Titanium enhances germination, fresh biomass accumulation and initial growth in tomato and stimulates stem and root length in a hormetic manner (#114472)

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Titanium enhances germination, fresh biomass accumulation and initial growth in tomato and stimulates stem and root length in a hormetic manner

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Background: Titanium (Ti) may induce biostimulant responses that can display hormetic curves, while supplied as titanium oxide nanoparticles (*n*TiO_2), it may enhance germination and plant growth.

Methods: Tomato (*Solanum lycopersicum* L.) seeds cv. Rio Grande were imbibed in solutions containing 0.0, 52.2, 104.4, 156.6, or 208.8 µM Ti supplied as *n*TiO_2. The germination experiment lasted 16 d, during which we estimated seed weight increase after imbibition; total germination percentage (TGP); germination speed coefficient (GSC); and the vigor indexes I and II (VI I and II). Seedling were grown for 30 days after sowing and at the end we measured lengths of stems and roots, number of roots and leaves, water content, as well as fresh and dry biomass weight. In addition, the potential hormetic effect of Ti on length of roots and stems was estimated.

Results: Applications of 156.6 and 208.8 µM Ti significantly increased vigor index I, root length and total water content in leaves, while applying 208.8 µM Ti significantly increased fresh biomass weight of roots. The hormetic analysis revealed that the application of 156.6 µM Ti stimulated the length of roots and stems, with different dose-response curves.

Conclusion: The application of *n*TiO_2 to tomato seeds improved some parameters of germination and plant growth during the initial growth stage, demonstrating its biostimulant effects in a hormetic manner.

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Short title: Ti biostimulates tomato

ABSTRACT

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Keywords: Beneficial elements, germination, hormesis, nanoparticles**,** Solanaceae, *Solanum lycopersicum*.

INTRODUCTION

Titanium is a transition metal found in a concentration of 0.57% of the Earth's crust, ranking ninth among the chemical elements of the periodic table in terms of abundance (*Gosen & Ellefsen, 2018*). Of the transition metals, Ti is mainly extracted from minerals such as rutile, composed of titanium dioxide (TiO_2), while other rare polymorphs of TiO_2 include akaogiite, anatase, and brookite. Other common Ti-containing minerals include perovskite ($CaTiO_3$), titanite ($CaTiSiO_5$), ilmenite ($FeTiO_3$), titanite ($CaTiSiO_5$) and leucoxene, the latter exhibiting an extremely variable chemical composition to be expressed as a chemical formula. Importantly, ilmenite, leucoxene, and rutile are the only commercially valuable Ti-containing minerals (*Kotova et al., 2016; Maldybayev et al., 2024*).

Ilmenite and rutile are the most important minerals for obtaining Ti. The former provides about 92% of the world's Ti supply, is typically more abundant in sands with transition metals, and naturally contains between 55 and 65% TiO_2. Rutile is the most abundant of naturally occurring form TiO_2 and has between 95 and 100% of this dioxide; however, its abundance is generally lower than that of ilmenite in the source deposits. Ilmenite is processed to produce Ti concentrates, either as synthetic rutile or titaniferous slag, which are transformed into TiO_2 powder (*Bedinger, 2018*). This material (TiO_2 powder) has important applications in chemicals, ceramics, coatings, inks, masterbatches, paints, paper, and plastics, since it increases brightness, gloss, opacity, reflective index, and whiteness, while acting as a photocatalytic antimicrobial compound. It is also employed in mining for the extraction of other metals, as well as in the jewelry, medical, aerospace and space, military and defense industries. The main producing countries of mineral titanium are Australia, South Africa, China, and Mozambique, and global production of this element amounts to more than 10.3 million megagrams (Mg), with annual increases of approximately 4% (*Gosen &*

Ellefsen, 2018). In the United States of America, the estimated value of Ti mineral concentrates consumed is greater than 670 million dollars (*Bedinger, 2018; Gosen & Ellefsen, 2018*). Given this expansion of the use of titanium, greater exposure of living beings and the environment to the metal is expected; therefore, it is necessary to have more detailed knowledge of its effects on various biological systems and the environment.

Since climate change and population growth (9.8 billion inhabitants by 2050, globally) represent two of the main challenges humanity is currently facing, agricultural systems require developing and adapting technologies to produce in more limiting environments and reduce the negative impact of stress factors of both biotic and abiotic nature. Consequently, in recent years, the use of nanotechnology has taken an important role in agriculture as a tool to improve crop production systems, allowing the controlled release of agrochemicals (nanoherbicides, nanofungicides, nanofertilizers, etc.) in order to improve productivity and food supply, since an increase of 56% in food demand is estimated by 2050 (*Chaud et al., 2021; van Dijk et al., 2021; Santás-Miguel et al., 2023*).

Titanium oxide nanoparticles ($n\mathrm{TiO_2}$) are made from the mixture of anatase and rutile in their crystalline forms, and have anticorrosive and photocatalytic properties (*Shi et al., 2013*), which allows them to be used in beauty products, food additives (E171), or as excipient in medicines, among other uses, without knowing any restriction regarding the maximum dose allowed by the World Health Organization, the Food and Agriculture Organization of the United Nations, or the European Union (*Lyu et al., 2017*). Due to its growing demand, the current production of titanium dioxide nanoparticles is close to 88,000 Mg year^{-1}, making it the nanomaterial with the greatest release into the environment. In Europe, an input of 0.13 µg $n\mathrm{TiO_2}$ kg^{-1} of soil yearly is estimated, with the potential to increase to 1200 µg kg^{-1} year^{-1} due to waste sludge discharges (*Abukabda et al., 2017; Tiwari et al., 2017; Radziwill-Bienkowska et al., 2018*).

In the agricultural, food, aerospace, cosmetics, and medical industries, the use of metals in the form of nanoparticles is very common due to the various properties that they provide, which contributes to their accumulation in waste sludge whose effects on the biota and the environment are not yet known in detail (*Tangahu et al., 2011; Tchounwou et al., 2012; Keller et al., 2013*).

The potential positive, neutral, or negative effects of non-essential elements, such as Ti, in higher plants are mediated by processes of hormesis, a dose-response relationship conditioned by environmental factors, which at low doses stimulate biological processes, but inhibit them at high doses. This natural phenomenon represents an evolutionary strategy of the species, restricted by biological plasticity that allows adaptive responses to environmental challenges (*Calabrese & Mattson, 2017; Lee et al., 2020*). Hormetic responses are considered normal within the physiological functions of an organism at the cellular level, which allows a pre-conditioning to a specific environment. The control of hormetic responses in plants can contribute to improving growth, development, and production indicators in agricultural systems (*Matson, 2008; Agathokleous & Calabrese, 2019*).

In plant biology, titanium is considered a beneficial element, which can trigger hormetic responses either applied as bulk material or as nanoparticle (*Hong et al., 2005; Lyu et al., 2027*). Just recently, *Trela-Makowej, Orzechowska & Renata Szymanska (2024)* performed an integrative analysis of the effects of various concentration of $n\mathrm{TiO_2}$ on different plant species. For instance, the application of $n\mathrm{TiO_2}$ to spinach (*Spinacia oleracea* L.) increased the enzymatic activities of catalase (CAT), superoxide dismutase (SOD), and peroxidase (POX), decreased the levels of malondialdehyde (MDA) and that of reactive oxygen species (ROS), while keeping the stability of plastid membranes under high light conditions (*Hong et al., 2005*). In spinach, it also stimulated the enzymatic activity of glutamate dehydrogenase (GDH), glutamine synthase (GS), glutamate-pyruvate transaminase (GPT) and nitrate reductase (NR) (*Fan et al., 2006*). In wheat (*Triticum aestivum* L.), Ti reduced the mean germination time and improved seedling development (*Feizi et al., 2012*). In fennel (*Foeniculum vulgare* P. Mill.), $n\mathrm{TiO_2}$ enhansed seed germination percentage and seedling growth rate (*Feizi et al., 2013*). In onion (*Allium cepa* L.), $n\mathrm{TiO_2}$ indiced the germination of seeds and the growth of seedlings, in addition to inducing the activity of hydrolytic and antioxidant enzymes (*Laware & Raskar, 2014*). In mung bean (*Vigna radiata* [L.] R. Wilczek), $n\mathrm{TiO_2}$ affected the germination mechanism and grain growth rate (*Mathew, Sunny & Shanmugam, 2021*). In tomato (*Solanum lycopersicum* L.), the application of a $n\mathrm{TiO_2}$ (80:20 anatase:rutile), with a 27 nm particle size had neither toxic nor stimulatory effects on seeds or plants (*Song et al., 2013*). Nevertheless, little has been explored about the hormetic effect of nanotitanium on the processes of seed germination and seedling growth, performing in-depth mathematical and statistical analyses. Herein we aimed to evaluate the effect of the application of $n\mathrm{TiO_2}$ at five doses (0, 52.2, 104.4, 156.6, and 208.8 μM Ti), on seed germination indicators, as well as on variables related to initial growth of tomato seedlings, in order to derive potential hormetic curves. The hypothesis to be tested was that $n\mathrm{TiO_2}$ may have biostimulant effects on the processes of seed germination and initial growth of tomato seedlings and that the variables evaluated may display dose-response hormetic curves.

MATERIALS AND METHODS

Plant material and treatments

Saladette type hybrid tomato seeds cv. Rio Grande were provided by Geneseeds (Mexico). Healty and homogeneous seeds (without observable physical damage) were selected, and then weighed in groups of ten. Subsequently, a stock solution of titanium dioxide nanoparticles (*n*TiO_2, 99.7% Sigma-Aldrich, <25 nm) with a concentration of 208.8 µM was prepared. Dilutions were made from the stock solution to 52.2, 104.4, and 156.6 µM *n*TiO_2; the control solution was distilled water. The seeds underwent an imbibition process for 24 h under controlled laboratory conditions, following the methodology described by *Dzib-Ek et al. (2021)*. Accordingly, 10 seeds were placed in bottles containing 30 mL of each of the solutions to be evaluated. After imbibition, the seeds were removed from the jars, dried with absorbent paper, and weighed again. With the difference in weights, the seed weight increase after imbibition (SWIAI) was estimated (*ISTA, 2010*).

Germination

After seed imbibition for 24 h in the different *n*TiO_2 solutions, groups of 10 seeds were placed in plastic containers with lids (12 × 11 × 7 cm). Each container was provided with an 11 x 10 cm piece of filter paper, in which 5 mL of distilled water were pured. During the time the experiment was carried out, the moisture of the experimental unit was maintained by adding 5 mL of distilled water every third day. The experimantal unit was represented by a container with 10 seeds. For seed germination measurements, records were taken every 24 h; once data was contant, we stoped taking recors and performed the calculations.

Germination kinetics

Germination recordings were done daily for 16 d. Any seeds that reached a radicle more than 2 mm long were considered germinated seeds.

Total Germination Percentage (TGP). This variable was estimated according to *Billard et al. (2014)*. The TGP measures the real percentage value of germinated seeds, and considers the maximum germination value reached in the kinetics (constant value), as follows:

$$\mathrm{TGP} = \frac{Sprouted\ seeds}{Total\ seeds} \times 100$$

Germination Speed Coefficient (GSC). It was estimated according to what was described by *Kader (2005)* as follows:

$$\mathbf{GSC} = \frac{\mathbf{Total\ number\ of\ germinated\ seeds\ per\ experimental\ unit}}{\mathbf{A1T1 + A2T2 + AXTX}}$$

Where A1, A2… = The number of seeds sprouted in a particular number of days. T1, T2… is the number of seed germination days after the start of incubation.

Vigor index I and II. They were determined according to the methodology proposed by Vashisth *& Nagarajan (2010)*, using the following formulas:

Vigor index I = (Percentage of Germination) × Seedling length (Root+Stem)

Vigor index II = (Percentage of Germination) × Seedling weight (Root+Stem)

Initial growth, biomass production, and total water content

Thirty-five days after sowing (das), the lengths of the shoot and main root were taken with a 10 cm long graduated ruler. The number of secondary roots was counted manually using a magnifying glass, and the number of leaves was also counted manually.

Weight of fresh and dry biomass of roots, stems, and leaves

After 35 d of sowing the seeds, the shoots and roots of each seedling were separated in a germination box. The samples were weighed on an Adventurer Ohaus Pro AV213C analytical balance (Parsippany, NJ, USA) to obtain the weight of fresh biomass. Subsequently, samples were depostdid in paper bags to be dried at 70 °C for 48 h in a forced air oven (Riossa HCF-125D; Guadalajara, Jalisco, Mexico). Finally, the weight of the dry biomass was obtained on the analytical balance.

Total Water Content (TWC). It was determined in roots, stems, and leaves, following the protocol described by *Jones & Turner (1978)*, as follows:

$$\text{TWC (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Hormetic effects

To test whether the shoot and root length variables displayed hormetic dose curves in response to $n\mathrm{TiO_2}$, data were fitted to the model developed by *Brain & Cousens (1989)*, which is defined as:

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

Where y_{ij} represents the response in the j^{th} repetition at the i^{th} concentration of $n\mathrm{TiO_2}$; X_{ij} is the i^{th} concentration level of $n\mathrm{TiO_2}$; *c* indicates the frequency response at infinite doses; *d* represents the average response of the untreated control; *f* and *e* designate the degree of increase in hormesis ($f > 0$ as a necessary condition for the presence of hormesis); and *b* is the size of hormesis. All these statistical analyses were run using the R statistical software (*Venables & Smith, 2024*) and the dcr library (*Ritz et al., 2015*).

Experimental design and statistical analysis

In this study we established a completely randomized experimental design with five treatments and three replicates. The experimental unit was a plastic container with 10 seeds. With the results obtained, we firstly performed an analysis of variance and then a mean comparison test using the LSD method ($p \leq 0.05$). For this, the SAS software was used (*SAS, 2011*).

RESULTS

Weight gain in tomato seeds

In the study, $n\mathrm{TiO_2}$ did not have significant effects on seed weight increase after imbibition (SWIAI) compared to the control. However, seeds imbibed with 104.4 µM Ti gained 114.5% more weight than seeds imbibed with 208.8 µM Ti, and this difference was significant (**Fig. 1**).

****Figure 1**

Germination variables

As observed in **Table 1**, the means of the TGP were similar among all the treatments tested. Likiweise, Ti doses had no effect on the germination speed coefficient (GSC) since all Ti treatments were statistically similar to the control. In the case of vigor indexes I (VI I) and II (VI II), similar trends were observed between them, but with a different statistical interpretation. Regarding VI I, the seedlings from seeds treated with 156.6 and 208.8 µM Ti showed a higher value compared to the control by 26 and 27%, respectively. For VI II, the application of 208.8 µM Ti resulted in higher means as compared to the application of 104.4 µM Ti, and though the highest dose of Ti applied (i.e., 208.8 µM Ti) surpassed the control by 21.7%, all means were similar to the control.

****Table 1**

Root and stem length, number of roots and leaves

The average values of the variables root length, and stem length, number of roots and number of leaves are displayed in **Table 2**. The greatest root length was observed in seedlings of the seeds exposed to 156.6 µM, with an increase of almost 31% respect to the control. Tough means of stem length were all statistically similar, the 156.6 µM Ti dose resulted in an increase of 16.3% as copared to the control. The treatments applied did not affect the number of lateral or secondary roots. However, the number of leaves decreased by 32.2, 37.1, and 26.6% when applying 52.2, 104.4, and 156.6 µM Ti, respectively, compared to the control.

****Table 2**

Total water content in tomato seedlings

The water content in roots, stems, and leaves were differently affected by the Ti treatments tested (**Fig. 2**). In roots and stems, no differences were detected among the treatments tested (**Fig. 2A and 2B**). In leaves, the water content in seedlings from seeds treated with 208.8 µM Ti was higher by 2.6%, when compared to the control treatment (**Fig. 2C**).

****Figure 2**

Fresh and dry biomass

Different responses were found in the means of fresh and dry biomass weight in seedlings from seeds treated with Ti. With the 208.8 µM Ti dose, the greatest amount of fresh biomass was produced in roots, with an average increase of 35.2% compared to the control. The 52.2 and 104.4 µM Ti doses reduced fresh stem biomass by 20.9 and 45.7% compared to the control. In leaves, the application of 208.8 µM Ti increased the fresh biomass weight by 23.9% as compared to the application of 104.4 µM Ti, though in the end, all Ti treatments were similar to the control. Similar results were observed for the total fresh biomass of the seedlings, since seeds imbibed with 208.8 µM Ti resulted in seedlings with 27.8% more biomass than those treated with 104.4 µM Ti, while all means were similar to the control.

The dry biomass weight of roots was statistically similar among treatments. In stems, the 104.4 µM Ti doses applied to the seed decreased the dry biomass weight of seedlings by 43.2%, compared to the control. Dry biomass weight of leaves decreased by 27.1% when seeds were treated with 104.4 µM Ti, as compared to the control. The total dry biomass of the seedlings was lower by 24.2% in seedlings from seeds imbibed with 104.4 µM Ti than those treated with 0 µM Ti (**Table 3**).

****Table 3**

Hormetic effect

According to our analyses, the application of Ti resulted in hormetic dose-response effects for root length (RL) and shoot length (SL), with a stimulatory behaviour at low or intermediate Ti doses, while at high Ti doses it was inhibitory (**Fig. 3**). The dose-response curves obtained were inverted U-shaped for SL and J-shaped for RL (*Kendig, Le & Belcher, 2010*). The dose with 104.4 µM Ti remained above the hormetic zone for SL. The dose of 156.6 µM Ti caused the highest stimulation value, while, with 208.8 µM Ti, toxicity was observed at the threshold. Regarding RL, it was observed that the dose of 104.4 µM Ti tends to decrease the value of this variable, while the doses of 156.6 and 208.8 tend to increase it.

****Figure 3**

DISCUSSION

The seed is considered the main reproductive organ in vascular plants. This is formed from the plant ovule after fertilization. Germination is a fundamental process by which the hydrated embryo of a seed develops an axis and culminates in the emergence of a new plant. During this process the state of latency is broken, and embryonic growth begins. Under optimal conditions of moisture, temperature, oxygenation, and luminosity, the germination process shows better responses (*ISTA, 2005; Ruiz-Nieves et al., 2021*).

The imbibition process is how seeds absorb water, allowing the level of moisture to be homogeneous and the processes of respiration, protein synthesis, and mobilization of reserves and germination to be activated. Imbibition in water or solutions improves germination in seeds of different species (*Balaguera-López, Deaquiz & Alvarez-Herrera, 2009; Pérez et al., 2016; Pompelli, Jarma-Orozco & Rodríguez-Páez, 2023*).

Titanium nanoparticles ($n\mathrm{TiO_2}$) can increase resistance to stress and promote water penetration to the seed, which causes greater weight gain (*Khot et al., 2012*). The effects of $n\mathrm{TiO_2}$ can be influenced by factors such as type of nanoparticle, dose, form of application, exposure time, and genotype of plant to be treated (*Missaoui et al., 2017; Chemingui et al., 2019; Missaoui et al., 2021a; Missaoui et al., 2021b*). In this study, it was observed that seeds imbibed with 208.8 µM Ti presented lower weight gain compared to those treated with 104.4 µM Ti, with a significant difference between them (**Fig. 1**). It is likely that the high dose of Ti (208.8 µM Ti) used in this study resulted in a pronounced oxidative stress that boosted the production of ROS (i.e., $O_2^{\bullet-}$, H_2O_2 or $HO^{\bullet}$), causing changes in the metabolism of the seed and thus affecting the penetrability of water and oxygen into it (*Feizi et al., 2013; El-Bakatoushi, 2017*).

Regarding total germination percentage (TGP; **Table 1**), there were not significant differences among treatmnets tested, though the application of 208.0 µM Ti resulted in the greatest mean (96.6%). These results coincide with those observed in cucumber (*Cucumis sativus* L.), canola (*Brassica napus* L.), tobacco (*Nicotiana tabacum* L.), and corn (*Zea mays* L.), in which stimulation of germination was observed with treatments from 1.25 to 62.6 mM $\mathrm{TiO_2}$ nanoparticles (99.5% purity); with 18.78 mM Ti from $n\mathrm{TiO_2}$ (20 nm); with 125.2 to 626 mM $\mathrm{TiO_2}$ nanoparticles (Sigma Aldrich, 99.5%; 25 nm); and with 12.5 mM ($n\mathrm{TiO_2}$, 99% purity), respectively (*Mushtaq, 2011; Mahmoodzadeh, Nabavi,& Kashefi, 2013; Frazier, Burklew & Zhang, 2014; Karunakaran et al., 2016*).

The germination speed coefficient (GSC) plays an important role when establishing a crop in the field, since, through this variable, a seed can be classified as fast or slow germinating (*Navarro, Febles & Herrera, 2015; Talská, 2020*). The GSC is the reciprocal of time average of a seed to germinate and independent of the percentage of total germination and its value ranges from 0 (no germination) to 1 (rapid germination) (*Ranal & García, 2006*). The doses of Ti used in this study did not show significant effects on the mean velues of GSC, though they tended to decrease as the Ti dose increased.

The values above the control observed in vigor index I in this study coincide with what was found in faba bean (*Vicia faba* L.) treated with 626 µM Ti (< 100 nm, Sigma Aldrich) (*Ruffini et al., 2016*). On the contrary, higher doses (18.78 mM Ti) decrease the vigor index of canola seedlings (*Mahmoodzadeh, Nabavi & Kashefi, 2013*). It is likely that Ti improves germination and seed vigor because it stimulates the translocation of nutrients and activates antioxidant enzymes that reduce the accumulation of ROS causing oxidative stress (*Fashui, Zhenggui & Guiwen, 2000; Sadeghi et al., 2011*).

The percentages obtained in germination help determine the vigor of the seeds. Plant species with germination between 60 and 80% are considered to have intermediate vigor, while values greater than 80% are considered to have high vigor (*Salinas et al., 2001; Jafari, Kordrostami & Ghasemi-Soloklui, 2024*). Consequently, the results shown in TGP indicate that the seeds have a high level of vigor. In addition to the high values obtained with the 156.6 and 208.8 µM Ti treatments in vigor index I, it was observed that the evaluated seeds have a potential for rapid emergence, which allows obtaining healthy and vigorous seedlings under adverse environmental conditions (*Navarro, Febles & Herrera, 2015*), thanks to the use of Ti.

It has been observed that *n*TiO_2 improves growth and differentiation processes in plants, since it induces the transcriptional activation of genes involved in photosynthesis and light capture in chloroplasts, which in turn allows better CO_2 fixation, sugar biosynthesis, and biomass accumulation (*Ze et al., 2011; Tumburu et al., 2017; Abdel et al., 2018*). In tomato, *Pérez-Velasco et al. (2023)* demonstrated that covered rutile-TiO_2 nanoparticles enhance yield and growth by modulating gas exchange and nutrient status. In our study, greater root length was observed with high doses (i.e., 156.6 and 208.8 µM Ti) (**Table 2**), coinciding with what was reported in cucumber, onion, and *Arabidopsis thaliana* seeds treated with 6.26 and 12.52 mM Ti (*Andersen et al., 2016; Szymanska et al., 2016*). On the contrary, tomato seedlings treated with increasing doses from 0.62 to 62.6 mM Ti did not significantly increase germination percentage or root length, but an increase in SOD activity was observed when a high concentration of Ti was applied (1000 mg Ti L^{-1}) (*Song et al., 2013*). Likewise, under our experimental conditions, an increase of 16.3% in stem length was observed with respect to the control when applying the 156.6 µM Ti dose, as compared to the control. In oat (*Avena sativa* L.) seeds treated with 3.12, 6.26, 12.52 or 25.04 mM Ti, no significant effect of Ti on shoot length was found (*Ramesh et al., 2014*). Under our experimental conditions, Ti had no significant effects on the number of lateral or secondary roots; however, in the treatments with 52.2 and 104.4µM Ti, a significant decrease of 32.2 and 37.1%, respectively, in the number of leaves was observed, compared to the control. In this case, it is likely that $n$$TiO_2$ induced autophagy in chloroplasts through oxidative stress, damaging photosynthetic activity and carbon fixation in the plant (*Shull, Kurepa & Smalle, 2019*).

Regarding total water content in the seedlings, $n$$TiO_2$ treatments did not result in significant changes in roots and stems (**Fig. 2A and 2B**). In leaves, however, the highest dose (*i.e.*, 208.8 µM Ti) resulted in a 2.6% increase as compared to the control. This is a benefit for crops, because plant growth is mediated by the amount of water absorbed by the root and by physical properties such as pressure potential or turgor at the cellular level, which trigger cell elongation and expansion (*Feng et al., 2016*).

The increase in biomass and yield are highly dependent on the stimulation of chlorophyll biosynthesis, enzymatic activity, and the increase in photosynthetic capacity promoted by Ti (*Cigler et al., 2010*). In our study, this effect became evident when evaluating fresh biomass (**Table 3**), since with 208.8 µM Ti the greatest amount of biomass was obtained in roots and leaves (35.2 and 12.8%, respectively, as compared to the control). However, the application of low doses (52.2 and 104.4 µM Ti) reduced fresh stem biomass by 17.3 and 31.3%, respectively. Coincidentally, tomato seedlings treated with doses equal to or greater than 0.62 mM Ti decrease the weight of fresh biomass (*Song et al., 2013*).

The application of 104.4 µM Ti decreased the dry biomass of the stems by 43.2% (**Table 3**), whereas this dose reduced the dry biomass of the leaves by 27.1%, as compared to the control. A similar result was observed in total biomass of seedling. Coinciedently, *Feizi et al.* (*2013*) reported that the application of 0.5 mM Ti to fennel decreases the dry weight of the roots, stems, and seedling.

In relation to the hormetic effect (**Fig. 3**) in the root (RL) and stem length (SL) variables, it was observed that *n*TiO_2 exerts different effects according to the curves obtained: inverted U-shaped for SL and J-shaped for RL. In the SL variable, the dose with 104.4 µM Ti remained above the hormetic zone, while the 156.6 µM Ti dose caused the highest stimulation value. Consequently, the stimulant response curve was observed at medium doses, although in other studies the response has been observed at low doses (*Trela-Makowej, Orzechowska & Szymańska, 2024*). When applying 208.8 µM Ti L^{-1}, toxicity was observed at the threshold. For RL, it was observed that the 104.4 µM Ti L^{-1} dose describes a phenomenon associated with biological dysfunction or toxic damage, while higher doses (i.e., 156.6 and 208.8 µM Ti) show stimulation on this variable (*Cox et al., 2016; Guzmán-Báez et al., 2021*).

In order to perform fair comparisons among different studies, key factors have to be taken into consideration. In addition to the experimental conditions in wich the studies were carried, other factors determining the final effect of the nanoparticles include: concentration, particle size, distribution, morphological shape, chemical composition, surface characteristics, and coating, as well as the plant species (*López-Herrera et al., 2024*). For instance, while *Song et al. (2013)* and *Acosta-Slane et al. (2024)* exposed seeds for 48 to 120 h to *n*TiO_2 of approximately 27 nm in size, provided by Evonik, at concentrations between 50 and 5000 mg L^{-1}, our seeds were exposed for only 24 h to *n*TiO_2 of <25 nm in size, provided by Sigma, at concentrations between 2.5 and 10 mg L^{-1}. When exposing tomato seeds to up to 750 mg L^{-1} *n*TiO_2 of □25 nm in size for 1 h, *Raliya et al. (2015)* found not effect on germination but aerosol mediated applications were found to be more effective than the soil mediated applications on the uptake of the nanoparticles and the stimulation of growth responses. Such different findings exploited different properties of nanoparticles with different dosages, exposure times and concentrations (*Chahardoli et al., 2022*). Hence, the fundamental mechanism behind the effects of nanoparticles on plant physiology and metabolism remain still an open question. These facts may explain, at least in part, the different results observed among these approaches.

Various studies have claimed that TiO_2 nanoparticles result in hormetic dose-response curves in plants and other organisms (i.e., *Iavicoli, Calabrese & Nascarella, 2010; Hartmann et al., 2010; Nascarella & Calabrese, 2012; Chahardoli et al., 2022; Trela-Makowej, Orzechowska & Renata Szymanska, 2024*). Nevertheless, no one has statistically estimated and demostrated such hormetic effects supported with validated mathematical models. To the best of our knowledge, this study represents the first attempt to provide an in-depth statistical evaluation of hormetic dose-response curves triggered by $n\text{TiO}_2$ in a so important crop species such as tomato.

As a consequence of the impact of global climate change, it has become evident that increases in temperatures, changes in precipitation patterns, incidence of extreme weather events, and reductions in water availability may all result in reduced agricultural productivity (*Yuan et al., 2024*). Since Ti can promote seed germination, plant growth and water content in plant tissues, as demonstrated in this study, it may also contribute to reduce the effects of global warming and climate change on the earth, thus it provides applications for sustainable agriculture, food safety and security.

CONCLUSIONS

Herewith we have demonstrated that $n\text{TiO}_2$ may improve some parameters of seed germination and initial vegetative growth of tomato seedlings in a hormetic manner. In particular, it stimulates vigor index of seeds, root and stem length, as well as the water content in leaves. Importantly, Ti increases fresh biomass weight in roots and leaves and the effects in root and stem length display hormetic dose response curves. Therefore, imbibition of tomato seeds with $n\text{TiO}_2$ can help promote seed germination and stimulate initial growth of tomato seedlings.

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Competing Interests

The authors have declared that no competing interests exist.

Author Contributions

Víctor Hugo Carbajal Vázquez performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
Libia Iris Trejo-Téllez conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, contributed to funding acquisition, and approved the final draft.
Josafhat Salinas-Ruiz analyzed the data, prepared figures, reviewed drafts of the article and approved the final draft.
Fernando Carlos Gómez-Merino conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the article, contributed to funding acquisition, supervised the whole experiment, and approved the final draft.

Data Availability

The raw data are available in the Supplemental File 1.

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FIGURE CAPTIONS

Figure 1 Weight increase in seeds of tomato (*Solanum lycopersicum* L.) cv. Rio Grande imbibed for 24 h with different concentrations of titanium (Ti) supplied as titanium dioxide nanoparticles (*n*TiO_2). Means ± SD with different letters denote statistical differences among treatments (LSD, $p \leq 0.05$).

Figure 2 Total water content in seedlings of tomato (*Solanum lycopersicum* L.) cv. Rio Grande exposed to different concentrations of titanium (Ti) supplied as titanium dioxide nanoparticles (*n*TiO_2) 30 days after sowing. A: Roots; B: Stems; C: Leaves. Means ± SD with different letters in each variable denote statistical differences among treatments (LSD, $p \leq 0.05$).

Figure 3 Hormetic response in the variable root and shoot length in seedlings of tomato (*Solanum lycopersicum* L.) cv. Rio Grande exposed to different concentrations of titanium (Ti) supplied as titanium dioxide nanoparticles (*n*TiO_2) 30 days after sowing. A: maximum stimulatory response; B: hormetic zone; C and D: toxic threshold.

Figure 1

Weight increase in seeds of tomato (*Solanum lycopersicum* L.) cv. Rio Grande imbibed for 24 h with different concentrations of titanium ($nTiO_2$).

Means ± SD with different letters denote statistical differences among treatments (LSD, $p \leq 0.05$).

Figure 1.

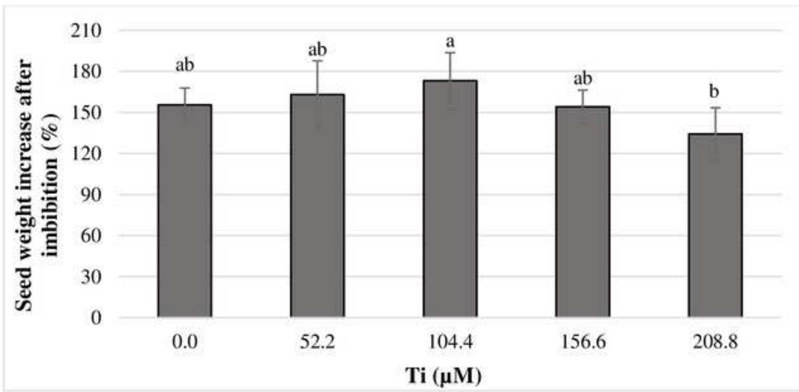
Seed weight increase after imbibition (%)
210
180
150
120
90
60
30
0
ab
ab
a
ab
b
0.0
52.2
104.4
156.6
208.8
Ti (μM)

Figure 2

Total water content in seedlings of tomato (*Solanum lycopersicum* L.) cv. Rio Grande exposed to different concentrations of titanium (nTiO$_2$) 30 days after sowing. A: Roots; B: Stems; C:

Means ± SD with different letters in each variable denote statistical differences among treatments (LSD, $p \leq 0.05$).

Figure 2

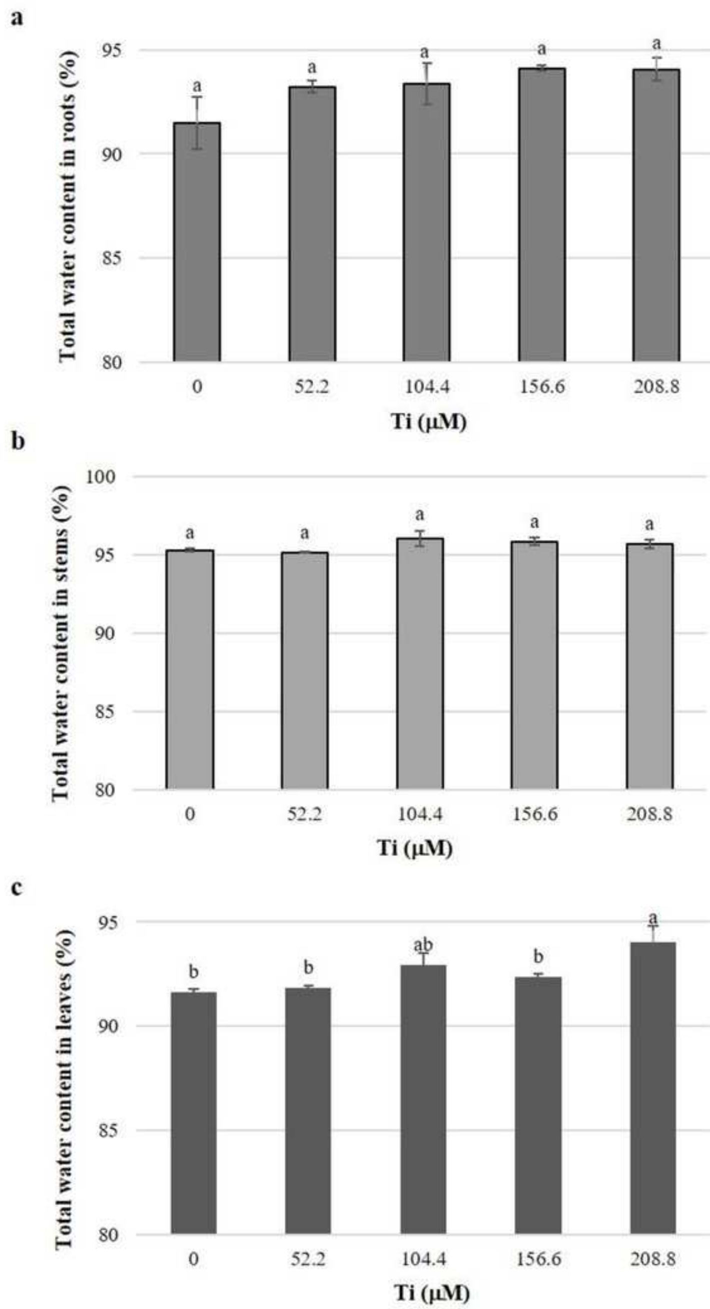
a
Total water content in roots (%)
95
90
85
80
a
a
a
a
a
0
52.2
104.4
156.6
208.8
Ti (μM)
b
Total water content in stems (%)
100
95
90
85
80
a
a
a
a
a
0
52.2
104.4
156.6
208.8
Ti (μM)
c
Total water content in leaves (%)
95
90
85
80
b
b
ab
b
a
0
52.2
104.4
156.6
208.8
Ti (μM)

Figure 3

Hormetic response in the variable root and shoot length in seedlings of tomato (*Solanum lycopersicum* L.) cv. Rio Grande exposed to different concentrations of titanium (Ti) supplied as titanium dioxide nanoparticles (nTiO$_2$) 30 days a

A: maximum stimulatory response; B: hormetic zone; C and D: toxic threshold.

Figure 3

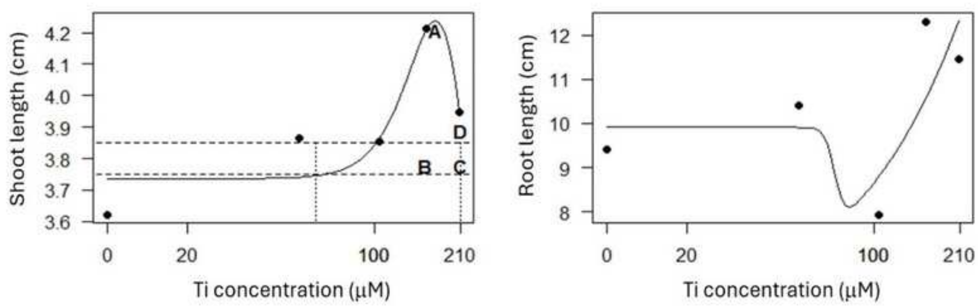
Shoot length (cm)
4.2
4.1
4.0
3.9
3.8
3.7
3.6
A
D
B
C
0
20
100
210
Ti concentration (µM)
Root length (cm)
12
11
10
9
8
0
20
100
210
Ti concentration (µM)

Table 1 (on next page)

Biostimulant effects of titanium (Ti) applied as titanium dioxide nanoparticles (*n*TiO 2) on germination parameters of tomato (*Solanum lycopersicum* L.) seeds cv. Rio Grande.

Tomato seeds were imbibed in solutions containing different concentrations of Ti supplied as titanium dioxide nanoparticles ($n\mathrm{TiO_2}$; 25 nm) for 24 h as described in Materials and Methods. Means ± SD with different letters in each variable indicate statistical differences among treatments (LSD, $p \leq 0.05$).

Table 1 Biostimulant effects of titanium (Ti) applied as titanium dioxide nanoparticles (*n*TiO_2) on germination parameters of tomato (*Solanum lycopersicum* L.) seeds cv. Rio Grande.

Ti concentration (µM)	Total germination percentage	Germination speed coefficient	Vigor index I	Vigor Index II
0.0	90.0±8.7 a	0.170±.006 a	1170.5±120.5 b	48.3±8.1 ab
52.2	86.7±2.9 a	0.168±.005 a	1228.9±49.0 ab	46.6±3.8 ab
104.4	80.0±5.0 a	0.177±.020 a	943.1±57.8 b	36.6±2.4 b
156.6	90.0±5.0 a	0.157±.031 a	1476.0±107.9 a	51.0±5.1 ab
208.8	96.6±2.9 a	0.151±.009 a	1485.6±47.6 a	61.7±6.8 a

Tomato seeds were imbibed in solutions containing different concentrations of Ti supplied as titanium dioxide nanoparticles (*n*TiO_2; 25 nm) for 24 h as described in Materials and Methods. Means ± SD with different letters in each variable indicate statistical differences among treatments (LSD, $p \leq 0.05$).

Table 2(on next page)

Biostimulant effects of titanium (Ti) applied as titanium dioxide nanoparticles (*n*TiO 2) on growth parameters of tomato (*Solanum lycopersicum* L.) seedlings cv. Rio Grande.

Tomato seedlings emerged from seeds imbibed in solutions containing different concentrations Ti supplied as titanium dioxide nanoparticles (*n*TiO_2; 25 nm) for 24 h, were grown for 30 days as described in Materials and Methods. Means ± SD with different letters in each variable indicate statistical differences among treatments (LSD, $p \leq 0.05$).

Table 2 Biostimulant effects of titanium (Ti) applied as titanium dioxide nanoparticles (*n*TiO_2) on growth parameters of tomato (*Solanum lycopersicum* L.) seedlings cv. Rio Grande.

Ti concentration (µM)	Root length (cm)	Stem length (cm)	Number of lateral roots	Number of leaves
0.0	9.40±0.46 bc	3.62±0.21 a	3.88±0.19 a	1.43±0.06 a
52.2	10.41±0.77 abc	3.86±0.32 a	3.54±0.29 a	0.97±0.17 bc
104.4	7.94±0.17 c	3.85±0.06 a	3.79±0.15 a	0.90±0.08 c
156.6	12.30±1.12 a	4.21±0.27 a	3.35±0.27 a	1.05±0.16 abc
208.8	11.44±0.59 ab	3.94±0.09 a	4.10±0.23 a	1.36±0.03 ab

Tomato seedlings emerged from seeds imbibed in solutions containing different concentrations Ti supplied as titanium dioxide nanoparticles (*n*TiO_2; 25 nm) for 24 h, were grown for 30 days as described in Materials and Methods. Means ± SD with different letters in each variable indicate statistical differences among treatments (LSD, $p \leq 0.05$).

Table 3 (on next page)

Fresh and dry biomass weight of tomato (*Solanum lycopersicum* L.) seedlings cv. Rio Grande treated with different concentrations of titanium (Ti) applied as titanium dioxide nanoparticles (*n*TiO 2).

Tomato seedlings emerged from seeds imbibed in solutions containing different concentrations of Ti supplied as titanium dioxide nanoparticles (*n*TiO_2; 25 nm) for 24 h, were grown for 30 days as described in Materials and Methods. Means ± SD with different letters in each variable indicate statistical differences among treatments (LSD, $p \leq 0.05$).

Table 3 Fresh and dry biomass weight of tomato (*Solanum lycopersicum* L.) seedlings cv. Rio Grande treated with different concentrations of titanium (Ti) applied as titanium dioxide nanoparticles ($nTiO_2$).

Ti concentration (µM)	**Fresh biomass weight (mg)**			
	Root	Stem	Leaves	Seedling
0.0	152.4±30.0 b	188.4±6.7 a	184.8±8.0 ab	525.7±44.3 ab
52.2	201.2±8.6 ab	155.8±7.8 bc	178.5±13.4 ab	535.3±29.6 ab
104.4	167.6±6.8 ab	129.3±8.4 c	161.2±6.5 b	458.1±18.7 b
156.6	197.6±20.5 ab	181.5±0.9 ab	184.9±9.2 ab	564.0±29.6 ab
208.8	235.2±31.6 a	187.3±12.5 a	212.0±13.2 a	634.5±53.6 a
Ti concentration (µM)	**Dry biomass weight (mg)**			
	Root	Stem	Leaves	Seedling
0.0	12.0±1.3 a	8.8±0.5 a	15.5±0.9 a	36.3±2.5 a
52.2	13.6±0.7 a	7.5±0.4 a	14.5±0.8 ab	35.7±1.8 ab
104.4	11.2±1.8 a	5.0±0.3 b	11.3±0.6 b	27.5±2.5 b
156.6	11.7±1.4 a	7.5±0.4 a	14.2±1.0 ab	33.3±2.7 ab
208.8	13.7±1.5 a	8.1±0.9 a	12.4±1.3 ab	34.2±2.4 ab

Tomato seedlings emerged from seeds imbibed in solutions containing different concentrations of Ti supplied as titanium dioxide nanoparticles ($nTiO_2$; 25 nm) for 24 h, were grown for 30 days as described in Materials and Methods. Means ± SD with different letters in each variable indicate statistical differences among treatments (LSD, $p \leq 0.05$).