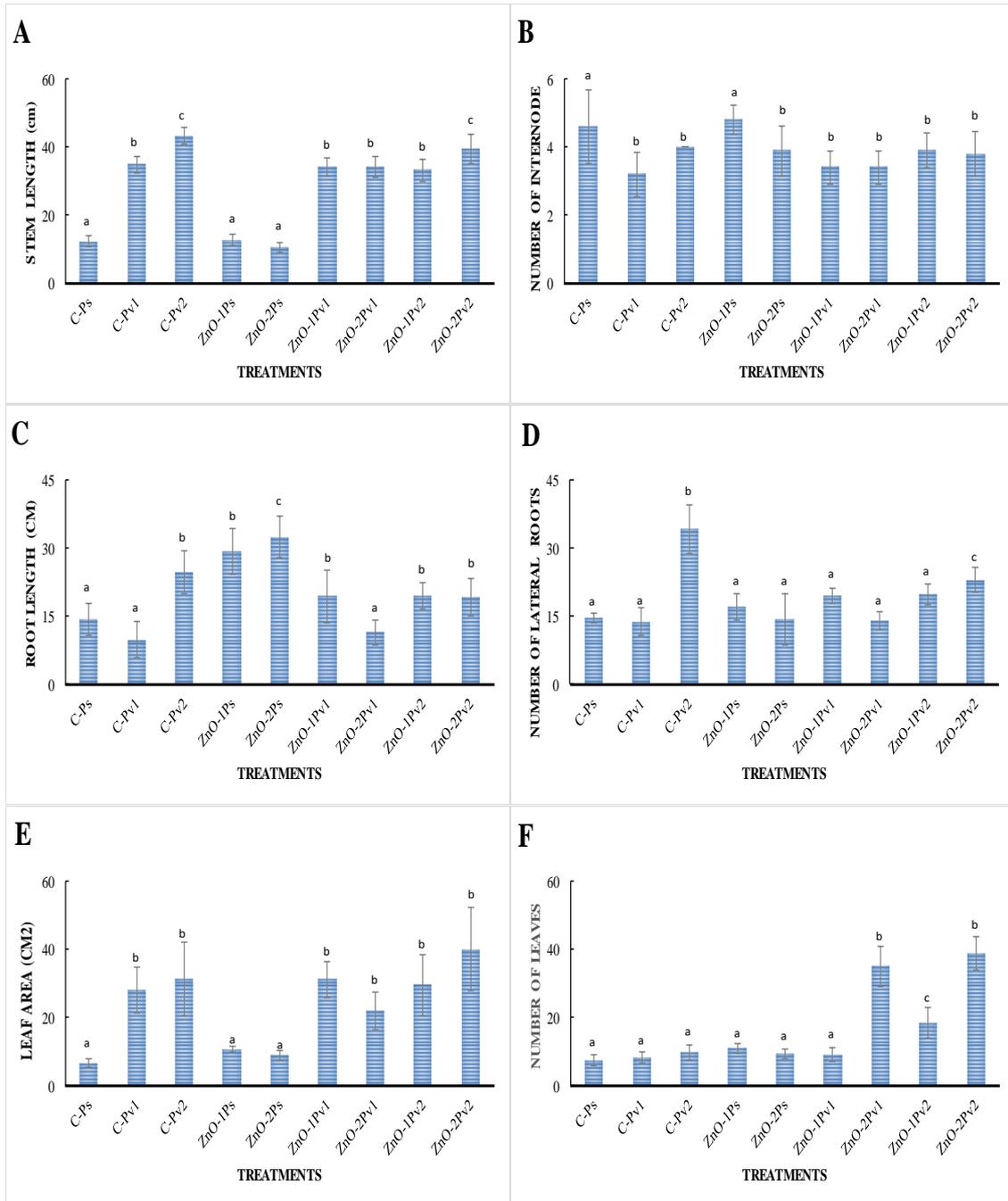


Figure 1. (A) Stem length, (B) Number of enter node, (C) Root length, (D) Number of lateral roots, (E) Leaf area and (F) Number of leaves of pea [*Pisum sativum* L. (Ps)] and beans [*Phaseolus vulgaris* L. (Pv1) and *Vigna angularis* (Pv2)] Grown in water (C) or exposed to 150 mg/L (ZnO-1) or 300 mg/L (ZnO-2) of Zinc oxide. Data are the means of 5 repetitions (\pm SE). Different letters represent significant differences at $p < 0.05$

Table 3. The effects of the ZnO concentrations on the growth

Independent variables	C-Ps	ZnO-1Ps	ZnO-2Ps	C-Ps*ZnO-1Ps	C-Ps*ZnO-2Ps	ZnO-1Ps*ZnO-2Ps	Error
df	5	5	5	25	25	25	
<i>Pisum sativum</i> L.	144.74**	588.65**	390.99**	319.06**	148.76**	1243.68**	MS=3

Independent variables	C-Pv1	ZnO-1Pv1	ZnO-2Pv1	C-Pv1*ZnO-1Pv1	C-Pv1*ZnO-2Pv1	ZnO-1Pv1*ZnO-2Pv1	Error
df	5	5	5	25	25	25	
<i>Phaseolus vulgaris</i> L. (Pv1)	485.03**	158.63**	242.91**	350.34**	765.98**	158.24**	MS=3

Independent variables	C-Pv2	ZnO-1Pv2	ZnO-2Pv2	C-Pv2*ZnO-1Pv2	C-Pv2*ZnO-2Pv2	ZnO-1Pv2*ZnO-2Pv2	Error
df	5	5	5	25	25	25	
<i>Vigna angularis</i> (Pv2)	485.03**	158.63**	242.91**	350.34**	765.98**	158.24**	MS=3

Asterisks indicate statistical significance ($P < 0.05$)

Table 4. The effects of the species on the growth.

Independent variables	Ps*Pv1	Ps*Pv2	Pv1*Pv2	Ps*Pv1*Pv2
df	25	25	25	125
Control	200.06**	675.23**	992.72**	1293.18***
Zno-1	802.75**	812.20**	430.70**	389.77***
ZnO-2	275.66**	422.88**	295.61**	199.06***
Error	MS=3	MS=3	MS=3	MS=2

Asterisks indicate statistical significance ($P < 0.05$)

Table 5. The effects of the ZnO concentrations on the productivity

Independent variables	C-Ps	ZnO-1Ps	ZnO-2Ps	C-Ps*ZnO-1Ps	C-Ps*ZnO-2Ps	ZnO-1Ps*ZnO-2Ps	Error
df	5	5	5	25	25	25	
<i>Pisum sativum</i> L.	4056.00**	-	-	4056.00**	4056.00**	-	MS=3

Independent variables	C-Pv1	ZnO-1Pv1	ZnO-2Pv1	C-Pv1*ZnO-1Pv1	C-Pv1*ZnO-2Pv1	ZnO-1Pv1*ZnO-2Pv1	Error
df	5	5	5	25	25	25	
<i>Phaseolus vulgaris</i> L. (Pv1)	29.36**	86.65**	25.20**	40.08**	15.01**	70.07**	MS=3

Independent variables	C-Pv2	ZnO-1Pv2	ZnO-2Pv2	C-Pv2*ZnO-1Pv2	C-Pv2*ZnO-2Pv2	ZnO-1Pv2*ZnO-2Pv2	Error
df	5	5	5	25	25	25	
<i>Vigna angularis</i> (Pv2)	31.63**	32.64**	48.75**	12.25**	25.49**	19.40**	MS=3

Asterisks indicate statistical significance ($P < 0.05$)

Table 6. The effects of the species on the productivity

Independent variables	Ps*Pv1	Ps*Pv2	Pv1*Pv2	Ps*Pv1*Pv2
df	25	25	25	125
Control	4056.00**	1767.65**	17.72**	17.72***
Zno-1	86.65**	32.64**	416.17**	416.17***
ZnO-2	25.20**	48.75**	79.59**	79.59***
Error	MS=4	MS=4	MS=3	MS=3

Asterisks indicate statistical significance (P < 0.05)

The results in *Figure 3* showed a significant reduction in chl a and b levels after treatment with nanoparticles (ZnO-NPs) compared to the control on 20-day-old pea leaves. Treatment of beans with ZnO-NPs (300 mg) maintains values of the controls. Chlorophyll metabolism (mg/L) showed significant changes under 0-300 mg/L of Zinc oxide (*Table 7*). The significant variations between species in response to nanoparticles shows that these responses are genetically controlled at the level of chlorophyll metabolism (*Table 8*).

In the stems, the results in *Figure 4* showed responses significantly changed according to the cell organelle (compartment) and the dose of nanoparticle. The cytosolic isoforms have the highest activity for ZnO treatment (150 mg/L), followed by the mitochondrial isoforms for ZnO treatment (300 mg/L). In leaves, significant responses are recorded after ZnO treatment in the different analyzed cell compartments. The ZnO treatment (300 mg/L) stimulated GPOX activity in the mitochondria and chloroplasts by 80% compared to the control. The ZnO treatment (150 mg/L) stimulated activity in the cytosol and mitochondria by 50%. The addition of ZnO nanoparticles in the roots gives the highest stimulation. The addition of ZnO-NPs induced GPOX activity in both bean varieties. The effect of ZnO-NPs varied according to the applied dose and the involved isoforms. The activities of the cytosolic and chloroplastic isoforms increased proportionally with increasing doses of NPs-ZnO. They are inversely proportional to the dose in the mitochondria. Several studies showed the role of peroxidases in the response to various stimuli. At the root level, peroxidases do not behave in the same way. These peroxidases were stimulated after treatment with ZnO-NPs in a dose-proportional manner in red beans, but inversely proportional in the white variety. In the root mitochondria we did not see any significant effects. Metabolic adaptation can be based either on the functioning of the primitive pathways which would have been preserved in the higher plants and which would allow an active metabolism (mitochondrial pathway) or, on the contrary, on the establishment of a slowed-down life (cytosolic pathway). In both cases, it is the maintenance of certain metabolic balances that must be fundamental to produce adaptation. Results of GPOX activities and their interactions with resistance and tolerance under 0-300 mg/L of Zinc oxide were presented in *Tables 9* and *10*. It appears that control of oxidative stress induced by ZnO nanoparticles varied significantly due to applicable dose and species.

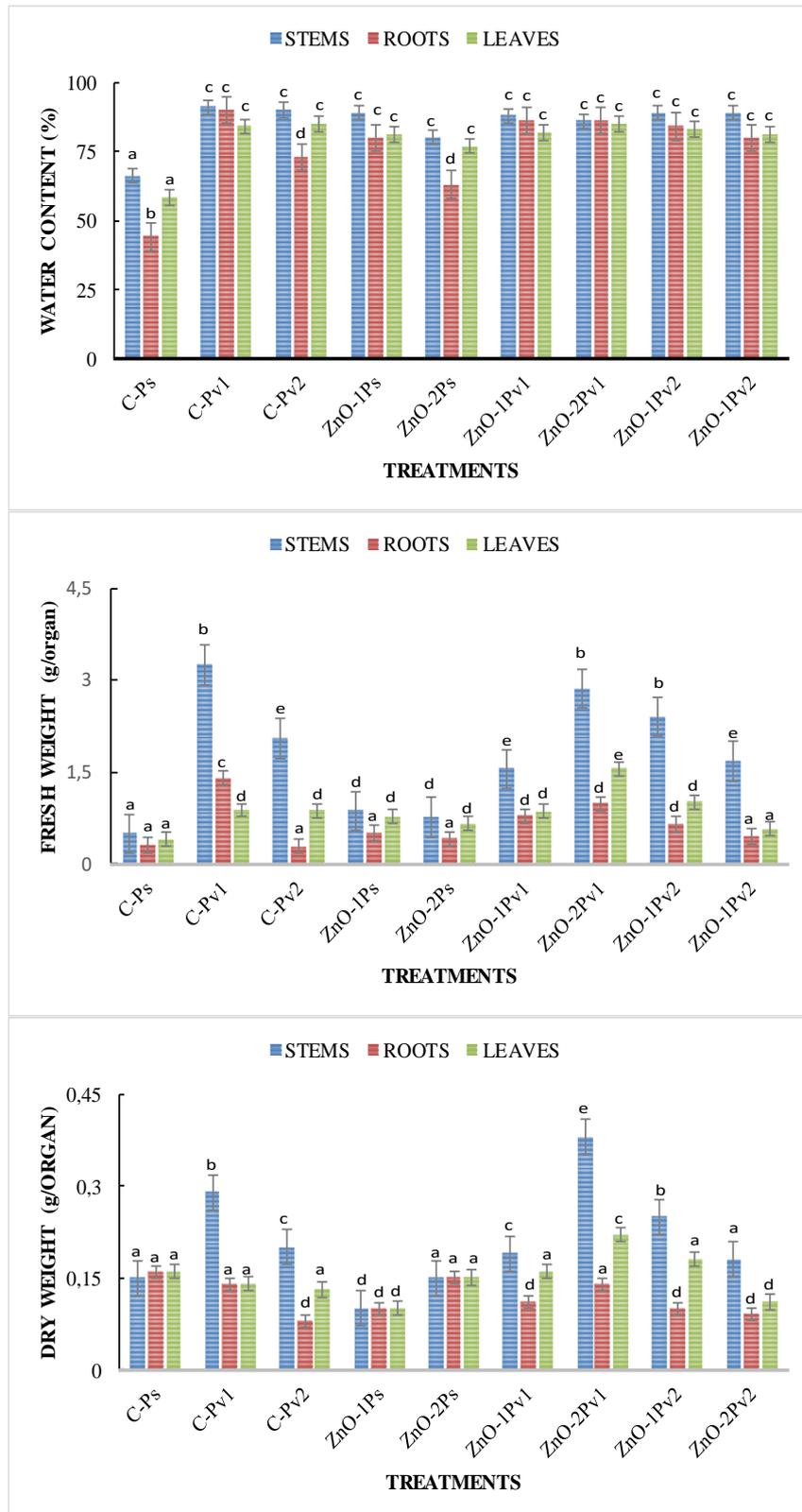


Figure 2. (A) Water content, (B) Fresh weight and (C) Dry weight of pea [*Pisum sativum* L. (Ps)] and beans [*Phaseolus vulgaris* L. (Pv1) and *Vigna angularis* (Pv2)] Grown in water (C) or exposed to 150 mg/L (ZnO-1) or 300 mg/L (ZnO-2) of Zinc oxide. Data are the means of 5 repetitions (\pm SE). Different letters represent significant differences at $p < 0.05$

The ZnO treatment (300 mg/L) stimulated catalase activity at the cytosol level by 115% compared to control (*Fig. 5*). The ZnO (150 mg/L) and ZnO (300 mg/L) treatment stimulates this activity in the chloroplast by 200%. At leaf level, cytosolic isoforms have the highest activity for ZnO treatment (150 mg/L), followed by mitochondrial isoforms for ZnO treatment (150 mg/L). At the root level, the addition of ZnO nanoparticles stimulates catalase activity, especially for the cytosolic and mitochondrial isoforms. ZnO-NPs treatment inhibited the cytosolic and chloroplastic catalase activities in a dose-dependent manner in both bean varieties, but inhibited the cytosolic catalase activity of the red variety and the mitochondrial catalase activity of the white variety. Catalase activity in roots was induced by ZnO-NPs. The cytosolic catalase activities were induced by 50-100% for the red variety and 75-170% in the white variety in the presence of ZnO-NPs. Mitochondrial catalase activities were induced after seed soaking or irrigation of plants in the white variety. Catalase activities are regulated by ZnO-NPs (*Tables 11 and 12*).

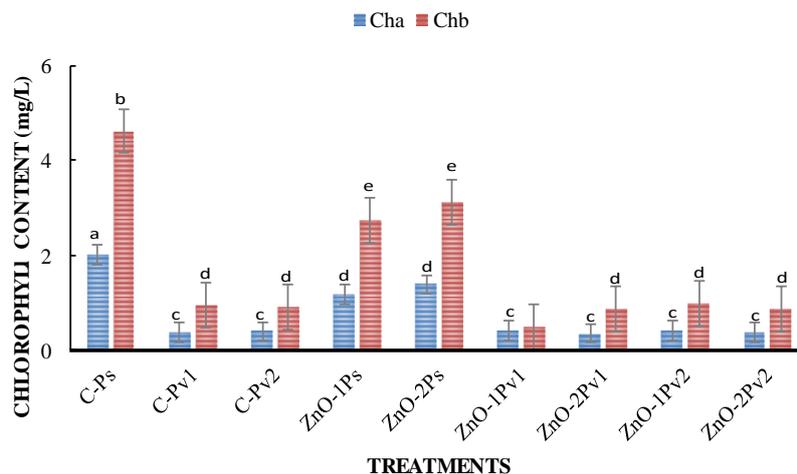


Figure 3. Chlorophyll a and b of pea [*Pisum sativum* L. (Ps)] and beans [*Phaseolus vulgaris* L. (Pv1) and *Vigna angularis* (Pv2)] Grown in water (C) or exposed to 150 mg/L (ZnO-1) or 300 mg/L (ZnO-2) of Zinc oxide. Data are the means of 5 repetitions (\pm SE). Different letters represent significant differences at $p < 0.05$

Table 7. The effects of the ZnO concentrations on the chlorophyll metabolism

Independent variables	C-Ps	ZnO-1Ps	ZnO-2Ps	C-Ps*ZnO-1Ps	C-Ps*ZnO-2Ps	ZnO-1Ps*ZnO-2Ps	Error
df	5	5	5	25	25	25	
<i>Pisum sativum</i> L.	31.59**	30.16**	32.34**	31.59**	31.59**	32.34**	MS=4
Independent variables	C-Pv1	ZnO-1Pv1	ZnO-2Pv1	C-Pv1*ZnO-1Pv1	C-Pv1*ZnO-2Pv1	ZnO-1Pv1*ZnO-2Pv1	Error
df	5	5	5	25	25	25	
<i>Phaseolus vulgaris</i> L. (Pv1)	27.77**	442.99**	27.57**	27.77**	27.77**	27.77**	MS=4
Independent variables	C-Pv2	ZnO-1Pv2	ZnO-2Pv2	C-Pv2*ZnO-1Pv2	C-Pv2*ZnO-2Pv2	ZnO-1Pv2*ZnO-2Pv2	Error
df	5	5	5	25	25	25	
<i>Vigna angularis</i> (Pv2)	30.37**	27.72**	29.92**	30.37**	30.37**	27.72**	MS=4

Asterisks indicate statistical significance ($P < 0.05$)

Table 8. The effects of the species on the chlorophyll metabolism

Independent variables	Ps*Pv1	Ps*Pv2	Pv1*Pv2	Ps*Pv1*Pv2
df	25	25	25	125
Control	31.59**	31.59**	27.77**	27.77***
Zno-1	30.16**	30.16**	27.72**	27.72***
ZnO-2	32.34**	32.34**	27.57**	27.57***
Error	MS=4	MS=4	MS=4	MS=4

Asterisks indicate statistical significance (P < 0.05)

Table 9. The effects of the ZnO concentrations on the oxidative stress metabolism

Independent variables	C-Ps	ZnO-1Ps	ZnO-2Ps	C-Ps*ZnO-1Ps	C-Ps*ZnO-2Ps	ZnO-1Ps*ZnO-2Ps	Error
df	5	5	5	25	25	25	
<i>Pisum sativum</i> L.	6495.47**	61.00**	70.13**	45750.20**	149808.10**	802.95**	MS=3

Independent variables	C-Pv1	ZnO-1Pv1	ZnO-2Pv1	C-Pv1*ZnO-1Pv1	C-Pv1*ZnO-2Pv1	ZnO-1Pv1*ZnO-2Pv1	Error
df	5	5	5	25	25	25	
<i>Phaseolus vulgaris</i> L. (Pv1)	84.53**	61.00**	70.13**	332.78**	130.38**	2579.92**	MS=3

Independent variables	C-Pv2	ZnO-1Pv2	ZnO-2Pv2	C-Pv2*ZnO-1Pv2	C-Pv2*ZnO-2Pv2	ZnO-1Pv2*ZnO-2Pv2	Error
df	5	5	5	25	25	25	
<i>Vigna angularis</i> (Pv2)	24.82**	37.31**	209.07**	1213.30**	216.46**	169.33**	MS=3

Asterisks indicate statistical significance (P < 0.05)

Table 10. The effects of the species on the oxidative stress metabolism

Independent variables	Ps*Pv1	Ps*Pv2	Pv1*Pv2	Ps*Pv1*Pv2
df	25	25	25	125
Control	23715.68**	3984.47**	70.44**	70.44***
Zno-1	336.22**	24.34**	365.24**	365.24***
ZnO-2	580.86**	1552.89**	143.98**	143.98***
Error	MS=3	MS=3	MS=3	MS=3

Asterisks indicate statistical significance (P < 0.05)

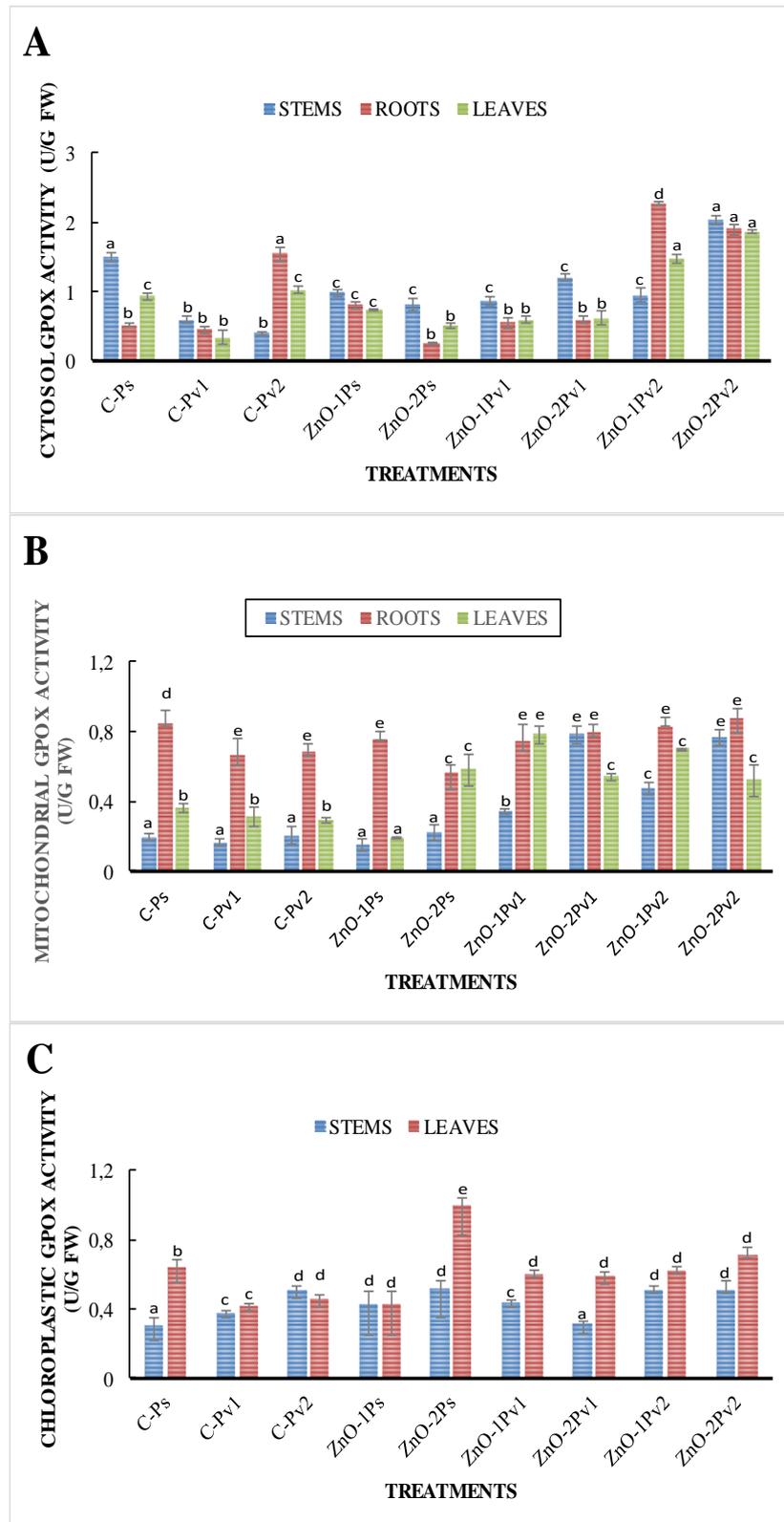


Figure 4. Guaiacol peroxidase activity in (A) Cytosol, (B) Mitochondria and (C) Chloroplast of pea [*Pisum sativum* L. (Ps)] and beans [*Phaseolus vulgaris* L. (Pv1) and *Vigna angularis* (Pv2)] Grown in water (C) or exposed to 150 mg/L (ZnO-1) or 300 mg/L (ZnO-2) of Zinc oxide. Data are the means of 5 repetitions (\pm SE). Different letters represent significant differences at $p < 0.05$

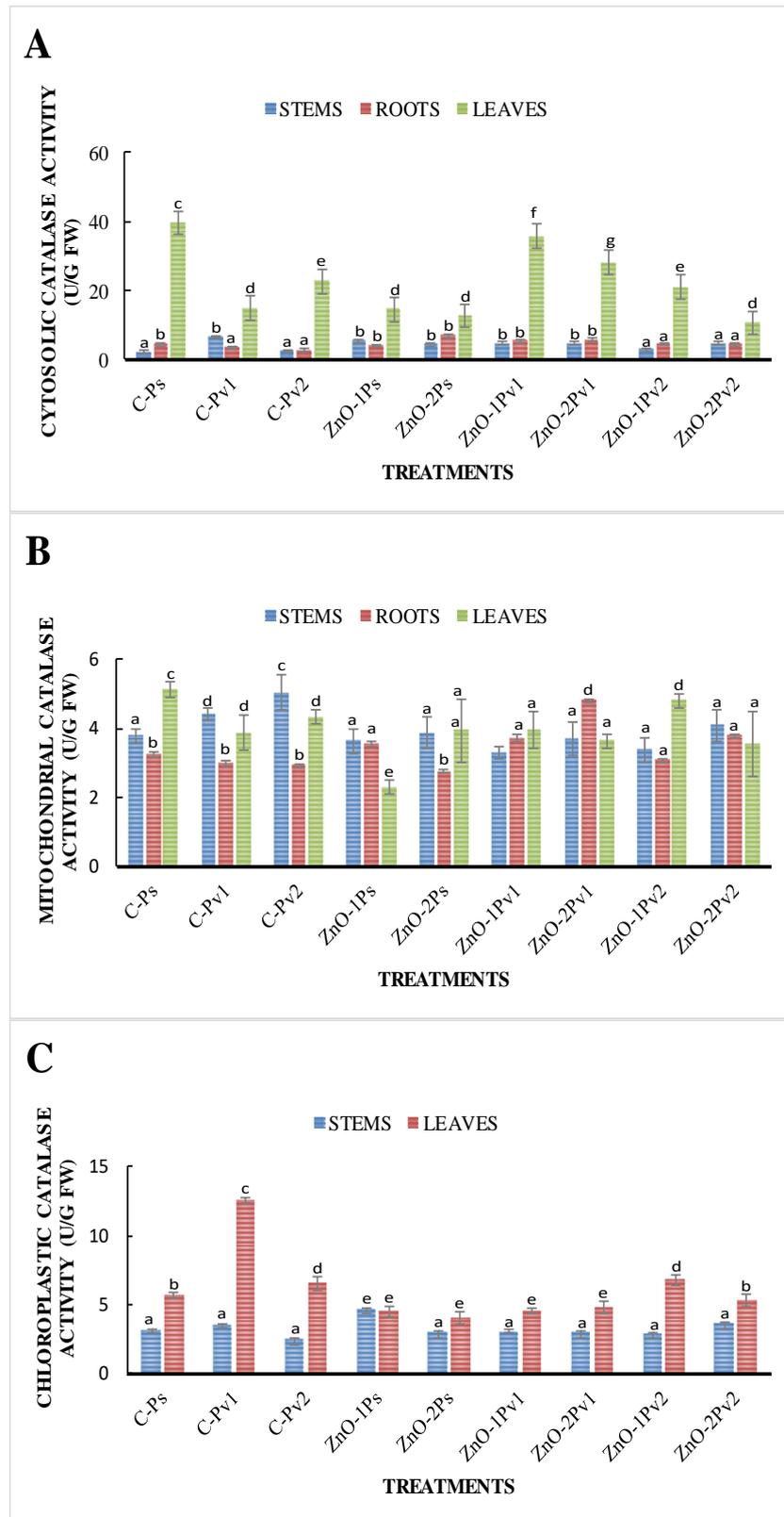


Figure 5. Catalase activity in (A) Cytosol, (B) Mitochondria and (C) Chloroplast of pea [*Pisum sativum* L. (Ps)] and beans [*Phaseolus vulgaris* L. (Pv1) and *Vigna angularis* (Pv2)] Grown in water (C) or exposed to 150 mg/L (ZnO-1) or 300 mg/L (ZnO-2) of Zinc oxide. Data are the means of 5 repetitions (\pm SE). Different letters represent significant differences at $p < 0.05$

Table 11. The effects of the ZnO concentrations on the antioxidant systems

Independent variables	C-Ps	ZnO-1Ps	ZnO-2Ps	C-Ps*ZnO-1Ps	C-Ps*ZnO-2Ps	ZnO-1Ps*ZnO-2Ps	Error
df	5	5	5	25	25	25	
<i>Pisum sativum</i> L.	7.27**	15.01**	9.48**	15.64**	9.41**	21.28**	MS=3

Independent variables	C-Pv1	ZnO-1Pv1	ZnO-2Pv1	C-Pv1*ZnO-1Pv1	C-Pv1*ZnO-2Pv1	ZnO-1Pv1*ZnO-2Pv1	Error
df	5	5	5	25	25	25	
<i>Phaseolus vulgaris</i> L. (Pv1)	45.70**	77.31**	10.66**	30.17**	80.40**	180.32**	MS=3

Independent variables	C-Pv2	ZnO-1Pv2	ZnO-2Pv2	C-Pv2*ZnO-1Pv2	C-Pv2*ZnO-2Pv2	ZnO-1Pv2*ZnO-2Pv2	Error
df	5	5	5	25	25	25	
<i>Vigna angularis</i> (Pv2)	17.49**	12.83**	72.59**	35.48**	72.59**	864.12**	MS=3

Asterisks indicate statistical significance (P < 0.05)

Table 12. The effects of the species on the antioxidant systems

Independent variables	Ps*Pv1	Ps*Pv2	Pv1*Pv2	Ps*Pv1*Pv2
df	25	25	25	125
Control	122.63**	87.56**	34.19**	34.19***
Zno-1	53.47**	5.82**	175.35**	175.35***
ZnO-2	4.76**	22769.41**	1213.21**	1213.21***
Error	MS=3	MS=3	MS=3	MS=3

Asterisks indicate statistical significance (P < 0.05)

Discussion

The obtained results showed that the sensitivity of the two varieties changed from one genotype to the other. This study was consistent with several studies on the differential sensitivity of legumes to metals (Siddiqui et al., 2015). These results suggested that enzymatic antioxidant system responses (GPOX and catalase) are carried out by isoforms specific to each compartment. The stimulation of the defense system after application of each stimulus could also explain the improved growth of pea plants.

Significant changes were due to genetic variability. All the results obtained show that this was a genetic control of the responses of pea and bean plants to the enrichment of the environment by nanoparticles. The dose of ZnO controlled intensity of the responses of each plant. In previous research, application of zinc oxide nanoparticles induced growth of *Brassica juncea* (Mazumder et al., 2020) and reduced genetic impairment under salt stress in tomato (*Solanum lycopersicum* L. 'Linda') (Hosseinpour et al., 2020). Researchers analyzed the impact of zinc oxide nanoparticles on cytotoxicity, genotoxicity and mRNA expression in tomato (Sun et al., 2020a, b) and barley

(*Hordeum vulgare* L.) seedlings Plaksenkova et al. (2020). Missaoui et al. (2021) showed that TiO₂ affected growth due to changes in nanoparticle availability and accumulation. Faizan et al. (2019) showed that effective use of zinc oxide nanoparticles through root dipping on the performance of growth, quality, photosynthesis and antioxidant system in tomato. Zinc oxide nanoparticles disturbed germination and seedling growth in *Allium cepa* L. (Tymoszuk and Wojnarowicz, 2020). Alabdallah and Alzahrani (2020) described the potential mitigation effect of ZnO nanoparticles on *Abelmoschus esculentus* L. metabolism under salt stress conditions.

Nanoparticles alter the plant's capacity to absorb and transport some nutrients. NPs produced increases in the contents of Mg, Zn and Mn, and a decline in the contents of Fe and Cu in leaves and stems (Missaoui et al., 2021). Physiological disturbances could be correlated with exposure to ZnO-NPs. The application of ZnO-NPs in agriculture has indicated variable impacts on plant growth (Rajpu et al., 2021). Biomass accumulation in the vegetative growth phase of a plant can therefore be regarded as the ultimate expression of its metabolic performance. Taking into account the various studies on the effects of zinc nanoparticles on plants, it appeared that there was an interaction between metabolism, growth and stress. The distribution of metabolites between growth, production of defense compounds and storage compounds therefore has to be very tightly regulated.

Conclusions

We evaluated the effectiveness of nanoparticles by studying the interaction of ZnO-NPS with plant production (growth and photosynthesis) and the defense system (oxidative stress and enzymatic antioxidant system: GPOX and CAT) of pea (*Pisum sativum* var. Alicia), white bean (*Phaseolus vulgaris* L.) and red bean (*Vigna angularis*) seedlings. We analyzed the responses on the basis of genetic variability (tolerance) and within the same species (resistance). We used the results of the analyses for the prediction of growth control, metabolism and defense mechanisms against the presence of ZnO-NPs in the three species.

An application of ZnO-NPs increased plant production. These responses vary according to ZnO-NPs doses. A positive dose effect in root and a negative dose effect in stem. We suggested that there was a specificity of responses for each organ. These responses could be due to the anatomical and functional properties of the different tested organs.

The antioxidant system responses were carried out by specific isoforms of each compartment. The responses of the cytosolic, chloroplast and mitochondrial isoforms varied according to ZnO-NPs doses. Stimulation of the defense system after application of ZnO-NPs could also explain the improved plant growth. ZnO-dependent root control varied in the two bean varieties. This work will be continued in the future with more genotypes of pea and beans. We used actually for the present work the genotypes available in the region.

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