Original Article

Silver Nanoparticles Increase Nitrogen, Phosphorus, and Potassium Concentrations in Leaves and Stimulate Root Length and Number of Roots in Tomato Seedlings in a Hormetic Manner

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Abstract

Background: Silver nanoparticles (AgNPs) display unique biological activities and may serve as novel biostimulators. Nonetheless, their biostimulant effects on germination, early growth, and major nutrient concentrations (N, P, and K) in tomato (*Solanum lycopersicum*) have been little explored.

Methods: Tomato seeds of the Vengador and Rio Grande cultivars were germinated on filter paper inside plastic containers in the presence of 0, 5, 10, and 20 mg/L AgNPs. Germination parameters were recorded daily, while early growth traits of seedlings were determined 20 days after applying the treatments (dat). To determine nutrient concentrations in leaves, a hydroponic experiment was established, adding AgNPs to the nutrient solution. Thirty-day-old plants were established in the hydroponic system and kept there for 7 days, and subsequently, leaves were harvested and nutrient concentrations were determined.

Results: The AgNPs applied did not affect germination parameters, whereas their application stimulated length and number of roots in a hormetic manner. In 37-day-old plants, low AgNP applications increased the concentrations of N, P, and K in leaves.

Conclusion: As novel biostimulants, AgNPs promoted root development, especially when applied at 5 mg/L. Furthermore, they increased N, P, and K concentration in leaves, which is advantageous for seedling performance during the early developmental stages.

Keywords

Solanaceae, *Solanum lycopersicum*, nanotechnology, silver nanoparticles, hormesis, biostimulation, root growth

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Introduction

Silver nanoparticles (AgNPs) have numerous applications in the food and agriculture sectors. These nanotechnologies show constant and accelerated growth in the generation of technological developments, patents, uses, and new markets.[1-3] However, their interactions with living organisms have not been studied in depth. Even though these nanoparticles can serve as stimulants of plant metabolism,[4-6] their effect tends to show dose–response hormetic curves,[7,8] where it is possible to observe stimulating or beneficial effects at low concentrations, and inhibitory or negative effects at high concentrations.[9,10] These hormetic effects of AgNPs require further study of their impact on living organisms and the environment. Consequently, the application of AgNPs in agricultural crops requires the determination of thresholds between beneficial and toxic effects in plants in order to estimate hormetic effects.

Regarding beneficial effects in plants, AgNPs have been shown to improve root length, leaf area, chlorophyll content, seed germination, and postharvest life of the fruit.[11-13] Also, AgNPs may increase the content of glutamine and asparagine, as well as the activity of superoxidase dismutase (SOD), catalase and peroxidase in shoots and roots.[14] However, in some plants the application of AgNPs decreased growth, biomass production, concentration of the ribulose-1,5-bisphosphate carboxylase/oxygenase, the mitotic index and cell division.[15-20] These findings imply that AgNPs may have different effects depending on plant genotypes. Therefore, it is of utmost importance to evaluate the effects of AgNPs in crops of agri-food importance, such as tomato, and to analyze the responses among different genotypes. With more than 5 million hectares cultivated and 180 million tons of fresh fruit production worldwide, tomato is considered one of the most important crops in the world, with the main producing countries being (in descending order): China, India, Turkey, the United States, Egypt, Iran, Italy, Spain, Mexico, and Brazil.[21] The tomato fruit is a source of vitamins B1, B2, and C, minerals (potassium, iron, sodium), antioxidant compounds (lycopene, carotenes), and organic acids, which give it a special value in the diet and foods of most of the world's countries.[22,23]

In tomato, the application of AgNPs (from 50 mg/L AgNPs) had no significant effects on seed germination, but it did decrease root elongation, chlorophyll content, and fruit yield, and increased oxidative stress and accumulation of nanoparticles in roots, shoots, leaves, and fruits. This was attributed to the small hydrodynamic diameter of AgNPs of just 10-15 nm.[24] General increases in the activity of antioxidant enzymes and free amino acids in tomato have been shown to be part of a strategy to modulate oxidative stress induced by AgNPs of 20 nm size, with ~18-22 m^2/g specific surface area.[14] Still, the application of 12-36 mg/kg AgNPs with a particle diameter of 2-15 nm to the soil only decreased the dry biomass weight of shoots, but increased the root length of tomato plants, compared to their respective controls in the Brandywine tomato variety.[25] Also in the Brandywine tomato variety, the application of 10-30 mg/L AgNPs with a diameter of 46-48 nm decreased the total biomass weight compared to the control, which was due to severe damage to the structure of vascular tissues that impacted the gene expression of membrane transporters related to proton flux and P and S transport.[26] When applying 50 mg/L AgNPs with a mean diameter of 50 nm and spherical shape to 7 tomato varieties (Primo Early, Primo Early CH, Cal.j.n3, Early Urbana VF, King Stone, Super Stone, and Super Strain B), 5 of them (Early Urbana VF, Primo Early, Primo Early CH, Super Strain B, and Cal.j.n3) increased their germination percentages, while seedling vigor, root and shoot length, and general growth of seedlings decreased proportionally to the concentrations of AgNPs applied (0-100 mg/L AgNPs).[27] Since seed germination is a pivotal process for plant reproduction and survival, a plethora of measurements to evaluate this phenomenon have been developed and validated, including germination velocity, coefficient of velocity of germination, and time to germination.[28,29] The germination velocity (also known as speed of germination, germination rate index, index of velocity of germination, or emergence rate) refers to the rate of germination in terms of the total number of seeds that germinate in a time interval. The coefficient of velocity of germination (also known as coefficient of rate of germination or Kotowski's coefficient of velocity) considers the time from the start of the experiment to a determined interval, the number of seeds germinated in the time interval (not the accumulated number, but the number corresponding to the i-th interval), and the total number of time intervals.[30] We hypothesized that changes in the supply of AgNPs in different tomato cultivars would produce differential responses in relation to seed germination, initial growth and nutrient status of seedlings. To address our hypothesis, we proposed the following specific aims: (1) To establish a germination experiment under controlled conditions using tomato seeds to determine if the application of AgNPs had a hormetic effect on the coefficient of velocity of germination, germination velocity, and mean time to germination; (2) to analyze whether the application of AgNPs at certain concentrations to seedlings grown in a hydroponic system had a stimulatory or inhibitory effect on plant growth, biomass production, and the concentrations of the 3 major essential plant nutrients (N, P, and K) and the cultivars tested. In both cases (eg, germination and hydroponic approaches), the AgNPs we used had an average diameter of 35 nm determined by transmission electron microscopy.[31]

Methods

Silver Nanoparticles

Agrovit® brand AgNPs sold by Bionag (Tijuana, BC, Mexico) were used in this study. The commercial product is a solution of spherical AgNPs containing 12 mg/mL metallic silver and

188 mg/mL polyvinylpyrrolidone (PVP, 15-30 kD) in water, with an average content of 20% AgNPs (200 mg/mL AgNPs). The hydrodynamic diameter of metallic silver with PVP is 70 nm on average. A detailed characterization of the nanoparticles has already been published.[31] PVP is a strongly hydrophilic polymer that can serve as a surface stabilizer and nanoparticle dispersant that prevents the aggregation of nanoparticles.[32]

Germination

Tomato (*Solanum lycopersicum*) seeds from Vengador (Syngenta) and Rio Grande (Caloro) cultivars were used in this research. The experiment was performed inside plastic containers (12 cm long, 11 cm wide, 7 cm high), provided with a lid to reduce water loss. Once the plastic containers were disinfected with 70% ethanol, seeds were placed on sterile filter paper inside them. Each treatment was applied to 75 seeds, which were evenly distributed in 3 independent containers (25 seeds in each container). Then, 15 mL of one of the following treatments was added: 0, 5, 10, or 20 mg/L of AgNPs. The containers were closed and placed in the dark at 28°C for 3 days. After this time, seeds were exposed to natural light under laboratory conditions. Inside the closed plastic containers, the mean temperature was 26 C/22°C (day/night), relative humidity was 98%, and a standard 12 hours light/12 hours dark cycle was used. Every other day, seeds were watered with 3 mL of each treatment to maintain sufficient moisture in the plastic containers. Germination was recorded considering a seed whose radicle was more than 2 mm long as germinated. This observation was made from day 1 to day 20 after sowing. The calculations to determine germination velocity, coefficient of velocity of germination, and mean time to germination were done based on methodologies described elsewhere.[27,28,33]

Initial Plant Growth and Biomass Production

At 20 days after treatment application, the following variables were recorded in plants: height, root length, number of roots, fresh biomass weight, and dry biomass weight. To record the dry biomass weight, the plants were dried in a forced air oven (Riossa, HCF-125; Guadalajara, Jalisco, Mexico) at 72°C for 72 hours. The weights were recorded on an electronic scale (Ohaus, Adventure Pro AV213C; Pine Brook, NJ, USA).

Leaf Nutrient Concentrations

To measure the concentrations of essential nutrients N, P, and K in leaves, an open hydroponic system was established under greenhouse conditions. First, seeds were germinated inside 200-cavity plastic trays with agrolite as substrate. Once seedlings reached an age of 30 days, they were taken and transplanted into 3 L plastic containers that contained 100% Steiner's nutrient solution, which was completely replaced every 7 days for 2 weeks. The whole experiment in hydroponics lasted 2 weeks, with the first week considered as an acclimation period for the plants to the hydroponic system. During the second week, we applied AgNP treatments, exposing the plants to 0, 5, or 10 mg/L AgNPs for 7 days. The greenhouse had the following conditions: relative humidity of 75%, average temperature of 25°C, and photosynthetically active radiation of approximately 20 mol m^{-2} day^{-1}, with a 12/12 (day/night) photoperiod. Plants were harvested after 2 weeks of growth in hydroponics under greenhouse conditions. From the harvested plants, leaves were separated, placed in fully labeled paper bags, and dried at 72°C for 72 hours, in the previously described equipment. Once dried and at constant weight, the leaves were ground in a Wiley mill (Thomas Scientific; Swedesboro, NJ, USA) using a 2 mm sieve. To determine total N concentrations, the micro-Kjeldahl method was followed.[34] For P and K quantification, an acid digestion with HNO_3:$HClO_4$ (2:1, v:v) was performed, and for their determination, an inductively coupled plasma-optical emission spectrophotometer (ICP-OES 725-ES; Agilent; Santa Clara, CA, USA) was used.

Importantly, in order to ensure reliable, high-quality sources of purified water for our assays, we used a Milli-Q EQ 7000 Water Purification System, which offers a full qualification program, advanced data traceability, and accurate monitoring. In addition, water and nutrient solutions used in our studies were continuously monitored by ICP-OES. Multi Element Standards provided by Agilent were taken as references. Thus, we ensured that our solutions were not contaminated.

Statistical Analysis

For the variables of germination, plant height, root length, fresh and dry biomass weight of roots and shoots, as well as nutritional concentrations in leaves of both tomato cultivars, a linear mixed model was used with a normal response under the restricted maximum likelihood estimation method (REML). For the variable number of roots, the generalized linear mixed model with a Poisson distribution (λ_{ijk}) was used. As the name implies, counts refer to data with a nonnegative integer response variable and arise from studies tracking the number of occurrences, for example, number of diseased plants in a phytopathology study, number of insects or weeds in ecological or agricultural studies, etc. The Poisson distribution figures prominently in the modeling of count data. The experimental design used was completely randomized with a 2×4 factorial treatment arrangement. The linear mixed model for normal responses is given by

$$y_{ijk} = \alpha + b_k + g_i + \text{conc}_j + (g * \text{conc})_{ij} + \varepsilon_{ijk}$$

where y_{ijk} is the response observed in the k-th plant of the i-th cultivar under the j-th concentration of AgNPs, α is the intercept, b_k is the random effect due to container assuming

Table 1. Significance Analysis of The Effects of Cultivar, Concentration of AgNPs, and Their Interactions (Cultivar × AgNPs) on Germination Variables of Tomato (*Solanum Lycopersicum*) Seeds Exposed To Different Concentrations of AgNPs for 20 Days.

Study factor	Coefficient of velocity	Velocity of germination	Average time to germination
Cultivar	*	ns	*
AgNP concentration	ns	ns	ns
Cultivar × AgNPs	ns	ns	ns

Significant difference at $P \leq 0.05$ (*), non-significant difference (ns).
AgNPs, silver nanoparticles.

$b_k \sim N(0,\sigma_b^2)$, g_i is the effect of cultivar i, conc_j is the effect of concentration j of AgNPs, $(g * \text{conc})_{ij}$ is the effect of the interaction of both factors, and ε_{ijk} is the experimental error with mean zero and constant variance σ^2 $\{\varepsilon_{ijk} \sim iidN(0,\sigma^2)\}$.

Now, the linear predictor for non-normal response is given by

$$\eta_{ijk} = \alpha + b_k + g_i + \text{conc}_j + (g * \text{conc})_{ij}$$

where η_{ijk} is the linear predictor that relates the effects of factors $\alpha, b_k, g_i, \text{conc}_j$, and $(g * \text{conc})_{ij}$ as above. The link function is $\log(\lambda_{ijk}) = \eta_{ijk}$.

The maximum likelihood estimators were obtained using the REML and Laplace methods implemented by the GLIMMIX procedure of SAS 9.4. The comparison of means was done with Fisher's LSD test at a significance level of $\alpha = .05$.

Results are expressed as means of at least 3 independent experiments with either 75 seeds or 10 plants each, plus standard error of the mean (SEM).

Hormetic Effect

In order to test whether the root length response variable is hormetic, the data were adjusted to the Brain and Cousens model[35] defined as

$$E(y_{ij}) = c + \frac{d - c + fx_{ij}}{1 + (x_{ij}/e)^b}$$

where y_{ij} denotes the response in the j-th repetition at the i-th NP concentration, x_{ij} is the ij-th NP concentration level, c denotes the frequency response at infinite doses, d denotes the mean response of the non-treated control, f and e denote the degree of hormesis increase ($f > 0$ as a necessary condition for the presence of hormesis), and b is the size of the hormesis. For the statistical analysis, the R statistics software and the *dcr* library were used.

Results

Effect of Individual Factors and Their Interaction on Seed Germination

When analyzing the individual effects and the interactions of the factors, only the cultivar factor showed statistical significance on the coefficient of velocity of germination and mean time to germination (Table 1).

The coefficient of velocity was greater in seeds of the Vengador cultivar (15.05% higher than in Rio Grande), while the germination velocity was similar between the evaluated cultivars. In contrast, the Rio Grande cultivar showed the highest mean time to germination (17.76% more than Vengador) (Figure 1).

Effect of Individual Factors and their Interaction on Plant Growth Parameters and Biomass Production

The analysis of variance showed a significant effect of both the cultivar and the AgNP concentration individual factors, as well as the interaction of both factors (cultivar × AgNPs) on the variables of plant height, root length, number of roots, as well as fresh and dry biomass weight of shoots and roots (Table 2).

The effect of the cultivar as an individual factor demonstrated that Vengador showed higher means in the evaluated variables than did Rio Grande: 10.50% higher plant height; 11.66% longer root length, and 32.05% more roots (Figure 2).

When analyzing the single effect of the study factors (Figure 3), we observed that plant height decreased as the concentration of AgNPs in the nutrient solution increased; this reduction was 8.58, 13.53, and 22.11% when applying 5, 10, and 20 mg/L AgNPs, respectively, compared to the control. Contrarily, root length increased by 20.72 and 14.77% with 5 and 10 mg/L AgNPs, but decreased when applying 20 mg/L AgNPs. The number of roots improved by 81.82, 61.36, and more than 100% with the addition of 5, 10, and 20 mg/L of AgNPs compared to the control.

Due to the cultivar × AgNP interaction, average plant height did not vary between the control and the application of 5 mg/L AgNPs in the Vengador cultivar, while at this same concentration this variable decreased 11.30% in the Rio Grande cultivar (Figure 4). The application of 10 mg/L AgNPs decreased height by 20.74% in Vengador, while in Rio Grande, this variable was reduced by 17.34%, compared to the control. The concentration of 20 mg/L AgNPs decreased the height by more than 20% in both tomato cultivars evaluated, as compared to the control.

Root length showed an increase of 55.17% in Vengador when applying 5 mg/L AgNPs; however, in Rio Grande, this

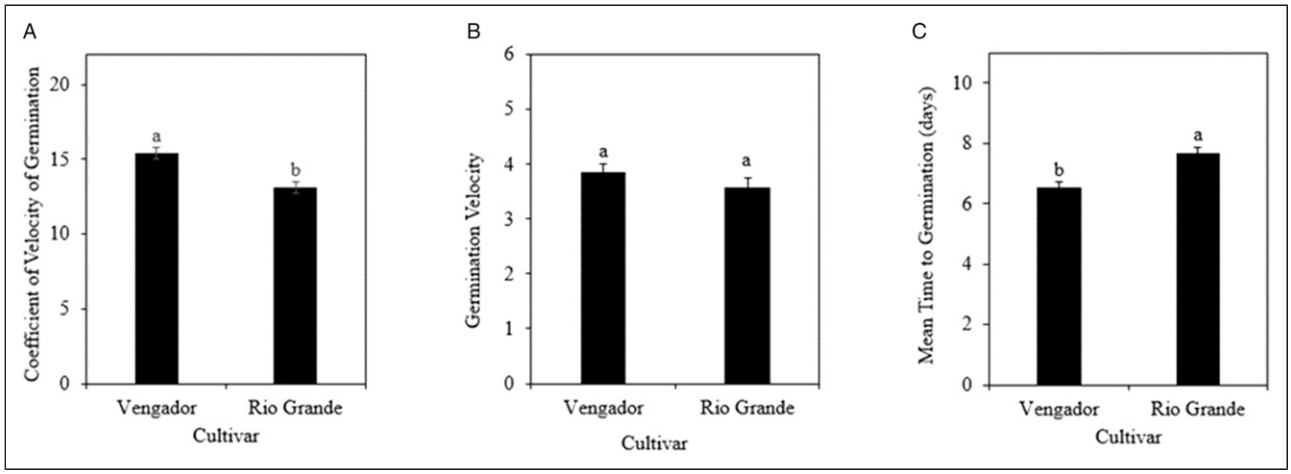

Figure 1. Individual effect of the cultivar factor on germination variables of tomato (*Solanum lycopersicum*) seeds treated with different concentrations of silver nanoparticles for 20 days. (A) Coefficient of velocity of germination; (B) germination velocity; (C) mean time to germination. Means ± SEM with different letters indicate significant differences (LSD, $\alpha = 0.05$).

Table 2. Significance Analysis of The Effects of The Cultivar and AgNP Concentration Factors and The Interactions Between Both Factors (Cultivar × AgNPs) on Initial Growth Variables of Tomato (*Solanum lycopersicum*) Plants Exposed to Different AgNPs Concentrations for 20 Days.

Study factor	Plant height	Root length	Number of roots	Fresh biomass weight of shoots	Fresh biomass weight of roots	Dry biomass weight of shoots	Dry biomass weight of roots
Cultivar	*	*	*	*	*	*	ns
AgNP concentration	*	*	*	*	*	*	ns
Cultivar × AgNPs	*	*	*	*	*	ns	ns

Significant difference at $P \leq 0.05$ (*), non-significant difference (ns). Data are means of 25 biological replicates. The experiment was repeated 3 times. AgNPs, silver nanoparticles.

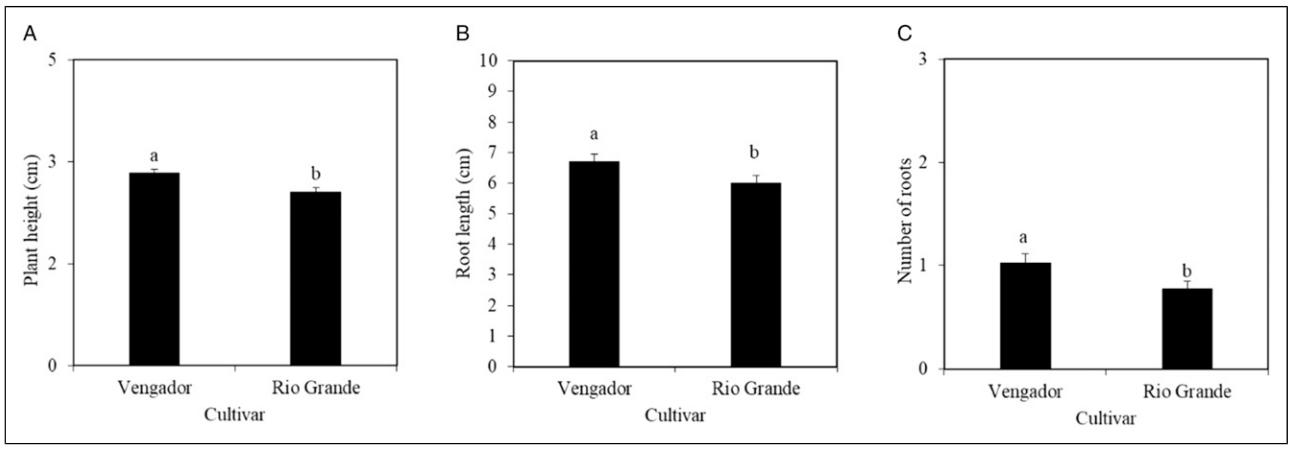

Figure 2. Individual effect of the cultivar factor on growth variables of tomato (*Solanum lycopersicum*) plants treated with different concentrations of AgNPs for 20 days. (A) Plant height; (B) root length; (C) number of roots. Means ± SEM with different letters indicate significant differences (LSD, $\alpha = 0.05$).

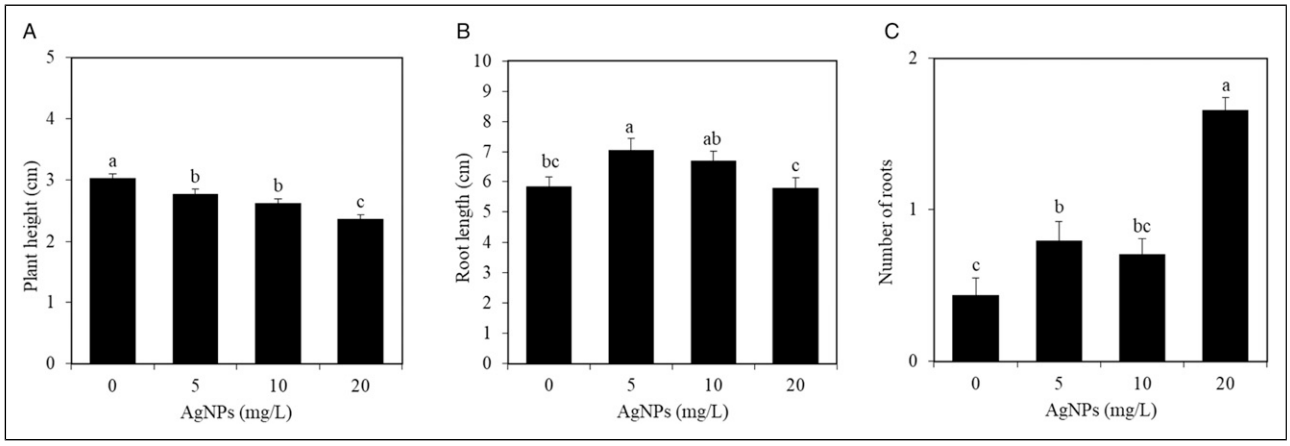

Figure 3. Individual effect of the AgNP factor on growth variables of tomato (*Solanum lycopersicum*) plants treated with different concentrations of AgNPs for 20 days. (A) Plant height; (B) root length; (C) number of roots. Means ± SEM with different letters indicate significant differences (LSD, $\alpha = 0.05$).

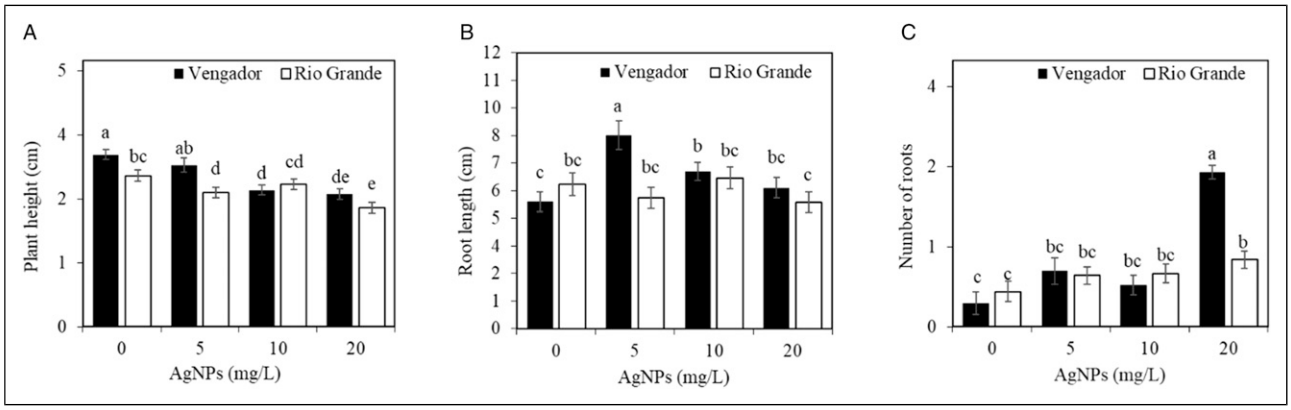

Figure 4. Effect of the interaction of cultivar × AgNPs on growth variables of tomato (*Solanum lycopersicum*) plants exposed to AgNPs for 20 days. (A) Plant height; (B) root length; (C) number of roots. Means ± SEM with different letters indicate significant differences (LSD, $\alpha = 0.05$).

variable decreased by 9.41%, both compared to the control. When applying 10 mg/L AgNPs, this variable increased 26.20% in Vengador and 4.47% in Rio Grande, also in both cases with respect to the control. When applying 20 mg/L AgNPs, the mean of this variable increased by 12.92% in Vengador, while in Rio Grande, it decreased by 12.26%, with respect to the control.

The number of roots in both tomato cultivars increased with the application of AgNPs, although only in the treatment with 20 mg/L AgNPs was this variable statistically different from the control, in both cultivars.

Regarding biomass production by effect of the cultivar factor, Vengador showed 26.40% greater fresh biomass weight and 26.80% more dry biomass weight of shoots as compared to Rio Grande. However, Rio Grande presented 16.26% higher fresh biomass weight and 15.09% greater dry biomass weight of roots. Nevertheless, significant differences between cultivars were only observed regarding the fresh biomass weight of the latter tissue (Figure 5).

The application of 5 mg/L AgNPs increased the fresh biomass weight of shoots by 11.60%, but when increased to 10 and 20 mg/L AgNPs, this variable decreased by 12.46 and 24.95%, compared to the control. In roots, this variable increased by 12.43 and 6.97% when 5 and 20 mg/L AgNPs were applied, respectively, with a decrease of 9.02% in plants treated with 10 mg/L AgNPs, compared to the control. Regarding the dry biomass weight of both shoots and roots, the treatments were statistically similar. However, important percentage variations were observed. The dry biomass weight of shoots increased 16.22% when applying 5 mg/L, and when applying 10 mg/L AgNPs, it decreased 18.02%, compared to the control. The dry biomass weight of roots increased by 54.54, 34.09, and 31.82% when applying 5, 10, and 20 mg/L AgNPs, respectively, compared to the control (Figure 6).

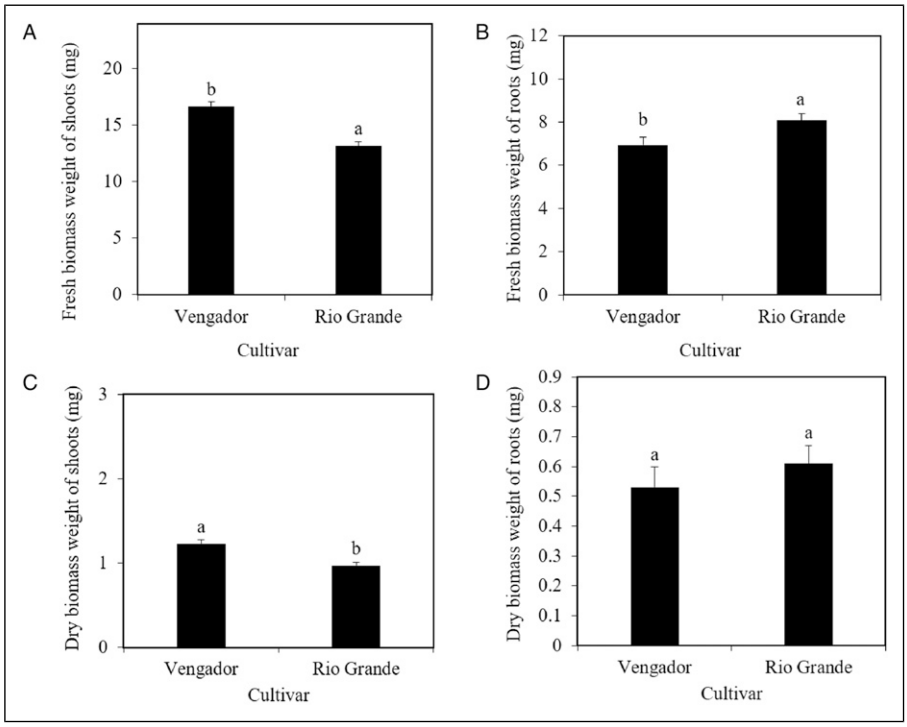

Figure 5. Individual effect of the cultivar factor on the biomass production of tomato (*Solanum lycopersicum*) plants treated with different concentrations of AgNPs for 20 days. (A) Fresh biomass weight of shoots; (B) fresh biomass weight of roots; (C) dry biomass weight of shoots; (D) dry biomass weight of roots. Means ± SEM with different letters indicate significant differences (LSD, $\alpha = 0.05$).

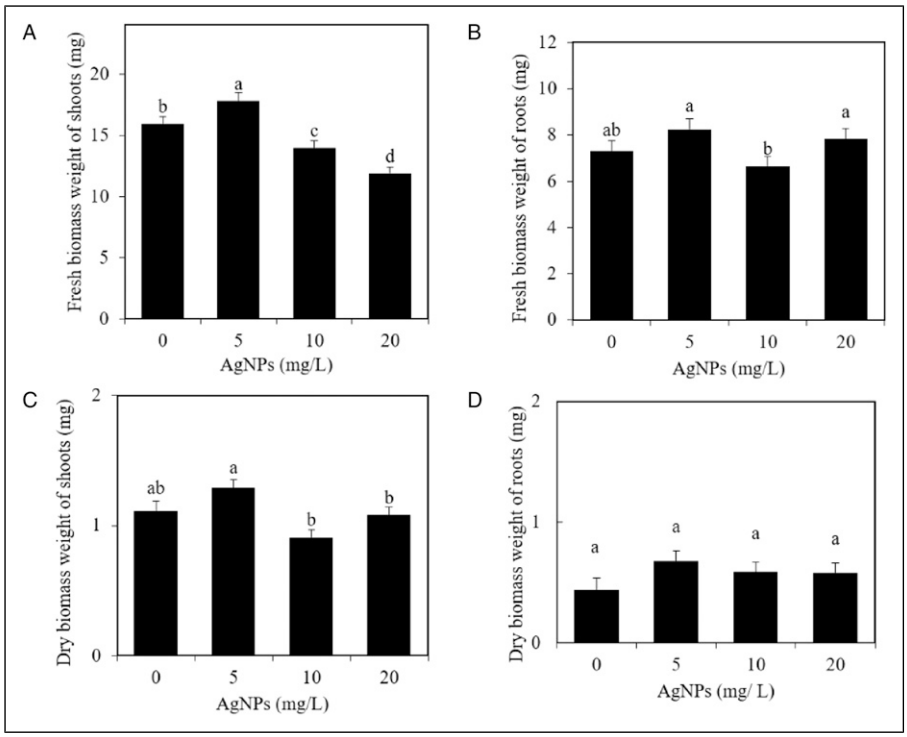

Figure 6. Individual effect of the AgNP factor on the biomass production of tomato (*Solanum lycopersicum*) plants exposed to AgNPs for 20 days. (A) Fresh biomass weight of shoots; (B) fresh biomass weight of roots; (C) dry biomass weight of shoots; (D) dry biomass weight of roots. Means ± SEM with different letters indicate significant differences (LSD, $\alpha = 0.05$).

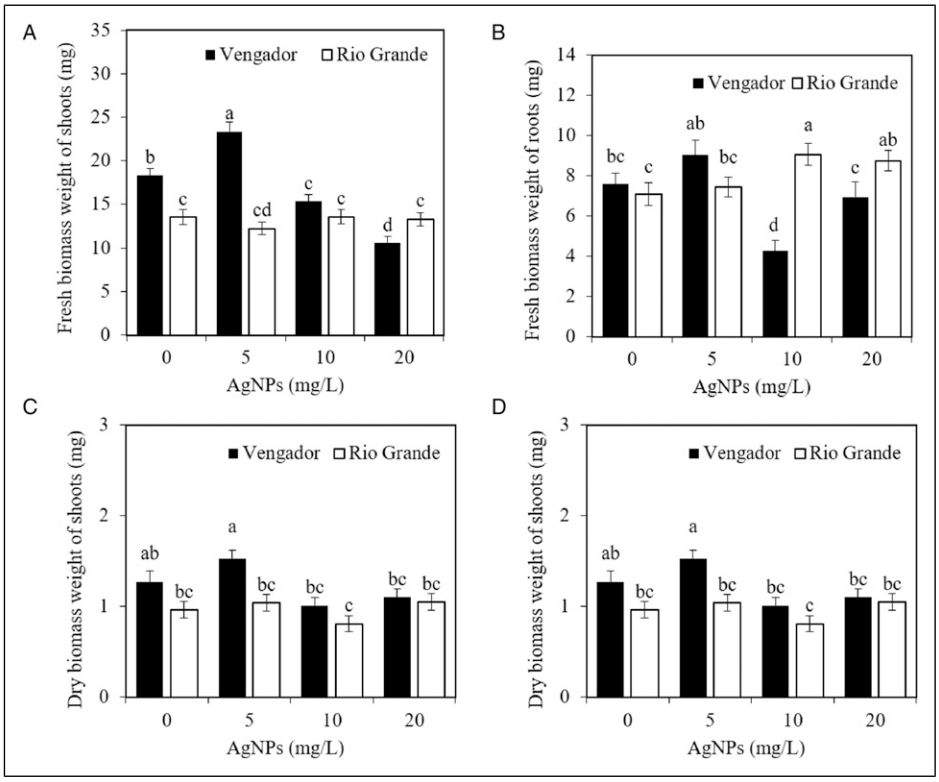

Figure 7. Effect of the interaction of cultivar × AgNPs on the fresh and dry biomass weight of shoots and roots of tomato (*Solanum lycopersicum*) plants exposed to AgNPs for 20 days. (A) Fresh biomass weight of shoots; (B) fresh biomass weight of roots; (C) dry biomass weight of shoots; (D) dry biomass weight of roots. Means ± SEM with different letters indicate significant differences (LSD, $\alpha = 0.05$).

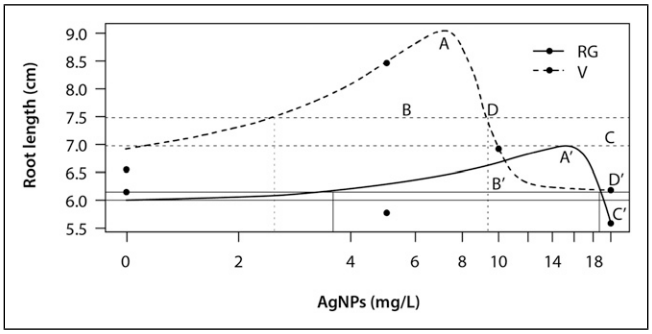

Figure 8. Hormetic response in the root length variable of tomato (*Solanum lycopersicum*) cv. Vengador (V) and Rio Grande (RG) plants exposed to different concentrations of AgNPs for 20 days. A and A': Maximum stimulatory response; B and B': hormetic zone, C and C': and D and D': toxic threshold. Letters without an apostrophe (A, B, C, and D) refer to Vengador, while letters with an apostrophe (A', B', C', and D') refer to Rio Grande.

The interaction between the study factors revealed an increase of 27.50% in the fresh biomass weight of shoots in Vengador when applying 5 mg/L AgNPs and a decrease of 21.82% when applying 10 mg/L AgNPs. This decrease was greater than 30% when applying 20 mg/L AgNPs, with respect to the control in this same cultivar (Figure 7). This same tendency was observed when evaluating the production of dry biomass of shoots in this cultivar. Nevertheless, it is important to highlight that the dry biomass weight of shoots increased by 20.47% in Vengador when adding 5 mg/L AgNPs. Regarding fresh biomass of roots in Vengador, the application of 5 mg/L AgNPs increased the average of this variable by more than 16%, compared to the control, although both treatments were statistically similar. However, the application of 10 and 20 mg/L AgNPs decreased this variable by 43.93 and 8.4%, respectively, compared to the control. In Rio Grande, the fresh biomass weight of shoots did not suffer any effect from the treatments, while fresh biomass of roots increased in all treatments with AgNPs, compared to the control, although only in the treatment with 10 mg/L AgNPs did this variable increase significantly, with respect to the control. The dry biomass weight of roots did not show significant changes between treatments, although Rio Grande exhibited higher means for this variable than Vengador did.

Hormetic Effect of AgNPs on Root Length

When analyzing the behavior of the root length variable, a dose–response hormetic effect was noted, characterized by stimulation at low doses and inhibition at high doses (Figure 8).

Table 3. Significance Analysis of the Effects of the Cultivar and AgNP Concentration Factors and Their Interactions (Cultivar × AgNPs) on the Concentrations of N, P, and K in the Leaves of Tomato (*Solanum Lycopersicum*) Plants Exposed to Different Concentrations of AgNPs for 7 Days.

Study factor	N	*P*	K
Cultivar	*	*	*
AgNP concentration	ns	*	*
Cultivar × AgNPs	*	*	*

Significant difference at $P \leq 0.05$ (*), non-significant difference (ns).

Table 4. Effect of the Cultivar Factor on the Concentrations of N, P, and K in the Leaves of Tomato (*Solanum lycopersicum*) Plants Exposed to Different Concentrations of AgNPs for 7 Days.

		P	
Cultivar	N	DBW (g/kg)	K
Vengador	22.24 ± 0.50 a	1.42 ± 0.06 b	7.39 ± 0.30 b
Rio Grande	17.06 ± 0.43 b	1.84 ± 0.05 a	8.64 ± 0.18 a

Means ± SEM with different letters indicate significant differences (LSD, α = 0.05).
AgNPs, silver nanoparticles; DBW, dry biomass weight.

Table 5. Effect of the AgNPs Factor on the Concentrations of N, P, and K in the Leaves of Tomato (*Solanum lycopersicum*) Plants Exposed to Different Concentrations of AgNPs for 7 Days.

		P	
AgNPs (mg/L)	N	DBW (g/kg)	K
0	18.98 ± 1.11 a	1.76 ± 0.05 a	7.71 ± 0.23 b
5	20.79 ± 0.85 a	1.77 ± 0.09 a	8.50 ± 0.45 a
10	19.17 ± 0.84 a	1.36 ± 0.08 b	7.85 ± 0.31 ab

Means ± SEM with different letters indicate significant differences (LSD, α = 0.05).
AgNPs, silver nanoparticles; DBW, dry biomass weight.

An inverted U-shaped dose–response curve was obtained. The hormetic response in the root length variable was more evident in Vengador than in Rio Grande. Vengador presented a greater increase in the mean value of this variable when applying 5 mg/L AgNPs, while the Rio Grande had a maximum response at 10 mg/L AgNPs. The mean value of this variable decreased when applying 20 mg/L AgNPs, in both cultivars evaluated.

Effect of Individual Factors and their Interaction on N, P, and K Concentrations in Leaves

The cultivar factor significantly affected the concentrations of N, P, and K in the leaves of tomato plants exposed to different concentrations of AgNPs (Table 3). The AgNP factor only affected the concentrations of P and K, while the interaction between the factors affected the concentration of the 3 macronutrients measured.

When analyzing the cultivar factor, it was observed that Vengador shows higher concentrations of N, while Rio Grande exhibits higher concentrations of P and K (Table 4).

The different levels of AgNPs did not affect the concentrations of N, while for P there is a decrease in its concentration when applying 10 mg/L AgNPs. Contrarily, K concentration increases in the plant leaves treated with both 5 and 10 mg/L AgNPs, although in the latter treatment the average is statistically similar to the control (Table 5).

The interaction of the study factors (cultivar × AgNPs) significantly affected the concentrations of the 3 essential elements analyzed (Table 6). Although statistically similar to the control, the application of 5 mg/L AgNPs increased the concentration of N in Vengador by approximately 6%. The highest concentration of P was found in the cultivar Rio Grande exposed to 5 mg/L AgNPs, a mean that was statistically similar to the respective control in this same cultivar. Likewise, the highest concentration of K was observed in the cultivar Rio Grande treated with 5 mg/L AgNPs.

Discussion

Silver nanoparticles have innumerable applications in agriculture, including their use as antiviral,[36] antibacterial,[37-39] and antifungal agents,[40-42] as well as preservatives of the shelf life of flowers, foliage, vegetables, and fruits,[13,43-45] and as a stimulant of plant metabolism and growth.[5,7,46,47] However, the responses to the application of AgNPs to plants can vary and depend on the type of plant species,[27,48] particle size,[49,50] composition, functionalization, concentration, and exposure time, among other factors.[51,52] For example, the effect of applying 0, 25, 50, 75, and 100 mg/L AgNPs (spherical, 50 nm average size) was analyzed in 7 tomato varieties and it was found that with 25 mg/L AgNPs the seed germination index is stimulated in only one genotype of the seven ones evaluated. Increasing to 75 and 100 mg/L AgNPs decreased this variable in 2 varieties, while the rest showed no change.[27] The decrease in the percentage of seed germination as a consequence of the application of AgNPs can be attributed to the destabilization of the plasma membrane[53] or the accelerated decomposition of reserve substances in seeds.[46] Since the reserve substances in tomato are lower than those of other seeds such as soybeans,[54] such accelerated consumption of these reservoirs promoted by AgNPs can negatively affect the germination process. In the present study, the germination indicators evaluated were not affected by the AgNP treatments tested, although differences were observed between cultivars with regard to the coefficient of velocity of germination and mean time to germination[28] (Table 1; Figure 1). Despite the absence of statistically significant differences from the application of AgNPs, percentage increases were observed in terms of

Table 6. Effect of The Interaction of Cultivar × AgNP Factors on the Concentrations of N, P, and K in the Leaves of Tomato (*Solanum Lycopersicum*) Plants Exposed to Different Concentrations of AgNPs for 7 Days.

			P	
Cultivar	AgNPs (mg/L)	N	DBW (g/kg)	K
Vengador	0	21.89 ± 1.14 a	1.67 ± 0.05 bc	8.08 ± 0.30 bc
Rio Grande	0	16.07 ± 0.87 c	1.85 ± 0.07 ab	7.34 ± 0.32 c
Vengador	5	23.16 ± 0.80 a	1.49 ± 0.03 c	7.10 ± 0.24 c
Rio Grande	5	18.42 ± 0.52 bc	2.05 ± 0.004 a	9.90 ± 0.25 a
Vengador	10	21.65 ± 0.60 ab	1.12 ± 0.04 d	7.00 ± 0.20 c
Rio Grande	10	16.69 ± 0.54 c	1.60 ± 0.06 c	8.70 ± 0.30 b

Means ± SEM with different letters indicate significant differences (LSD, $\alpha = 0.05$).
AgNPs, silver nanoparticles; DBW, dry biomass weight.

coefficient of velocity of germination and germination velocity, especially with 5 and 10 mg/L AgNPs.

Initial growth after the application of AgNPs showed differences among treatments (Figures 2-4). Plant height decreased significantly in both cultivars evaluated due to the application of AgNPs. Similarly, the addition of 1000 mg/L AgNPs to mung bean (*Vigna radiata*) plants decreased shoot length.[55] Contrastingly, the application of 40, 60, and 100 mg/L AgNPs to basil (*Ocimum basilicum*) plants improved plant height by 23.93, 33.79, and 28.52% compared to the control.[56]

Root length in the Vengador cultivar increased significantly with the addition of AgNPs, although in the Rio Grande cultivar the application of AgNPs showed no significant effects on this variable. In brown mustard (*Brassica juncea*), the application of 25 and 50 mg/L AgNPs increased root length by 100% compared to the control, which was attributed to the fact that AgNPs modulate the antioxidant activity of the plant in favor of cell growth and expansion.[57] Furthermore, in pepper (*Capsicum annuum*), the application of AgNPs increased the concentrations of zeatin,[18] a phytohormone involved in root growth.[58] Root growth promoted by AgNPs can also stimulate the synthesis of gibberellins,[59] phytohormones involved in seed germination, shoot elongation, growth, flowering, and fruit development, as well as in the growth of roots at very low concentrations.[60,61] In castor bean (*Ricinus communis*), the application of 500-4000 mg/L AgNPs did not alter root growth, but its application improved the activity of SOD.[62] In millet, the addition of AgNPs decreased the length of the roots and shoots, since the penetration of AgNPs into the root system interferes with intercellular components and negatively affects cell division.[12] In mung bean, the application of 100 and 1000 mg/L AgNPs decreased root length.[55] Furthermore, the application of 40 mg/L AgNPs to mung bean and sorghum plants decreased vegetative growth,[16] and its addition to water hyssop (*Bacopa monnieri*) plants reduced root length.[63] These effects may be due to the inhibition of the mitotic index, as well as a potential chromosomal adherence and alterations in the achromatic spindle of meristematic cells caused by the application of AgNPs.[20]

In our study, both evaluated cultivars treated with AgNPs showed a significant increase in the number of roots, compared to control plants. AgNPs can stimulate the biosynthesis of auxins,[64] phytohormones that promote the development of lateral roots,[65] which may partly explain our results. Lateral root development is antagonistically regulated by auxins and cytokinins. Auxins promote lateral root development, while high concentrations of cytokinins disrupt lateral root initiation and the regular pattern of cell division. The cells of the pericycle of the xylem pole (from which the lateral roots are formed) are sensitive to cytokinins, whereas the primordia of the young lateral roots are not.[66] Transactivation of the cytokinin oxidase 1 enzyme that degrades cytokinins in lateral root founder cells results in an increase in the formation of lateral roots.[67] Cytokinins disrupt *PIN* genes (pin-shaped inflorescences) expression in lateral root founder cells and prevent the formation of an auxin gradient that is required to control lateral root primordia.[68] Therefore, the stimulation of the number of roots and the length of roots in our study must have been mediated by a balance between auxins and cytokinins triggered by the presence of AgNPs. It has been also reported that abscisic acid (ABA) promotes root growth[69] by regulating the expression of genes involved in ABA signaling and inhibiting ethylene biosynthesis.[64] ABA accumulation maintains primary root elongation at low water potentials by restricting ethylene metabolism.[70] Hence, the hormetic effects of AgNPs on root growth that we observed under our experimental conditions may be the result of an efficient stimulation of ABA and auxin biosynthesis and signaling. The stimulation responses at low doses and inhibition at high doses by AgNPs in the root length variable (Figure 8) are related to the hormetic effect of AgNPs.[71] This response was different in the evaluated cultivars, possibly due to their biological plasticity.[72] Moreover, the hormesis induced by AgNPs showed a different behavior between the aerial part and the root, and this hormetic effect was greater in the roots.

Under our experimental conditions, the application of AgNPs resulted in a higher allocation of root biomass as compared to the shoot. In nature, plants assign more resources to organs facing more stressful challenges. In this case, AgNPs

may have acted as a stressor, reducing water uptake,[73] which in turn could lead to an increase in root biomass. Nevertheless, nanoparticles coated with PVP reduce the negative effect of AgNPs by giving them stability and reducing their negative effect.[74]

The application of 5 mg/L increased the fresh biomass weight of shoots in Vengador, but when increasing to 10 and 20 mg/L AgNPs, the value of this variable decreased. In Rio Grande, no significant effects of AgNPs were observed in any of the applied concentrations (Figure 5). In ryegrass (*Lolium multiflorum*), the application of AgNPs improves the biomass weight of shoots by 55%.[75] The application of AgNPs exerts biostimulant effects on plant growth. These effects are due to the fact that AgNPs regulate the antioxidant capacity and the expression of genes involved in cell proliferation, photosynthesis, and hormonal signaling, especially of auxins, ABA, and ethylene.[64]

In our study, fresh biomass weight of roots increased in the Vengador cultivar when applying 5 mg/L AgNPs, although this value was statistically similar to the control. When increasing the concentration to 10 and 20 mg/L AgNPs, the average value of this variable decreased in Vengador. In Rio Grande, on the contrary, the fresh biomass weight of roots increased with 10 and 20 mg/L AgNPs, while with 5 mg/L AgNPs the mean was similar to the control (Figure 5). In *Arabidopsis thaliana*, the application of 3 mg/L AgNPs decreased biomass weight.[73] In pepper, the addition of AgNPs also decreased the fresh biomass weight of roots, shoots, and leaves, which could be attributed to a decrease in chlorophyll content and photosynthesis.[18] In duckweed, the addition of AgNPs decreases the chlorophyll content, while altering the transfer of electrons from the light collection complexes to the reaction centers, as well as the oxygen evolution complex in photosystem II. This, in turn, affects ATP and NADPH synthesis.[17] When accumulating in leaves, AgNPs can alter the structure of the thylakoid membrane and decrease chlorophyll content and biomass production.[73,76]

Dry biomass weight of shoots increased in plants exposed to 5 mg/L AgNPs in Vengador, although the value was statistically similar to that observed in the control plants (Figures 6 and 7). In this same cultivar, the concentrations of 10 and 20 mg/L AgNPs decreased the mean value of the dry biomass weight of shoots. In Rio Grande, no significant differences were observed between treatments with respect to this variable. In wheat (*Triticum aestivum*), the application of AgNPs decreased the biomass weight when applying from 20 to 100 mg/L.[77]

Dry biomass weight of roots suffered no effect from the application of AgNPs in either of the evaluated cultivars. In cucumber (*Cucumis sativus*), the addition of 50 and 100 mg/L AgNPs reduced this variable, due to the toxic effect from the amount of nanoparticles applied.[77] Similar reductions in the biomass weight of roots have been observed in common beans (*Phaseolus vulgaris*) and maize (*Zea mays*) when adding 60 mg/L AgNPs.[78]

The concentrations of N, P, and K were differentially affected by the study factors (Table 3). The highest concentration of N was observed in Vengador treated with 5 mg/L AgNPs (Tables 4-6). Phosphate and potassium showed their highest concentrations in Rio Grande treated with 5 mg/L AgNPs. In lily (*Lilium* spp.) cv. Mona Lisa, the concentrations and accumulations of N and K increased in plants treated with 25 and 50 mg/L AgNPs, but decreased when raising AgNPs to 100 and 150 mg/L, while those of P were not affected by the addition of AgNPs.[5] In common beans, the application of AgNPs to the soil considerably increases the chlorophyll content in leaves, as well as the absorption of N and P, the accumulation of proteins, and the expression of genes that encode the nitrate reductase and ferredoxin enzymes.[79] In wheat, the application of 25 mg/L AgNPs increased the absorption and efficiency in the use of N, P, and K, which improved growth and yield indicators.[80] Since AgNPs can affect the fluidity and permeability of the cell membrane, they influence water and nutrient uptake.[81] Inside plant cells, AgNPs may cause inhibition of apoplastic trafficking by clogging of pores and barriers in the cell wall or the nano-sized plasmodesmata, thereby effectively inhibiting the apoplastic flow of water and nutrients.[82,83]

Silver nanoparticles with a different size and shape may display equal cytotoxicity, but have different effects in the organism.[84] Indeed, the final effects induced by AgNPs are highly dependent on the physical and chemical properties of the particular batch of nanoparticles, which can vary considerably even if obtained from the same supplier. The shape of the nanoparticles can be a key factor in determining their effects on an organism.[85] The AgNPs we tested were spherical in shape, 35 nm in diameter, 70 nm in hydrodynamic diameter of metallic silver with PVP, and contained 12 mg/mL metallic silver and 188 mg/mL PVP (15-30 kD) in water, with an average content of 20% AgNPs (200 mg/mL AgNPs). Hence, our results may be comparable to those using similar nanoparticles but may differ from those displaying different physical and chemical properties.

Conclusion

We conclude that the AgNPs tested stimulate initial growth and biomass production of tomato and improve the nutrient status of the plants, while germination was not significantly affected under our experimental conditions. Although plant height was negatively affected by the application of AgNPs, root growth, number of roots, and production of fresh and dry biomass of shoots and fresh biomass of roots increase when applying AgNPs. Furthermore, the concentrations of the 3 main essential nutrients measured, N, P, and K, increased with the addition of 5 mg/L AgNPs, with highest mean of N in Vengador, and of P and K in Rio Grande. Thus, the cultivars we evaluated displayed different dose–response curves to the application of AgNPs.

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