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In vitro genotype-specific responses of tomato to ZnO nanoparticles: Impacts on growth and nutrient uptake

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Abstract

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The urgent need for sustainable and efficient methods to enhance plant resistance to abiotic stresses, along with the growing demand for high-quality, nutrient-rich food, has intensified the search for innovative agronomic solutions. One of the most actively researched approaches involves the use of nanoparticles, which, due to their small size and enhanced absorpion, have a potential to improve plant physiological processes.

This study aimed to evaluate the effects of zinc oxide (ZnO) nanoparticles (NPs) on growth and micronutrient accumulation in two Bulgarian tomato varieties (*Ideal* and *Rozovo sartse*) grown *in vitro*. Plants were cultured on Murashige and Skoog (MS) medium, supplemented with 18 nm ZnO NPs at four concentrations (0, 0.5, 1.5, and 2.5 mg/L). The results demonstrated a clear genotype-dependent response to nanoparticle exposure. While the *Ideal* variety showed limited growth improvement and no significant increase in microelement content, *Rozovo sartse* exhibited enhancements in both growth and nutrient accumulation, particularly at the highest concentration of 2.5 mg/L.

The highest accumulation of Zn, Fe, and B in both roots and shoots of *Rozovo sartse* occurred at 2.5 mg/L, with Zn levels in shoots reaching 249.9 ppm, which is more than twice the control. In contrast, Cu levels decreased in both genotypes, likely due to competitive uptake with Zn. Manganese levels were not significantly affected. Biometric indicators such as plant height, root length, fresh weight of shoot and roots, and number of roots were also significantly improved in *Rozovo sartse*, suggesting that ZnO NPs can be a potential tool for plant growth stimulation.

These findings highlight the importance of genotype selection when applying nanomaterials in plant biotechnology and support the use of ZnO NPs as a promising strategy for enhancing micronutrient content and growth in responsive tomato genotypes.
Keywords: zinc; *Solanum lycopersicum* L.; NPs; microelements

Introduction

Industries ranging from cosmetics and agriculture to textiles, medicine, and paints increasingly recognize nanoparticles (NPs) as valuable raw materials, thanks to their unique properties, including nanoscale size and exceptionally large surface area. These particles, derived from various elements, often enter the soil through industrial waste, raising ongoing concerns about their potential positive or negative effects on plants and animals (Rajput et al., 2020). However, it has been demonstrated that the application of nanoparticles in agriculture leads to a number of beneficial effects. For instance, under specific ecological conditions, nanoparticles have been shown to enhance the content of essential nutrients, such as iron (Fe), boron (B), zinc (Zn), and manganese (Mn) (Kralova and Jampilek 2022).

Among these, zinc oxide nanoparticles (ZnO-NPs) have shown significant influence on plant growth and health, offering both advantages and potential risks related to accumulation and phytotoxicity. Studies on the phytotoxicity of metal nanoparticles have demonstrated negative effects on growth and physiological processes across various plant species (Li et al., 2015; de la Rosa et al., 2021). Although findings are often contradictory, there is a clear consensus that the toxicity threshold of nanoparticles in plants is species-dependent, and each case should be assessed individually. Nevertheless, the use of optimal low concentrations of nanoparticles and the selection of safe and biodegradable nanomaterials could avoid harmful effects. The benefits of using nanoparticles are far greater. They improve photosynthesis, shoot development, and nutrient uptake, leading to increased biomass and productivity in plants. Studies have shown that ZnO-NPs enchance chlorophyll content and stimulate photosynthetic enzymes such as RuBisCO, thereby boosting plant vigor (Chen et al., 2024). At low concentrations, ZnO-NPs stimulate antioxidant enzyme activity, helping plants combat oxidative stress (Srivastava et al., 2021). In addition, ZnO-NPs possess antimicrobial properties that can help protect plants from pathogens and soil-borne diseases (Thounaojam et al., 2021; Vasileva et al., 2024; Vasileva et al., 2025).

Furthermore, zinc-based nanomaterials hold promise for the development of nanotubes and nanocomposites, aimed at boosting agricultural productivity and improving human nutrition. In recent years, the positive role of nanoparticles in helping plants with stand environmental stress, has received growing attention within the plant science community (Zhou et al., 2025). Overcoming challenges related to both abiotic and biotic stresses, in order to ensure sustainable agriculture on a global scale, necessitates the search for new and stable methods that nanobiotechnology can provide. Increasing global crop production by 70% is among the primary scientific goals and simultaneously challenges (FAO, 2009).

Long-term zinc deficiency in tomatoes impairs growth, photosynthesis, and root development, leading to stunted plants, chlorotic and necrotic leaves, poor nutrient uptake, and reduced yield and fruit quality. As zinc is crucial for enzyme activation and immune response, the deficient plants are also more vulnerable to infections. This is a widespread issue, as zinc deficiency is one of the most common micronutrient deficiencies in soils worldwide. If left uncorrected, zinc deficiency can significantly reduce crop yield and economic returns for growers (Srivastav et al., 2022).

ZnO-NPs have been extensively studied for their effects on tomato plants, and they tend to offer unique advantages compared to other nanoparticles. ZnO-NPs have been shown to significantly improve growth, biomass, and yield in tomatoes, often outperforming conventional zinc fertilizers (Ahmed et al., 2023). They enhance zinc absorption and increase its concentration in both leaves and fruits along with other elements such as K and Fe (Pejam et al., 2021). A study using biopolymer-coated ZnO-NPs demonstrated their effectiveness in controlling bacterial speck disease in tomatoes, while also improving photosynthesis and antioxidant enzyme activity (Dich et al., 2025). In our previous studies, ZnO nanoparticles (60–70 nm in size) have been shown to inhibit mycelal growth the *Verticillium dahliae* and *Fusarium oxysporum* at a concentration of 1.5 mg/L (Vasileva et al., 2024; Vasileva et al., 2025).

It has been found that ZnO-NPs significantly improved growth, biomass, yield, stress tolerance, and nutrient uptake in tomato (Pejam et al., 2021). Compared to bulk ZnO, they are more effective at upregulating stress-related genes and improving physiological traits (Ahmed et al., 2021). The optimal concentration of ZnO-NPs for plants depends on the species, application method, and environmental conditions. Studies have shown that low concentrations (5–50 ppm) promote seed germination, root elongation, and photosynthesis without toxicity (Pejam et al., 2021).

To complement current knowledge, our experiment focused on evaluating the effects of ZnO-NPs on the growth and micronutrient accumulation of two Bulgarian tomato varieties grown *in vitro*, by examining their physiological and biological parameters and assessing the potential for growth stimulation and biofortification.

Material and Methods

Chemicals and reagents used

Zinc oxide nanoparticles (ZnO NPs) were purchased from Nanografi Nanotechnology, with a purity of 99.99% and an average particle size of 18 nm. Suprapur grade 65% (m/m) HNO_3 and 30% (m/m) H_2O_2 (used for sample mineralization), and the multielement standards (ICP Multielement Standard IV) were purchased from Merck, Germany. Ultra-pure water was prepared in an in-house instrument (resistivity > 18 MΩ cm^{-1}). All other chemicals used were of analytical grades. The purity of the plasma torch argon was greater than 99.99%. All labware was carefully cleaned, rinsed with high-purity water, and dried under clean-air conditions at ambient temperature.

Experimental conditions, treatments, and design

The experimental work was conducted in 2023, using two tomato varieties, *Rozovo sartse* and *Ideal*, both a part of the Maritsa Vegetable Crops Research Institute's collection. Tomato seeds were surface-sterilized in a 5% calcium hypochlorite solution for 1 hour and rinsed 3 times with sterile distilled water (dH_2O). Following sterilization, the seeds were sown on a basal Murashige and Skoog (1962) medium, supplemented with macro- and micronutrients, Gamborg's vitamins (Gamborg et al., 1968), 3% sucrose, and 0.7% agar. The pH of the medium was adjusted to 5.8 using 0.1 M NaOH or 0.1 M HCl before autoclaving.

Seven days after germination, seedlings, after root removal, were transferred to vessels containing 25 mL of MS medium supplemented with ZnO-NPs. At four different concentrations: 0 mg/L (Control), 0.5 mg/L, 1.5 mg/L, and 2.5 mg/L. The vessels with plants were incubated for 20 days in a growth chamber maintained at 25 ± 1°C, with a photosynthetic photon flux density (PPFD) of 200 µmol m^{-2} s^{-1} and a 16/8 h light/dark photoperiod.

Determination of the biomass of tomato plants grown in vitro

The experiment was conducted in three replications, using 20 plants per genotype and treatment. To evaluate the influence of ZnO-NPs on *in vitro* plant growth, several biometric parameters were measured, according to standard protocols after 20 days of cultivation and ZnO-NPs exposure. These included plant height (cm), number of leaves, root length (cm), number of roots, fresh weight of shoot and roots (g), and dry weight of shoot and roots (g).

Determination of nutrient content of plants

Following biometric analysis, all plant samples were separated into roots and shoots (stems and leaves) and stored at –32°C. The samples were lyophilized and then mechanically ground using a mortar until a fine, homogeneous powder was obtained.

From each sample, approximately 0.250 g (weight was recorded for each sample) was weighed using an analytical balance, and transferred to Teflon digestion vessels. Each sample was treated with 0.5 mL of 99% HNO_3 and 2 mL of 99% H_2O_2. The mixtures were allowed to stand for 30 minutes at room temperature before undergoing microwave-assisted digestion in an Anton Paar system for 90 minutes.

The concentrations of Zn, Fe, Mn, Cu, and B in the resulting solutions were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 7000, PerkinElmer), with a dual-view configuration. The lines that exhibited low interference and high analytical signal and background ratios were selected. The employed emission lines for each mineral were B 249.667, Cu 327.393, Fe 259.939, Mn 257.610, Zn 206.200.

Data analysis

The experiment was carried out in three replicates, and the data were subjected to one-way analysis of variance (ANOVA), and expressed as mean values. Duncan's multiple range test (significance level 5%) was used, to calculate the differences between each concentration level of ZnO-NPs and compared to the control variants.

Results and Discussion

Tomato growth as influenced by ZnO-NPs

The growth response to ZnO-NPs showed genotype-dependent variation across biometric parameters of the two Bulgarian tomato varieties, *Ideal* and *Rozovo sartse* (Table 1). In *Ideal*, all tested ZnO-NP concentrations reduced shoot fresh weight compared to the control, with the lowest value (0.37 g) at 2.5 mg/L. Plant height also declined slightly with increasing nanoparticle concentration, although the differences were not statistically significant. The number of leaves remained relatively stable across treatments, with a slight increase at 1.5 mg/L. Root length and root number exhibited minor fluctuations, but no clear dose-dependent trend was observed. Conversely, *Rozovo sartse* showed a positive growth response to ZnO-NPs. Shoot fresh weight increased progressively from control to 2.5 mg/L, reaching a maximum of 0.69 g. Root biomass (both fresh and dry weight) also increased significantly under ZnO-NP treatments, alongside increased root length and number. Notably, the highest shoot dry weight was recorded at 0.5 mg/L for *Rozovo sartse*, suggesting enhanced shoot biomass accumulation at lower nanoparticle concentration. The number of leaves in this variety slightly decreased with nanoparticle treatment, but remained within a narrow range. Overall, *Rozovo sartse* demonstrated greater tolerance and biomass enhancement in response to ZnO-NPs compared to *Ideal*, which showed a tendency toward reduced growth metrics under higher nanoparticle concentrations.

Treatment with ZnO-NPs has shown potential to significantly improve growth parameters, biomass accumulation, and yield in tomato plants (Pejam et al., 2021). Several studies have reported increases in stem height and diameter, dry weight of plants (leaves, stem, and roots), as well as enhanced photosynthetic activity, antioxidant capacity, and protein accumulation in tomato plants, treated with Zn-NPs compared to untreated controls (Perez-Velasco et al., 2020; Faizan et al., 2020). According to Gurmani et al. (2012), the application of Zn increases the chlorophyll content in tomato plants, and plays an important role in plant metabolism by changing the action of key enzymes, such as carbonic anhydrase. The mechanism behind the increased chlorophyll content is the role of Zn as an important nutrient for the plants (Faizan et al., 2020). The observed improvement in photosynthetic traits following ZnO-NP exposure may also be attributed to increased light absorption, which contributes to chloroplast protection from senescence and prolongs the functional lifespan of the chloroplasts. This ultimately leads to enhanced photosynthesis and, consequently, promotes biomass accumulation (Yang et al. 2006). In contrast, El-Mahdy et al. (2019) observed that increasing concentrations of Zn and ZnO nanoparticles stimulated root production but inhibited biomass accumulation.

In the current study, the number of roots reflected differential responses between both varieties. While *Ideal* maintained relatively constant root numbers across treatments, *Rozovo sartse* exhibited slight increases in root numbers with ZnO treatment. This supports the observation that *Rozovo sartse* is more adaptable or responsive to Zn supplementation via nanoparticles.

Overall, the data highlight a concentration-dependent and genotype-specific effect of ZnO nanoparticles on tomato plant development. While low concentrations may stimulate growth in sensitive genotypes, higher doses can exert inhibitory effects, emphasizing the need for precise optimization when applying nanomaterials in agriculture.

Accumulation of essential nutrients in tomato plants

The data reveal distinct genotype-specific responses to ZnO-NPs treatments in terms of micronutrient accumulation in the roots (Table 2). In the *Ideal* variety, iron (Fe) content was highest in the control (748.2 ppm), and decreased significantly with ZnO-NPs application, reaching the lowest value (221.0 ppm) at 2.5 mg/L. Similarly, manganese (Mn) content also declined notably with increasing ZnO-NPs concentrations. Zinc (Zn) levels remained relatively stable across treatments, but showed no clear dose-dependent trend. Interestingly, copper (Cu) content peaked at 0.5 mg/L (17.6 ppm), suggesting some stimulation of Cu uptake at lower ZnO-NPs doses. Boron (B) levels remained relatively unchanged. These results indicate a possible genotype-specific limitation in the uptake or transport of trace elements in *Ideal*, consistent with observations by Raliya et al. (2016), who noted that nanoparticle efficiency often depends on plant genotype and physiological traits.

In contrast, the variety *Rozovo sartce* demonstrated a consistent increase in the accumulation of Zn, Fe, and B in the roots at all tested ZnO-NPs concentrations, compared to the control plants. These findings are supported by Dimkpa et al. (2017), who also reported enhanced micronutrient uptake in crops, treated with ZnO-NPs, particularly Zn and Fe. Notably, the highest accumulation of these elements was observed at the concentration of 2.5 mg/L ZnO-NPs, confirming a dose-dependent relationship, as previously described by Wang et al. (2016). The Mn levels showed a decreasing trend with increasing nanoparticle concentration, although the differences were not statistically significant. Similar non-significant changes in Mn uptake after ZnO-NPs application were reported by López-Millán et al. (2009), suggesting that competition or antagonism among micronutrients may play a role. A more pronounced decrease was observed for Cu levels, which declined at all ZnO-NPs concentrations. This inverse response could be attributed to competitive uptake between Zn and Cu.

Genotype-specific responses to ZnO-NPs are observed again in the shoot micronutrient accumulation (Table 3). Regarding Zn levels, the *Ideal* variety showed an increase in Zn accumulation, only at the highest concentration of 2.5 mg/L. In the *Rozovo sartce* variety, a positive tendency was observed for Zn accumulation, which increased gradually with the nanoparticle concentration from 115.5 ppm in the Control to 249.9 ppm at 2.5 mg/L. These results are consistent with reports by Wang et al. (2016), who demonstrated that zinc oxide nanoparticles can significantly improve Zn bioavailability and uptake, especially in *in vitro* plant cultures, and under nanoparticle-supplemented hydroponic conditions.

Table 1. Impact of ZnO-NPs on the biometrical parameters of tomato plants grown *in vitro*

Genotype	Variant	Fresh weight of shoot (g)	Plant height (cm)	Number of leaves	Fresh weight of roots (g)	Root length (cm)	Number of roots	Dry weight of shoot (g)	Dry weight of roots (g)
Ideal	Control	0.50^{b-d}	12.83^{a}	3.80^{ab}	0.13^{bc}	7.33^{ab}	5.94^{a-c}	0.15^{d}	0.06^{c}
	0.5 mg/L	0.43^{cd}	11.79^{ab}	3.90^{ab}	0.09^{c}	6.25^{b}	5.07^{bc}	0.24^{bc}	0.08^{c}
	1.5 mg/L	0.42^{cd}	11.23^{a-c}	4.33^{a}	0.11^{bc}	6.34^{b}	5.15^{bc}	0.20^{cd}	0.07^{c}
	2.5 mg/L	0.37^{d}	9.94^{bc}	3.76^{ab}	0.12^{bc}	6.30^{b}	4.88^{c}	0.19^{cd}	0.08^{c}
Rozovo sartce	Control	0.53^{a-d}	9.40^{c}	3.84^{ab}	0.12^{bc}	7.11^{ab}	5.28^{bc}	0.19^{cd}	0.07^{c}
	0.5 mg/L	0.58^{a-c}	10.43^{bc}	3.27^{b}	0.18^{ab}	7.24^{ab}	6.40^{ab}	0.36^{a}	0.16^{a}
	1.5 mg/L	0.62^{ab}	9.50^{c}	3.80^{ab}	0.18^{ab}	7.55^{ab}	6.09^{a-c}	0.23^{bc}	0.09^{bc}
	2.5 mg/L	0.69^{a}	11.30^{a-c}	3.37^{b}	0.23^{a}	7.92^{a}	7.29^{a}	0.25^{bc}	0.12^{a}

a,b,c… – Duncan's Multiple Range ($p \leq 0.05$) Test

Source: Authors' own elaboration

Table 2. Impact of ZnO-NPs on the accumulation of Zn, Fe, Mn, Cu, and B in roots of tomato plants grown *in vitro*

Roots						
Genotype	Variant	Fe, ppm	Zn, ppm	Mn, ppm	Cu, ppm	B, ppm
Ideal	Control	748.2 [a]	446.3 [ab]	454.7 [a]	10.3 [d]	10.1 [ab]
	0.5 mg/L	319.6 [bc]	407.3 [bc]	268.0 [bc]	17.6 [bc]	11.1 [ab]
	1.5 mg/L	308.7 [bc]	313.0 [bc]	220.5 [c]	13.3 [b-d]	10.6 [ab]
	2.5 mg/L	221.0 [c]	417.3 [bc]	225.2 [c]	10.9 [d]	11.8 [ab]
Rozovo sartce	Control	317.8 [bc]	239.8 [c]	474.8 [a]	42.1 [a]	8.1 [b]
	0.5 mg/L	461.6 [a-c]	382.0 [bc]	367.5 [ab]	12.2 [cd]	7.1 [b]
	1.5 mg/L	398.5 [bc]	424.6 [b]	404.4 [a]	12.6 [cd]	10.0 [ab]
	2.5 mg/L	605.6 [ab]	607.7 [a]	389.7 [ab]	16.5 [c]	15.9 [a]

a,b,c… – Duncan's Multiple Range ($p \leq 0.05$) Test
Source: Authors' own elaboration

The highest Fe levels in shoots were found in the *Ideal* variety, treated with the highest concentration of ZnO-NPs (2.5 mg/L), reaching 240.3 ppm, which represents an increase of approximately 34% compared to the control plants (102.3 ppm), and suggests a positive effect of ZnO-NPs on Fe accumulation (Table 3). A similar trend was observed by Raliya et al. (2016), who reported that ZnO-NPs enhance Fe mobilization and uptake, particularly under *in vitro*, controlled conditions. In contrast, Fe content in the *Rozovo sartce* variety did not exhibit significant variation across treatments and remained consistently lower in the *Ideal* variety, further emphasizing the genotype-specific nature of micronutrient responses to ZnO-NP exposure.Table 3:

Regarding Mn, all recorded values were statistically similar, ranging from 224.2 to 306.0 ppm, suggesting that the applied ZnO-NPs nanoparticle concentrations did not significantly influence Mn uptake in the shoots of either of the tomato varieties. This finding aligns with results from Dimkpa et al. (2017), who also reported minimal effects on Mn uptake following ZnO-NPs nanoparticle exposure.

Copper levels in shoots were somewhat variable, but tended to decrease under ZnO-NPs treatments, with the lowest value at 0.5 mg/L (8.1 ppm). In fact, a slight decline was observed, where ZnO-NPs negatively affected Cu uptake. In the case of B, a genotype-dependent response was evident. In the *Ideal* variety, B levels remained consistent, with only minor fluctuations, whereas in *Rozovo sartce*, B content increased with ZnO-NP concentration, reaching a peak at 1.5 mg/L, suggesting a potential synergistic effect between ZnO-NPs and B uptake in this variety.

Zinc deficiency can cause physiological stress in plants, given that Zn plays a fundamental role in many metabolic processes. Significant decreases in growth and fruit yield under Zn-deficient conditions have been widely reported (Karimi et al., 2019; Obrador et al., 2021). The results from the current experiment reinforce that ZnO-NPs influence the uptake and translocation of essential micronutrients in a concentration- and genotype-dependent manner, supporting earlier findings on the complex interactions between nanomaterials and plant nutrient homeostasis, which is in agreement with data presented by Salama et al. (2019). In the current study, *Rozovo Sartse* responded positively to ZnO-NPs treat-

Table 3. Impact of ZnO NPs on the accumulation of Zn, Fe, Mn, Cu, and B in shoots of tomato variety Ideal and variety Rozovo sartce grown *in vitro*

Shoots						
Genotype	Variant	Fe, ppm	Zn, ppm	Mn, ppm	Cu, ppm	B, ppm
Ideal	Control	102.3 [c]	154.5 [b-d]	224.2 [a]	17.4 [a]	33.7 [a]
	0.5 mg/L	186.3 [b]	141.6 [cd]	228.9 [a]	11.7 [bc]	28.4 [ab]
	1.5 mg/|L	194.9 [b]	124.1 [d]	265.5 [a]	12.8 [a-c]	29.5 [ab]
	2.5 mg/L	240.3 [a]	196.1 [a-c]	271.8 [a]	13.9 [ab]	28.7 [ab]
Rozovo sartce	Control	115.2 [c]	115.5 [d]	284.9 [a]	13.0 [ab]	21.9 [b]
	0.5 mg/L	113.6 [c]	150.9 [b-d]	302.5 [a]	8.1 [c]	25.5 [ab]
	1.5 mg/L	117.0 [c]	210.9 [ab]	302.8 [a]	10.4 [bc]	33.1 [a]
	2.5 mg/L	105.2 [c]	249.9 [a]	306.0 [a]	12.3 [bc]	32.7 [ab]

a,b,c… – Duncan's Multiple Range ($p \leq 0.05$) Test
Source: Authors' own elaboration

ments in terms of Zn accumulation in shoots, and showed enhanced accumulation of other key micronutrients (Fe and B) at higher ZnO-NPs concentrations, suggesting improved nutrient uptake and potential biofortification capacity. *Ideal* did not exhibit the capacity to accumulate elevated levels of Zn, but exhibited a decline in micronutrient levels, particularly Fe and Mn at 0.5 and 1.5 mg/L, indicating a less favorable or possibly stress-related response to ZnO-NPs.

These contrasting responses between both tomato genotypes highlight the importance of genetic background in determining plant tolerance, and the efficiency, with which they utilize nanoparticles as a nutrient source. Similar genotype-dependent differences in Zn uptake and utilization. In contrast, an efficiency have been observed in other crops, in which certain cultivars demonstrated enhanced Zn accumulation and improved growth, while others were more sensitive to higher ZnO-NP concentrations (Dimkpa et al., 2017; Rossi et al., 2019). The observed decline in micronutrient levels in the *Ideal* variety may be attributed to antagonistic interactions between Zn and other elements, such as Fe, Mn, and Cu, which compete for common uptake and transport pathways (Broadley et al., 2007). This antagonism can impair the balance of essential micronutrients and negatively affect plant physiological performance.

Overall, the findings of the present study emphasize the dual role of ZnO nanoparticles: they can serve as effective nanofertilizers that improve micronutrient content and biomass accumulation, and potentially enhance the nutritional value of tomato plants, but their effects are strongly dependent on genotype and applied concentration. Therefore, optimizing nanoparticle application strategies requires careful evaluation of crop-specific responses, to ensure beneficial outcomes without compromising plant health or nutritional quality.

These results suggest that ZnO-NPs could be integrated into sustainable agricultural practices as targeted nanofertilizers, particularly for varieties such as *Rozovo sartse*, that shows enhanced micronutrient and biomass accumulation. Their application could contribute to biofortification strategies, aimed at increasing the nutritional quality of tomato fruits, while also reducing reliance on conventional fertilizers. However, careful optimization of concentration, timing, and genotype selection is crucial to minimize potential stress responses, such as those, observed in the *Ideal* variety. Thus, the practical implementation of ZnO-NPs in crop production should be guided by genotype-specific responses to ensure both agronomic benefits and food safety. Future studies should focus on long-term field evaluations, fruit quality assessments, and potential environmental and food safety implications, in order to better define the safe and effective use of ZnO nanoparticles in sustainable agriculture.

Conclusion

This study highlights the genotype-specific responses of two Bulgarian tomato varieties, *Ideal* and *Rozovo sartse*, to zinc oxide nanoparticle treatments under *in vitro* conditions. While the variety *Ideal* exhibited limited capacity to accumulate zinc, or demonstrate significant biometric improvement across all applied ZnO-NPs concentrations, the variety *Rozovo sartse* responded positively. Specifically, *Rozovo sartse* showed enhanced accumulation of Zn, Fe, and B in both roots and shoots, with the strongest effect observed at the 2.5 mg/L treatment level. Biometric parameters, such as shoot and root fresh weight and root length, also improved under this treatment.

These findings highlight the importance of genotype selection when applying nanomaterials in plant biotechnology, and support the use of ZnO-NPs as a promising strategy for enhancing micronutrient content and growth in responsive tomato genotypes. Further studies are recommended to evaluate the effects under *in vivo* conditions, and to clarify the mechanisms underlying nutrient uptake and translocation influenced by nanoparticles.

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Conflicts of Interest

The authors declare no conflict of interest.

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