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Research Article

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Enhancing jalapeño pepper seedling quality for transplantation and potential stress tolerance through seed nanopriming

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Abstract
The use of nanoparticles as a tool for improving horticultural seedling production has generated growing interest, but their overall impact on seedling physiology still needs further exploration. This study evaluated the effect of nanopriming with ZnO, SiO_2, and ZnO+SiO_2 on the physiological, photosynthetic, and biochemical profile of Capsicum annuum L. seedlings, with the aim of strengthening their quality for transplanting. Growth parameters such as root biomass, shoot biomass, total biomass, stem length and diameter, and number of leaves were evaluated, as well as the content of photosynthetic pigments (chlorophyll a, b, total, carotenoids, SPAD index) and nitrate reductase (NR) activity. Seedlings treated with nanoparticles, especially ZnO and ZnO+SiO_2, showed more robust root development, higher levels of total chlorophyll and SPAD, and higher NR activity compared to treatments with commercial biostimulants, hydropriming, and the control. These results show that the use of nanoparticles not only enhances a specific variable but also strengthens key processes in seedlings in a comprehensive manner, generating a more balanced and functional physiological profile for transplanting. Multivariate analysis using heat mapping, principal component analysis (PCA), and radar charts confirmed the positive interaction between the different variables, highlighting the ZnO+SiO_2 treatment as the most effective strategy for improving the physiological quality of jalapeño seedlings. This work provides new evidence on the potential of nanopriming as a biotechnological tool in horticulture.

Keywords: jalapeño pepper; nanopriming, nitrogen assimilation, photosynthetic efficiency, seedlings, ZnO+SiO_2.

1. Introduction

During the initial stages of plant development, low physiological vigor can significantly compromise photosynthetic efficiency and nitrogen assimilation, processes that are essential for the growth and productivity of horticultural crops such as Capsicum annuum L. [1, 2]. These processes are closely related, as more than 50% of leaf nitrogen is used for proteins involved in photosynthesis, including the enzyme Rubisco, whose efficient functioning is vital for CO_2 carboxylation and RuBP regeneration [3, 4].

In turn, the enzyme nitrate reductase (NR) is an early physiological marker of nitrogen nutritional status, participating in the reduction of NO_3^- to NO_2^-, a critical step for the subsequent synthesis of amino acids and functional proteins [5, 6]. Limitations in nitrogen availability or internal metabolism lead to a drop in photosynthetic efficiency and Rubisco activity, limiting growth even under optimal irrigation and light conditions [7].

To address these limitations, nanopriming has emerged as a promising tool. This technique consists of treating seeds with metal or metalloid nanoparticles (such as ZnO, SiO_2, and ZnMo), which have high bioavailability and surface reactivity, thereby improving water absorption, activating antioxidant pathways, inducing the expression of functional genes, and modulating energy metabolism from early stages (Kumar et al., 2022; Nile et al., 2022).

Nanopriming with ZnO has been effective in improving Rubisco enzyme activity, chlorophyll content, and photosynthetic efficiency under stress and normal conditions [1, 8]. SiO_2 nanoparticles have been shown to improve water retention capacity and nitrogen assimilation, as well as drought resistance [9, 10]. In addition, combinations such as ZnMo have been associated with increased nitrate reductase enzyme activity and better metabolic integration between photosynthesis and nitrogen nutrition [11].

However, most of this research has been conducted on cereals, legumes, or model species. Few studies have comprehensively evaluated these effects on jalapeño pepper seedlings, representing a gap in applied physiological knowledge.

Therefore, the objective of this research was to evaluate the effect of nanopriming with ZnO, SiO_2, and ZnMo, as well as commercial biostimulants, on key indicators of physiological performance in *Capsicum annuum* L., including photosynthetic efficiency, chlorophyll biosynthesis, and nitrate reductase enzyme activity, promoting a more vigorous and functional early establishment of the crop.

2. Materials and Methods

2.1 Experimental site and plant material

The experiment was carried out during January-April 2025 at the Center for Food and Development Research A.C. (CIAD) (Delicias, Chihuahua, Mexico). The commercial variety Imperial F1 of jalapeño pepper seeds (*Capsicum annuum* L.), originally from China, was used. This variety is characterized by being a hybrid seed with high vigor and uniformity, designed for intensive production systems with high fruit demand. The surface of the seeds was sterilized with a 4% sodium hypochlorite (NaClO) solution, under constant agitation for five minutes; they were then washed three times with distilled water [12].

2.2 Characterization of zinc oxide and silicon dioxide nanoparticles

The zinc oxide (ZnO) and silicon dioxide (SiO2) NPs used in this study were synthesized using the wet chemistry method. For ZnO, a wurtzite-type crystalline structure with a purity of 99.7% and an average size of 50 nm was obtained, with no contaminants present.

As for SiO2, a purity of 99.9% was obtained, and a physical appearance of a light-colored fine powder with an average size of 80 nm. Both NPs were structurally and morphologically characterized by transmission electron microscopy (TEM) (Fig. 1), confirming their homogeneity and stability. The NPs were supplied by the company Investigación y Desarrollo de Nanomateriales S.A. de C.V., located in San Luis Potosí, Mexico. The nanofertilizer was a commercial product, BROADACRE® ZnMo, from Agrichem Fluagri, Guadalajara, Jalisco, Mexico. Its composition was 62% Zn, 5% Mo, and a 5% seaweed extract-based chelating agent, whose function is to prevent the precipitation of the nanofertilizer in suspension. According to the manufacturer, the particle size is 200 nm.

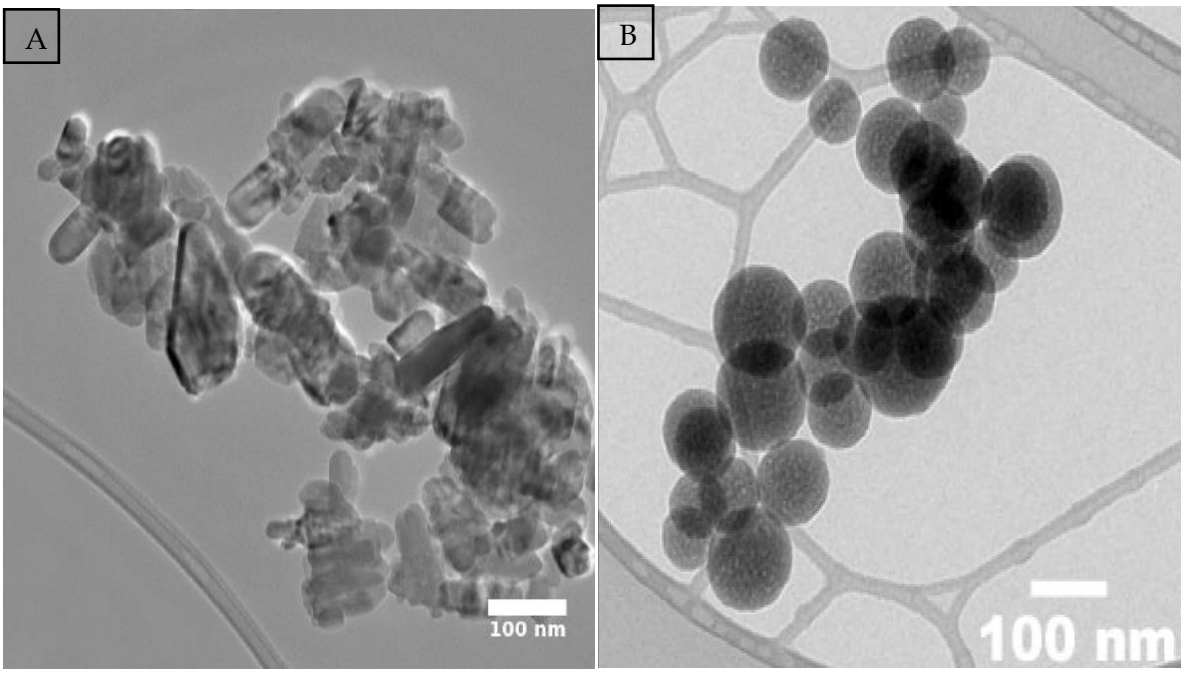


Figure 1. Morphology of the sample by Transmission Electron Microscopy (TEM), (A) ZnO, (B) SiO_2.

2.3 Experimental design and nanopriming treatments

A completely randomized design was used with eight treatments, three replicates, and 100 experimental units per treatment. A detailed description of the treatments and concentrations used is presented in Table 2. In all treatments, triple-distilled water was used as the solvent and chitosan (Quitofyt® Poly-D-glucosamine) as the stabilizing agent in the formulations with ZnO, SiO_2 and ZnO + SiO_2 nanoparticles (NPs), prepared in a final volume of 1 L per treatment.

To ensure adequate dispersion and homogenization of the nanoparticles, two consecutive stages were applied. In the first stage, a premix was prepared by magnetic stirring using a VMS-C7 Advanced plate (VWR®, Radnor, PA, USA) at 700 rpm for 20 minutes. In the second stage, ultrasound was applied at 40 kHz for 30 minutes using a Vevor® ultrasonic cleaner (Ultrasonic Cleaner, Cleveland, OH, USA), with the aim of improving the colloidal stability of the suspensions.

Table 1. Treatment, chemical composition of priming's and doses applied on *Capsicum annuum* L. seeds.

Treatment	Chemical Composition	Doses
Control	Not applicable	Not applicable
Hydropriming	Tridistilled water	Not applicable
NPs ZnO + Ch	<50 nm, 99.9% and Poli-D-glucosamine	(100 and 100 mg L^{-1})
NPs SiO_2 + Ch	<80 nm, 99.9% and Poli-D-glucosamine	(10 and 100 mg L^{-1})
NPs ZnO + SiO_2 + Ch	<50 nm, 99.9%, <80 nm, 99.9% and Poli-D-glucosamine	(100, 10 and 100 mg L^{-1})
NPs Zn + Mo	Liquid solution composed of 62% Zn, 5% Mo, and 5% of an algae extract-based chelating agent.	(124 and 10 mg L^{-1})
Osmoplant®	Liquid solution composed of 6% free amino acids, 2.4% nitrogen and 3.3% potassium.	(2000 mL L^{-1})
Codasil®	Liquid solution with a high concentration of soluble silicon composed of 20% silicon, 4% free amino acids and 11.20% potassium.	(2000 mL L^{-1})

2.4 Crop management

After drying at room temperature for 24 hours, the seeds were sown in polystyrene trays with 338 cavities each, using a substrate mixture in a 1:1:1 (v/v/v) ratio of vermiculite, perlite, and sphagnum peat moss (Premier® Sphagnum Peat Moss), previously moistened with distilled water. The seeds were sown at a depth of 3 mm. The trays were then placed under controlled germination conditions for 7 days in total darkness at a temperature of 25–30°C and relative humidity of 60–80%. After this period, the seedlings were transferred to a greenhouse under controlled conditions of 12–33°C and 30–45% relative humidity.

During the leaf development phase (days 1–30), automated foliar fertilization was applied with a volume of 500 mL of nutrient solution per tray, directly onto the foliage. The formulation of this solution was designed using NutriNet® Haifa Group software [14] and consisted of commercial fertilizers from the Haifa brand: 6 mM total nitrogen (from Haifa Poly-Feed 17-10-27+ME), 1.6 mM phosphorus, 0.3 mM additional potassium, 4 mM calcium (Haifa Cal GG), and 1.4 mM magnesium (Haifa Mag). Chelated micronutrients were also added: 5 µM iron (Haifa Micro Fe 13%), 2 µM manganese (Haifa Micro Mn 13%), 0.25 µM copper (Haifa Micro Cu 15%), 0.3 µM molybdenum, and 0.5 µM boron. This solution was applied at three-day intervals during the first 30 days of vegetative development.

2.5 Plant Sampling

After 30 days of cultivation, 10 jalapeño pepper seedlings were randomly selected per treatment for subsequent physiological and biochemical analysis. The samples were carefully washed three times with distilled water, followed by an additional wash with a 1% solution of nonionic detergent to remove surface residues and ensure the cleanliness of the plant tissue prior to analysis [15].

2.6 Plant analysis

2.6.1 Morphological parameters

Morphological variables were evaluated on day 31 after sowing. Ten seedlings were selected per treatment, and the following variables were measured: root length, stem length, number of leaves, and stem diameter, expressed in millimeters (mm). Length and thickness measurements were made using a 153 mm digital caliper (model HER-411; Steren®, Mexico City, Mexico) [16]. Root length was determined from the base of the hypocotyl to the apex of the radicle, while stem length was measured from the radicle-hypocotyl junction to the base of the cotyledons.

The fresh weight of the root system, the aerial part, and the entire seedling was quantified using a high-precision analytical balance (model HR-120-C; A&D Weighing®, San José, CA, USA). From these measurements, the total fresh biomass per seedling was calculated, expressed in grams per plant (g plant^{-1}).

2.6.2 Photosynthetic pigments

The assay was performed in triplicate, using 10 fresh leaf discs 7 mm in diameter obtained from photosynthetic tissue without physical damage or the presence of central veins. The discs were placed in glass test tubes, to which 10 mL of 99% methanol (v/v) was added as an extraction agent. The openings of the tubes were sealed with Parafilm®, and the samples were left to rest for 24 hours in complete darkness at a constant temperature of 25 ± 2 °C.

The photosynthetic pigments were quantified according to the methodology described by Wellburn [17], using a UV-VIS spectrophotometer model 10S (Thermo Fisher Scientific, Madison, WI, USA).

Absorbance readings were taken at 666 nm, 653 nm, and 470 nm, corresponding to the specific wavelengths for chlorophyll a, chlorophyll b, and carotenoids, respectively. The results were expressed in micrograms per gram of fresh weight (mg g^{-1} FW). Pigment concentrations were calculated using the following equations:

$$chl\ a = [15.65\ (A666) - 7.34(A653)] \quad (1)$$

$$chl\ b = [27.05\ (A653) - 11.21(A666)] \quad (2)$$

$$Carotenoids^{*} = ([(1000(A470)) - 2.86(chla) - 129.2(chlb)])/((221)) \quad (3)$$

$$Total\ chorophyll = chl\ a + chl\ b \quad (4)$$

2.6.3 Chlorophyll index

The relative chlorophyll content was determined using the SPAD (Soil Plant Analysis Development) index, using a portable SPAD-502 chlorophyll meter (Konica Minolta Sensing, Inc., Osaka, Japan). This instrument allows non-destructive measurements of leaf greenness, which is considered an indirect indicator of chlorophyll content [18, 19]. For each experimental unit, five fully expanded leaves per plant were selected, without physical damage or the presence of midribs, and five random readings were taken per plant during hours of high light radiation in order to standardize the evaluation conditions. The results were expressed in SPAD units. The measurement was carried out 31 days after sowing, specifically on April 2, 2025.

2.6.4 Nitrate reductase activity "in vivo"

The “in vivo” activity of the nitrate reductase enzyme (NR, EC 1.7.1.1) was quantified following the methodology described by Sánchez [20]. 0.5 g of 7 mm long leaf segments without physical damage or central veins were placed in test tubes with 10 mL of incubation solution composed of 100 mM potassium phosphate buffer (pH 7.5) and 1% (v/v) propanol as a permeabilizing agent. The samples were then infiltrated at a controlled pressure of 0.8 bar and kept in incubation at 30°C in complete darkness for 1 hour to promote nitrite accumulation mediated by enzymatic activity.

To stop the reaction, the tubes were placed in a water bath at 100°C for 20 min. Next, 1 mL of the reaction medium was extracted, to which 2 mL of a 1% (w/v) solution of sulfanilamide in 1 M HCl was added sequentially.5 M and 2 mL of a 0.02% (w/v) solution of N-naphthylethylenediamine dihydrochloride (NED) in 0.2 M HCl were added sequentially. The formation of the colored (azoic) complex allowed the quantification of the accumulated nitrite (NO_2^-) by spectrophotometry at 540 nm, using a standard nitrite curve as a reference.

2.7 Pearson Correlation Heatmap

To evaluate the relationships between physiological and biochemical variables, a Pearson correlation matrix was developed using the averages per treatment. The results were represented graphically using a heatmap generated in Python (v 3.11), where the intensity and direction of the correlations were expressed using a color scale. Correlation values close to +1 indicate a strong positive association, while values close to -1 reflect a strong negative correlation. Correlations close to 0 are interpreted as weak or non-existent, allowing the identification of functional or independent relationships between the variables evaluated [21].

2.8 Principal Component Analysis (PCA)

To identify multivariate variation patterns and reduce the dimensionality of the dataset, Principal Component Analysis (PCA) was applied. This analysis allowed the original variables to be transformed into a new set of orthogonal variables (principal components) that capture as much variance as possible from the system [22]. The results were visualized using a two-dimensional scatter plot, where the samples were projected onto the first two principal components, facilitating the identification of clusters and trends inherent to the treatments evaluated.

This procedure was implemented using the Python programming language (v 3.11).

2.9 Radar Chart: Multivariate Comparison by priming treatment

To compare multiple physiological and biochemical variables simultaneously between the treatments evaluated, a Radar Chart (also known as a spider chart or star chart) was used. This type of visualization allows multivariate data to be represented in a two-dimensional space, where each radial axis corresponds to a different variable. The magnitudes of the variables are projected from a central point toward the periphery and connecting the points corresponding to a treatment forms a polygon that facilitates visual comparison between treatments.

The Radar Chart was implemented using the Python programming language (v 3.11), which allowed for a clear and effective representation of the differences and similarities between treatments based on the measured variables [23].

2.10 Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA), followed by Fisher's LSD test for comparison of means ($p \leq 0.05$), using SAS® statistical software version 9.0 to validate significant differences between treatments [24]. In addition, multivariate analysis tools were applied, such as Principal Component Analysis (PCA), Pearson correlation matrices, and Radar Charts, whose detailed methodological description is presented in the previous sections.

3. Results and discussion

3.1 Morphological parameters

3.1.1 Stem length

The stem length of *Capsicum annuum* L. seedlings showed statistically significant differences ($p \leq 0.05$) between the treatments evaluated. The Control treatment achieved the longest stem length, with an average of 22.4 mm, closely followed by Hydropriming, with 21.8 mm; both treatments were grouped in statistical category "a."

In contrast, the NPs ZnO (17.1 mm), NPs SiO_2 (16.8 mm), and Codasil (17.0 mm) treatments recorded the shortest stem lengths, with reductions of between 22% and 25% compared to the control, and were classified in the statistical group "c."

The treatments NPs ZnO+SiO_2 (19.1 mm), Nanofertilizer ZnMo (18.5 mm) and Osmoplant (19.3 mm) showed intermediate values, with reductions of between 13% and 18% compared to the control, and were grouped in the statistical group "b."

These results reflect a differential impact of priming treatments on stem elongation in jalapeño seedlings (Fig. 2).

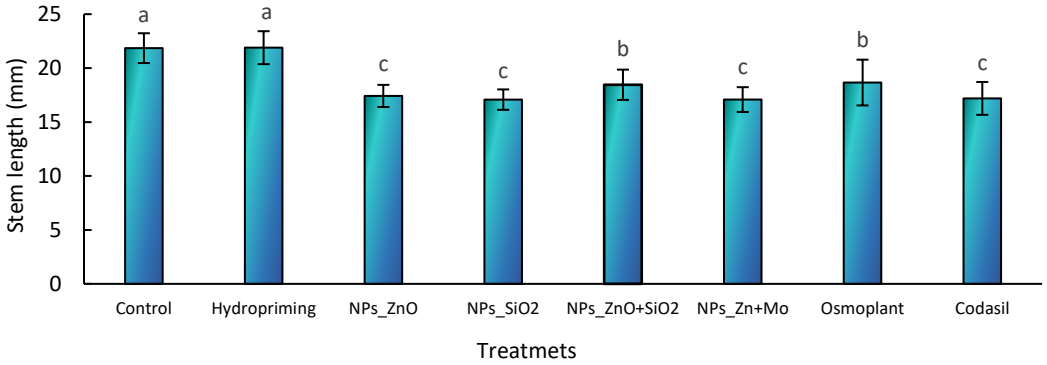


Figure 2. Effect of different priming treatments on the stem length of *Capsicum annuum* L. seedlings. The bars represent the mean ± standard error. Different letters indicate statistically significant differences ($p \leq 0.05$) according to Fisher's LSD test.

3.1.2 Stem diameter

The stem diameter of *Capsicum annuum* L. seedlings showed statistically significant differences ($p \leq 0.05$) between the treatments evaluated. The Hydropriming treatment recorded the highest average stem diameter (3.1 mm), classifying it in statistical group "a."

This result indicates greater radial expansion of the conductive and supporting tissue in seedlings subjected to controlled imbibition with water, without additives.

The control and treatment with ZnO NPs had average diameters of 2.9 mm, grouping them in statistical group "b," with an approximate reduction of 6.4% compared to Hydropriming.

The treatments with SiO_2 NPs (2.7 mm), ZnO+SiO_2 NPs (2.8 mm), and ZnMo nanofertilizer (2.9 mm) showed intermediate values, with reductions of between 6.4% and 12.9% compared to Hydropriming, classified in statistical groups "bc" and "b."

Osmoplant, on the other hand, had an average diameter of 2.6 mm, classified in the "cd" group, representing a decrease of 16.1% compared to Hydropriming.

Finally, the Codasil treatment showed the smallest stem diameter, with an average value of 2.5 mm, grouped in the statistical group "d" and registering a reduction of 19.4% compared to the Hydropriming treatment (Fig. 3).

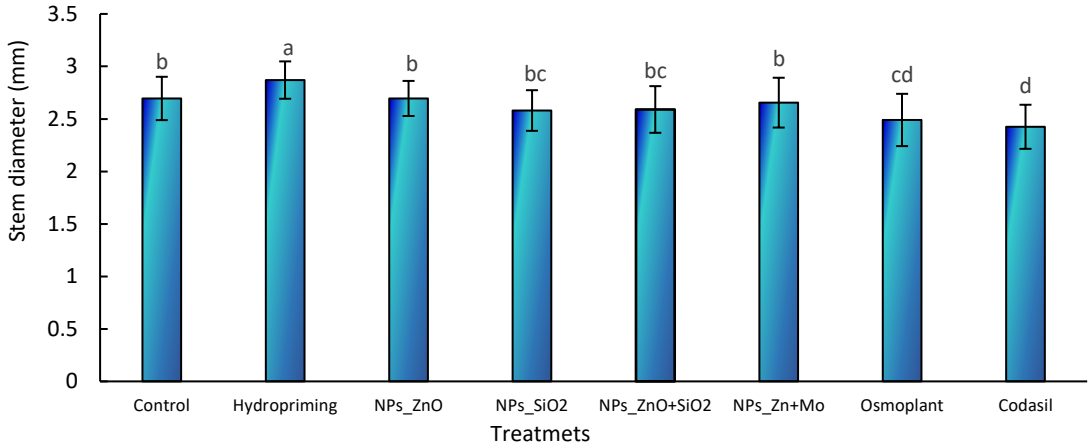


Figure 3. Effect of different priming treatments on the stem diameter of *Capsicum annuum* L. seedlings. Bars represent the mean ± standard error. Different letters indicate statistically significant differences ($p \leq 0.05$) according to Fisher's LSD test.

3.1.3 Number of leaves

The number of leaves in *Capsicum annuum* L. seedlings showed significant differences ($p \leq 0.05$) between treatments. The Hydropriming treatment had the highest average number of leaves (9.1), grouping in statistical category "a."

The Control, NPs ZnO, and NPs SiO_2 treatments showed slightly lower values (between 8.0–8.5 leaves) and were grouped in statistical category "b."

The NP ZnO+SiO_2 and Nanofertilizer Zn+Mo treatments recorded averages of approximately 7.9–8.0 leaves, forming the "bc" group, which represents a moderate reduction (~10–13%) compared to Hydropriming. The Osmoplant (7.5 leaves) and Codasil (7.0 leaves) treatments showed the lowest values, with reductions of 17% and 23%, respectively, compared to Hydropriming, and were grouped into the statistical categories "cd" and "d" (Fig. 4).

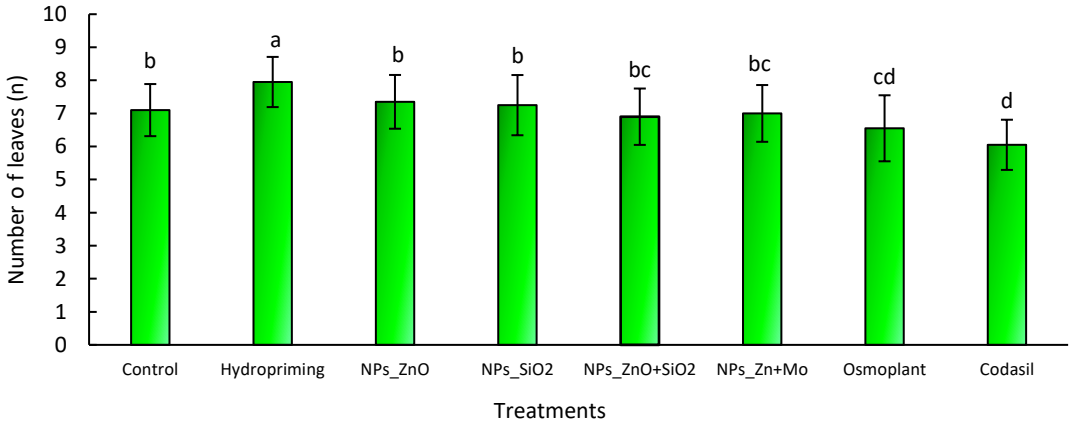


Figure 4. Effect of different priming treatments on the number of leaves (n) of *Capsicum annuum* L. seedlings. Bars represent the mean ± standard error. Different letters indicate statistically significant differences ($p \leq 0.05$) according to Fisher's LSD test.

3.2 Biomass

3.2.1 Total fresh weight

The ZnO NPs treatment showed the highest total fresh biomass with 3400 mg (100%, group "a"). The Control, Hydropriming, ZnO NPs+SiO_2, and Osmoplant showed values between 3000 and 3200 mg, representing approximately 88–94% of the maximum value, grouped in group "ab."

On the other hand, SiO_2 NPs, ZnMo nanofertilizer, and Codasil recorded the lowest total fresh biomass, with values between 2700–2800 mg, corresponding to a reduction of 17–20% compared to ZnO NPs, and were classified in group "b" (Fig. 5).

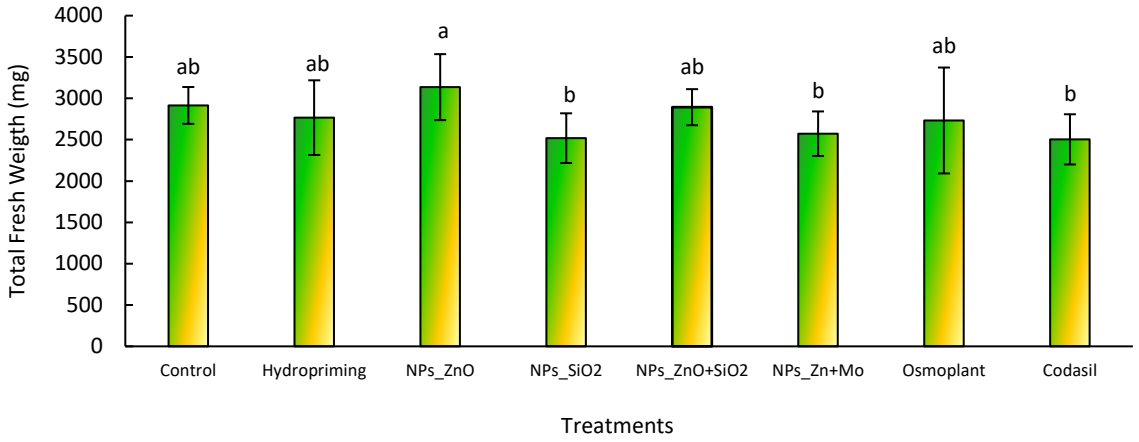


Figure 5. Effect of different priming treatments on total fresh biomass (mg) of *Capsicum annuum* L. seedlings. Bars represent the mean ± standard error. Different letters indicate statistically significant differences ($p \leq 0.05$) according to Fisher's LSD test.

3.2.2 Aerial fresh weight

The Control achieved the highest aerial biomass, with approximately 2100 mg (group "a"). The Hydropriming, NPs ZnO, NPs SiO_2, NPs ZnO+SiO_2 and Codasil treatments presented lower values, between 1700 and 1900 mg, representing between 81–90% of the maximum value, grouped in group "ab".

Finally, the ZnMo nanofertilizer and Osmoplant had the lowest values, which were statistically significant compared to the control, with values 17 to 19% lower (Fig. 6).

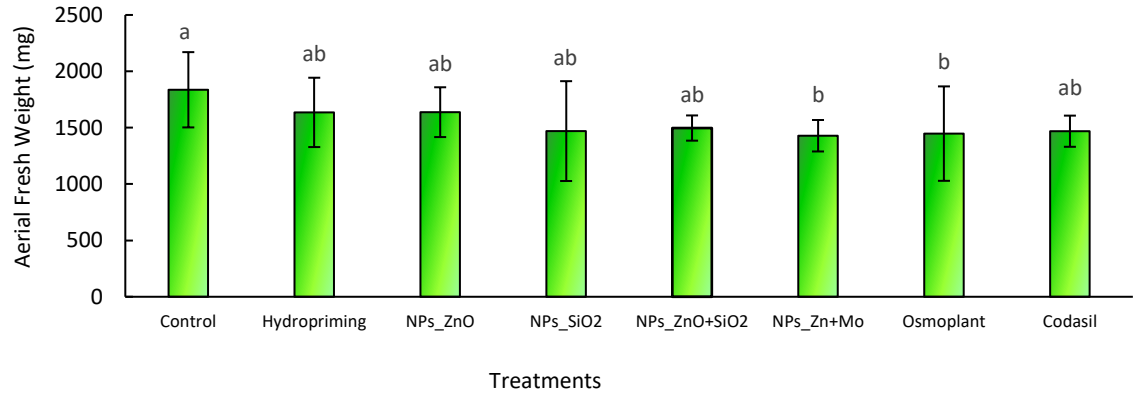


Figure 6. Effect of different priming treatments on total fresh biomass (mg) of *Capsicum annuum* L. seedlings. Bars represent the mean ± standard error. Different letters indicate statistically significant differences ($p \leq 0.05$) according to Fisher's LSD test.

3.2.3 Fresh root weight

The fresh root weight measured in the control treatment was approximately 1100 mg (Fig. 8a). Pretreatment with water (hydropriming) produced a slight increase, reaching about 1150 mg, representing an approximate increase of 4.5% compared to the control (Fig. 8b). However, this improvement was not statistically significant, as hydropriming shared the same grouping ("bc") as the control.

Zinc oxide nanoparticles (ZnO NP) showed the most prominent effect (Fig. 8c), with an increase in fresh root weight to approximately 1500 mg, i.e., an increase of around 36% compared to the control. This value was significantly higher than all treatments labeled "bc" or "c," placing it in the highest statistical category ("a"). Similarly, the combination of ZnO and SiO_2 reached 1400 mg (Fig. 8e), equivalent to a 27% increase

over the control. This result was also grouped in category "a," indicating that ZnO nanoparticles, both alone and in mixture with SiO_2, promoted significantly greater root development than the other treatments.

When silicon dioxide nanoparticles (SiO_2 NPs) were used individually (Fig. 8d), the fresh root weight was around 1200 mg, i.e., 9% above the control. However, this improvement was statistically similar to that of the control (both recorded the grouping "bc"), so it cannot be stated that SiO_2 NPs have a substantial effect compared to soaking in water. On the other hand, the zinc-molybdenum nanofertilizer (Fig. 8f) and the commercial biostimulant Osmoplant (Fig. 8g) reached values close to 1300 mg, equivalent to an approximate increase of 18% compared to the control. Both treatments were classified in intermediate groups ("bc" for Zn+Mo NPs and "ab" for Osmoplant), indicating that their increases, although notable, were not significantly lower than the 'a' treatments or higher than all the "bc" treatments.

Finally, treatment with Codasil resulted in a decrease in root development (Fig. 8h), registering 1000 mg, i.e., 9% less than the control. This value was in the lowest statistical category ("c"), confirming that Codasil significantly reduced fresh root weight compared to the most effective treatments (Fig. 7).

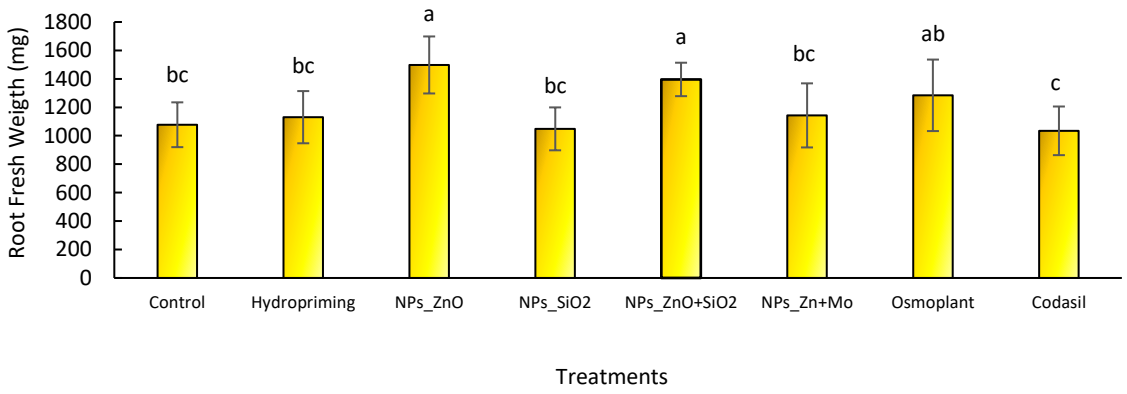


Figure 7. Effect of different priming treatments on fresh root biomass (mg) of *Capsicum annuum* L. seedlings. Bars represent the mean ± standard error. Different letters indicate statistically significant differences ($p \leq 0.05$) according to Fisher's LSD test.

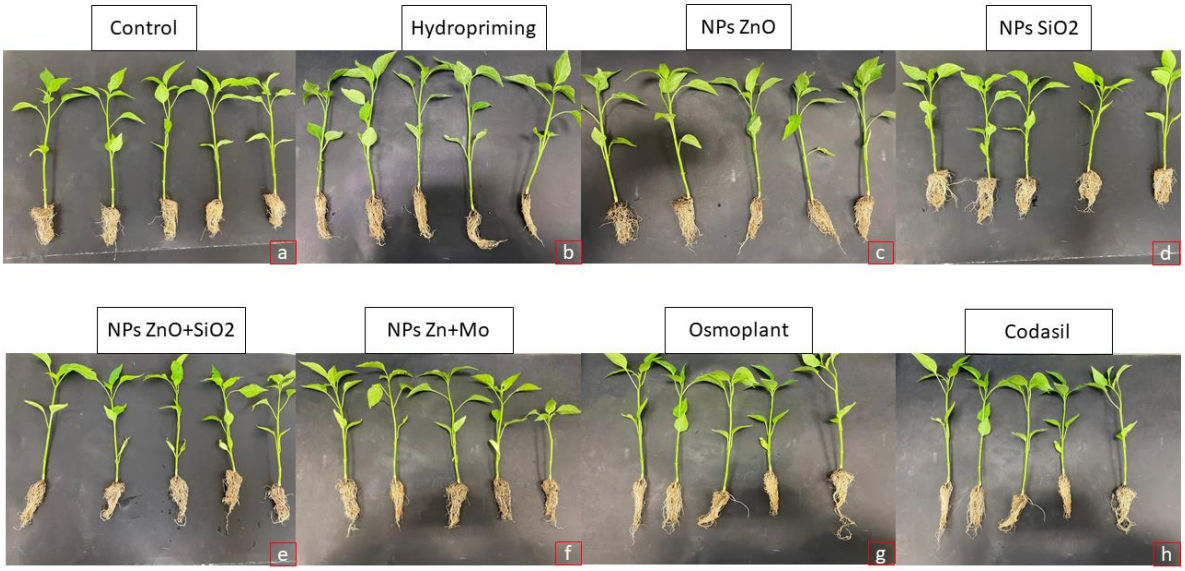


Figure 8. Representative view of the development of jalapeño pepper seedlings (*Capsicum annuum* L.) under different priming treatments. Each box shows five seedlings obtained after the germination and early growth period. From left to right and top to bottom, the treatments are: (a) Control: seeds without prior treatment. (b) Hydropriming: seeds soaked in water for the established time (primed with water). (c) ZnO NPs: seeds treated with zinc oxide nanoparticles. (d) SiO_2 NPs: seeds treated with silicon dioxide nanoparticles. (e) ZnO+SiO_2 NPs: seeds treated with a mixture of ZnO and SiO_2 nanoparticles. (f) Zn+Mo NPs: seeds treated with zinc and molybdenum nanoparticles. (g) Osmoplant: seeds treated with the commercial biostimulant Osmoplant. (h) Codasil: seeds treated with the commercial product Codasil. The roots and aerial parts of the seedlings are shown to illustrate the differences in vigor and root development associated with each treatment.

3.3 Photosynthetic Pigments

3.3.1 Chlorophyll a

The chlorophyll a content (mg g^{-1} FW) showed significant differences ($p \leq 0.05$) between treatments. Hydropriming obtained the highest value (2.53 mg g^{-1} FW) and was classified in category "a." Osmoplant reached 2.45 mg g^{-1} FW, also falling into category "a" and just 3% below Hydropriming.

The NP ZnO+SiO_2 (2.05 mg g^{-1} FW) and NP Zn+Mo (1.96 mg g^{-1} FW) treatments were grouped into "b," representing decreases of approximately 19% and 22% compared to Hydropriming, respectively. On the other hand, ZnO NPs (1.88 mg g^{-1} FW), SiO_2 NPs (1.72 mg g^{-1} FW), and Codasil (1.80 mg g^{-1} FW) formed group "bc," with reductions of 26%, 32%, and 29% compared to Hydropriming. Finally, the Control recorded the lowest value (1.55 mg g^{-1} FW; category "c"), which is approximately 39% less than Hydropriming (Fig. 9).

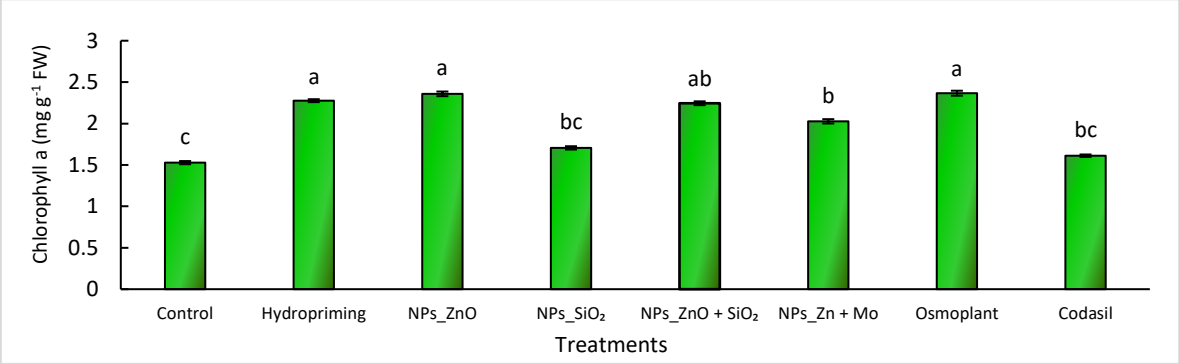


Figura 9. Efecto de diferentes tratamientos de priming en la clorofila a (mg g^{-1} FW) de plántulas de *Capsicum annuum* L. Las barras representan la media ± error estándar. Letras diferentes indican diferencias estadísticas significativas ($p \leq 0.05$) según la prueba LSD de Fisher.

3.3.2 Chlorophyll b

The chlorophyll b content (mg g^{-1} FW) varied significantly ($p \leq 0.05$) among the treatments evaluated. Seedlings exposed to zinc oxide nanoparticles (ZnO NPs) showed the highest value, with 1.22 mg g^{-1} FW, placing them in statistical category "a." In a second level were Hydropriming (1.05 mg g^{-1} FW), the combination ZnO+SiO_2 NPs (1.05 mg g^{-1} FW), the nanofertilizer ZnMo (0.96 mg g^{-1} FW), and Osmoplant (1.07 mg g^{-1} FW), all grouped under the letter "b." This means that, compared to the ZnO NPs treatment, these four treatments showed reductions of between 12% and 23%.

The treatment with SiO_2 nanoparticles recorded an intermediate content of 0.83 mg g^{-1} FW, placing it in the "bc" category and showing an approximate decrease of 32% compared to ZnO NPs. Finally, both the Control (0.75 mg g^{-1} FW) and Codasil (0.72 mg g^{-1} FW) obtained the lowest chlorophyll b values, sharing the "c" category and registering almost 38% less than the maximum observed in ZnO NPs (Fig. 10).

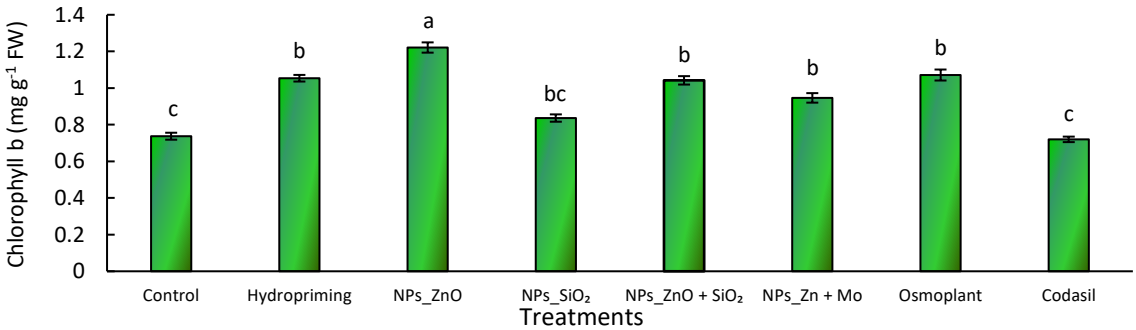


Figura 10. Efecto de diferentes tratamientos de priming en la clorofila b (mg g^{-1} FW) de plántulas de *Capsicum annuum* L. Las barras representan la media ± error estándar. Letras diferentes indican diferencias estadísticas significativas ($p \leq 0.05$) según la prueba LSD de Fisher.

3.3.3 Total chlorophyll

The total chlorophyll content (mg g^{-1} FW) showed significant differences ($p \leq 0.05$) between treatments. Seedlings treated with zinc oxide nanoparticles (ZnO NPs) recorded the highest value, with 3.58 mg g^{-1} FW, and were grouped in category "a." Similarly, Hydropriming reached 3.33 mg g^{-1} FW ("a") and Osmoplant presented 3.45 mg g^{-1} FW ("a"), with no significant differences between these three.

The combination of ZnO+SiO_2 NPs had a content of 3.27 mg g^{-1} FW, placing it in category "ab," indicating that it does not differ significantly from the maximum values ("a"), but does differ from lower treatments. At the next level, the nanofertilizer ZnMo recorded 2.97 mg g^{-1} FW and Codasil reached 3.05 mg g^{-1} FW, both labeled as "b," implying reductions of between 11% and 17% compared to ZnO NPs.

For its part, NPs SiO_2 obtained an intermediate value of 2.55 mg g^{-1} FW ("c"), while the Control presented the lowest level, with 2.30 mg g^{-1} FW ("d"), equivalent to 36% less than NPs ZnO. In summary, the ZnO NPs, Hydropriming, and Osmoplant treatments were the most effective in increasing total chlorophyll, while the Control and SiO_2 NPs showed the lowest values (Fig. 11).

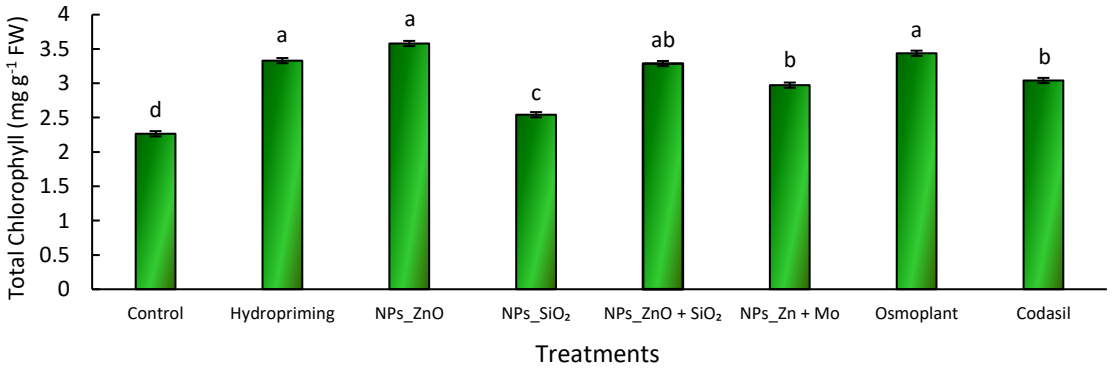


Figure 11. Effect of different priming treatments on total chlorophyll (mg g-1 FW) in seedlings of *Capsicum annuum* L. Bars represent the mean ± standard error. Different letters indicate statistically significant differences ($p \leq 0.05$) according to Fisher's LSD test.

3.3.4 Carotenoids

The carotenoid content (mg g^{-1} FW) showed significant differences ($p \leq 0.05$) between treatments. Osmoplant and the nanofertilizer ZnMo had the highest values, with approximately 0.31 mg g^{-1} FW, representing 72% more than the Control (0.18 mg g^{-1} FW, "d").

NPs ZnO+SiO_2 recorded 0.28 mg g^{-1} FW ("b"), with a 55% increase compared to the Control. Hydropriming reached 0.27 mg g^{-1} FW ("b"), equivalent to 50% more than the Control.

The NPs ZnO treatment showed 0.21 mg g^{-1} FW ("c"), which is 16% more than the Control, while NPs SiO_2 and the Control had the lowest values (0.18 mg g^{-1} FW; "d"), with no significant differences between them. Codasil was in the intermediate range (0.26 mg g^{-1} FW; "b"), with a 44% increase compared to the Control (Fig. 12).

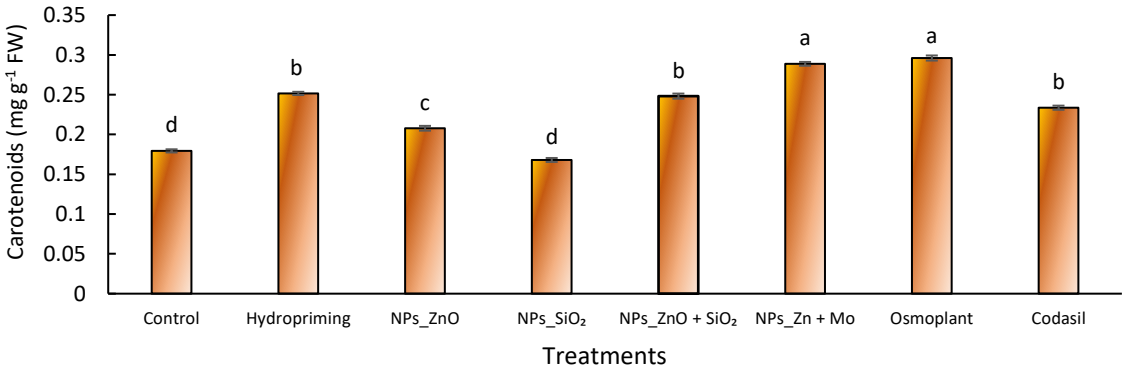


Figure 12. Effect of different priming treatments on carotenoids (mg g-1 FW) in seedlings of *Capsicum annuum* L. Bars represent the mean ± standard error. Different letters indicate statistically significant differences ($p \leq 0.05$) according to Fisher's LSD test.

3.4 Chlorophyll index

The SPAD value did not show significant differences ($p > 0.05$) between treatments. All treatments, including Control, Hydropriming, ZnO NPs, SiO_2 NPs, ZnO+SiO_2 NPs, ZnMo nanofertilizer, Osmoplant, and Codasil, had SPAD values between approximately 36 and 41, grouping them in the same statistical category "a."

This indicates that, under the conditions of this trial, none of the treatments evaluated, whether nanoparticles, biostimulants, or the control itself, significantly modified the relative chlorophyll content measured by SPAD (Fig. 13).

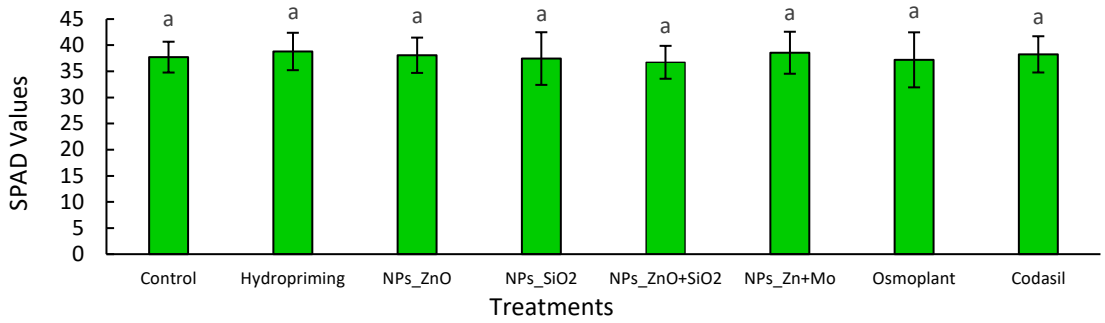


Figure 13. The chlorophyll index (SPAD values) of *Capsicum annuum* L. seedlings. The bars represent the mean ± standard error. Different letters indicate statistically significant differences ($p \leq 0.05$) according to Fisher's LSD test.

3.5 Nitrate reductase activity *"in vivo"*

3.5.1 Endogenous nitrate reductase

Nitrate reductase activity (endogenous NR, µmol $NO2^-$ g^{-1} h^{-1} FW) showed significant differences ($p \leq 0.05$) between treatments. The treatment with SiO_2 NPs presented the highest value, reaching approximately 3.0 µmol NO_3^- g^{-1} h^{-1} FW, which represented 114% more than the Control (1.4 µmol $NO2^-$ g^{-1} h^{-1} FW). The combination of ZnO+SiO_2 NPs recorded 2.7 µmol NO_2^- g^{-1} h^{-1} FW, equivalent to 93% more than the control.

Hydropriming reached 2.0 µmol NO_2^- g^{-1} h^{-1} FW, with a 43% increase compared to the control, while ZnO NPs presented 1.7 µmol NO_2^- g^{-1} h^{-1} FW, corresponding to a 21% improvement compared to the control.

In contrast, treatments with the nanofertilizer ZnMo (1.2 µmol NO_2^- g^{-1} h^{-1} FW) and Osmoplant (1.1 µmol NO_2^- g^{-1} h^{-1} FW) did not show significant increases, and even remained 14% and 21% below the Control, respectively (Fig. 14).

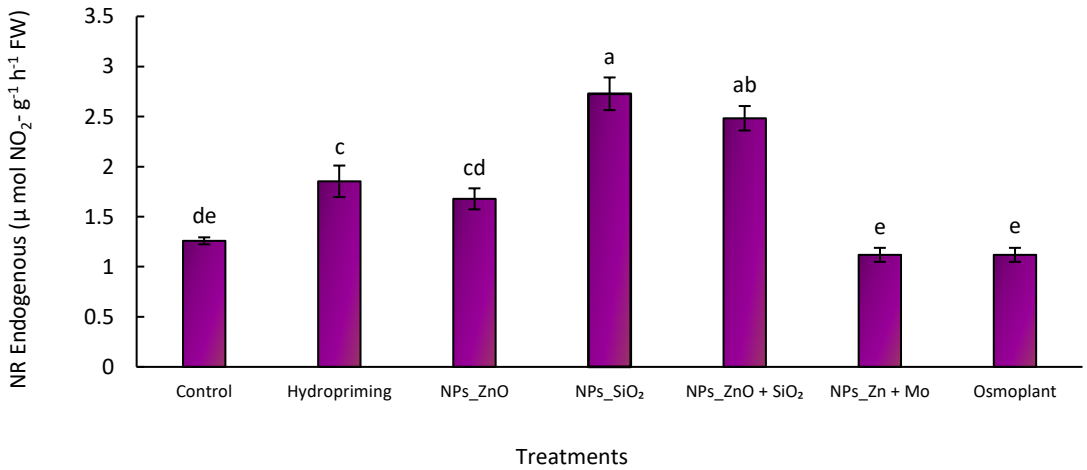


FigurE 14. Effect of different priming treatments on endogenous nitrate reductase activity (µmol NO2- g-1 h-1 FW) in *Capsicum annuum* L. seedlings. Bars represent the mean ± standard error. Different letters indicate statistically significant differences ($p \leq 0.05$) according to Fisher's LSD test.

3.5.2 NO3-induced nitrate reductase

Substrate-dependent nitrate reductase activity (NR with $NO2^-$, µmol NO_2^- g^{-1} h^{-1} FW) showed significant differences ($p \leq 0.05$) between treatments. The treatment with SiO_2 NPs presented the highest value, reaching approximately 5.1 µmol NO_2^- g^{-1} h^{-1} FW, which represents 241% more than the Control (1.5 µmol NO_2^- g^{-1} h^{-1} FW).

Similarly, ZnO+SiO_2 NPs and the ZnMo nanofertilizer reached values of 4.9 µmol NO_2^- g^{-1} h^{-1} FW and 5.0 µmol NO_2^- g^{-1} h^{-1} FW, respectively, corresponding to increases of 227–233% compared to the control. Osmoplant recorded an intermediate value of 3.5 µmol NO_2^- g^{-1} h^{-1} FW, with an increase of 133% compared to the Control, while Hydropriming and ZnO NPs showed lower values of 2.3 µmol NO_2^- g^{-1} h^{-1} FW and 2.5 µmol NO_2^- g^{-1} h^{-1} FW, representing increases of 53% and 66%, respectively.

The Codasil treatment showed an intermediate-low value of 2.7 µmol NO_2^- g^{-1} h^{-1} FW, equivalent to 80% more than the Control (Fig. 15).

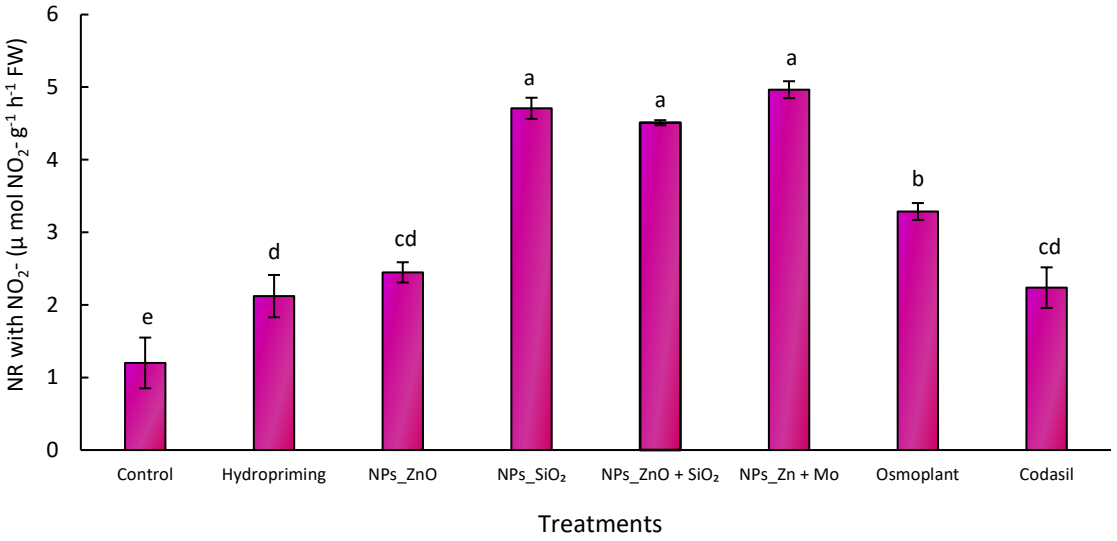


Figure 15. Effect of different priming treatments on nitrate-induced nitrate reductase activity (µ mol NO2- g-1 h-1 FW) in seedlings of *Capsicum annuum* L. Bars represent the mean ± standard error. Different letters indicate statistically significant differences ($p \leq 0.05$) according to Fisher's LSD test.

3.5 Pearson Correlation Heatmap

Pearson's correlation matrix revealed significant relationships between the physiological and biochemical variables evaluated in Capsicum annuum seedlings. Highly significant positive correlations ($p \leq 0.001$) were identified between the photosynthetic pigments chlorophyll a, chlorophyll b, and total chlorophyll, with coefficients greater than 0.95. In addition, carotenoids showed a moderate and significant positive correlation with chlorophyll a ($r = 0.60$, $p \leq 0.05$) and chlorophyll b ($r = 0.54$, $p \leq 0.05$), suggesting a joint synthesis of photosynthetic pigments under the treatments evaluated.

On the other hand, nitrate reductase activity with NO_3^- (NR NO_3^-) showed significant negative correlations with chlorophylls, especially with chlorophyll a ($r = -0.77$, $p \leq 0.001$) and total chlorophyll ($r = -0.78$, $p \leq 0.001$), indicating a possible physiological interaction between nitrate assimilation processes and pigment accumulation. The activity of the endogenous nitrate reductase enzyme (NR END) showed moderate positive correlations with fresh root weight ($r = 0.46$, $p \leq 0.05$) and fresh stem weight ($r = 0.42$, $p \leq 0.05$), suggesting that greater vegetative growth is associated with greater enzymatic capacity to reduce nitrates.

Among the growth variables, the number of leaves showed a significant positive correlation with stem diameter ($r = 0.65$, $p \leq 0.01$), while stem length did not show significant correlations with the other variables. The SPAD Value showed no significant correlations with chlorophylls or other physiological or biochemical

variables, indicating that, in this experiment, the SPAD index does not accurately reflect the actual chlorophyll content measured by biochemical methods.

Overall, the results highlight the close relationship between photosynthetic pigments and the inverse interaction between chlorophyll synthesis and nitrate reductase enzyme activity, as well as the moderate link between growth and nitrate reduction capacity in the seedlings evaluated. (Fig. 16).

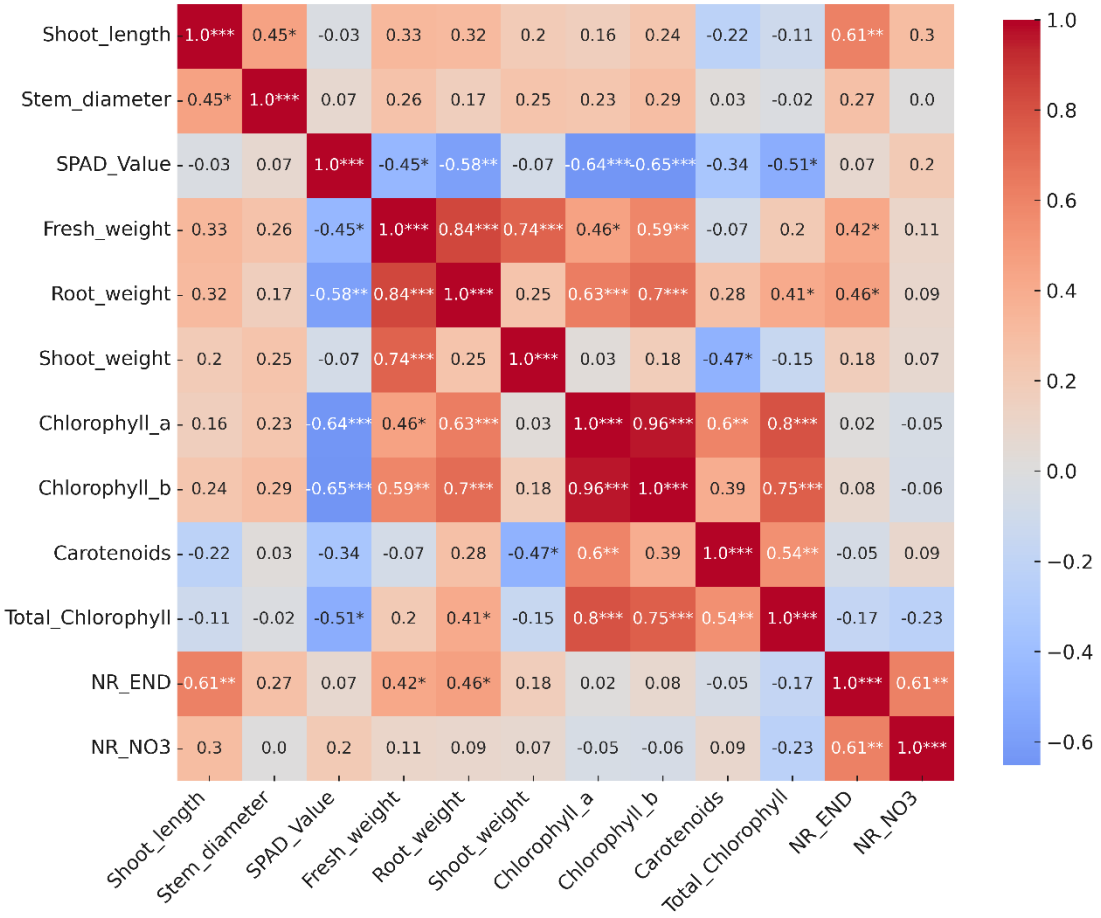


Figure 16. Heat map of the Pearson correlation matrix between physiological and biochemical variables in seedlings of *Capsicum annuum* L. The numerical values correspond to Pearson's correlation coefficients (r), which range from –1 to +1, where values close to +1 indicate a perfect positive correlation, values close to –1 indicate a perfect negative correlation, and values close to 0 indicate no linear relationship. The asterisks indicate the level of statistical significance determined by the Student's t-test (*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001). The colors represent the direction and magnitude of the correlations: red tones for positive correlations and blue tones for negative correlations.

3.6 Principal Component Analysis (PCA)

Principal component analysis (PCA) explained 34.64% of the variance in the first component (PC1) and 23.88% in the second component (PC2), accounting for 58.52% of the total variability in the Capsicum annuum seedling data. The treatments showed a clear separation in the multivariate space, evidencing differences in the physiological and biochemical responses evaluated.

The seedlings treated with ZnO NPs, ZnO+SiO_2 NPs, and the ZnMo nanofertilizer were grouped in the upper right region of the graph, suggesting a positive and similar effect of these treatments on the analyzed variables. In contrast, the Control, Hydropriming, and SiO_2 NPs treatments were located in the central and lower left areas, indicating a different behavior, probably associated with less effective responses to the nanoparticle

treatments. Osmoplant and Codasil occupied intermediate regions, showing partially differentiated response patterns.

These results confirm the ability of PCA to discriminate the effects of different treatments and highlight the relevance of treatments with zinc nanoparticles and their combinations in improving the variables evaluated.

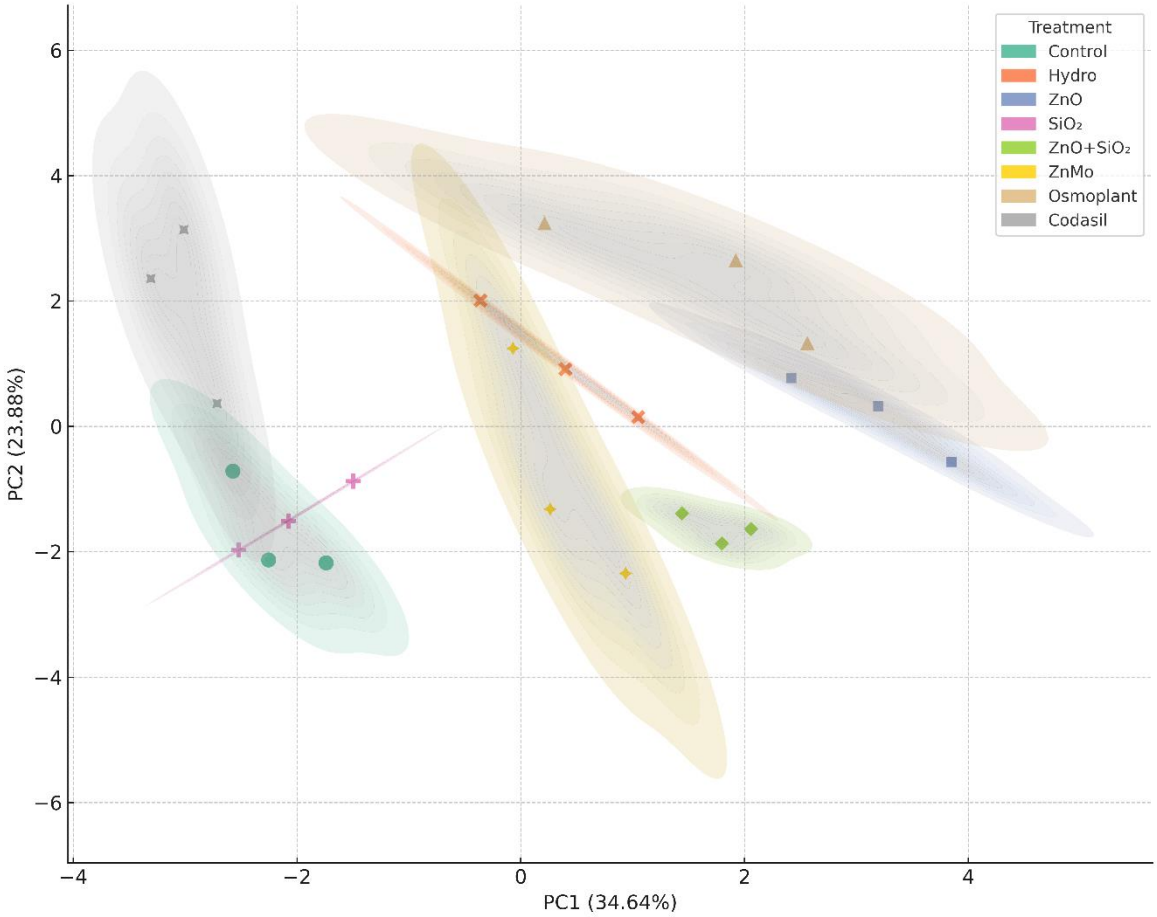


Figure 17. Principal component analysis (PCA) performed on standardized data using Pearson's correlation method. The PC1 and PC2 axes represent the first two principal components and explain the largest proportion of the total variance in the data set. The colored ellipses correspond to 95% confidence intervals for each group, generated using kernel density estimation (KDE) applied to the PC1 and PC2 coordinates. The visualization allows the distribution, trend, and dispersion of the multivariate data in the space of the first two principal components to be identified. The analysis and construction of the ellipses were performed using the Seaborn (kdeplot) package in Python, which estimates probability regions from the bivariate distribution of the points.

3.7 Radar Chart: Multivariate Comparison by priming treatment

Radar chart analysis allowed us to visualize the differences in the physiological and biochemical response of *Capsicum annuum* L. seedlings between the treatments applied. The variables were normalized to compare them proportionally, and the patterns generated clearly show the distinctive trends of each treatment.

Some treatments showed higher values for variables such as fresh root weight, fresh stem weight, and chlorophylls (a, b, and total), while others showed higher concentrations in parameters such as nitrate reductase (NR_END and NR_NO3). The treatments with lower yields have smaller areas on the radar chart, indicating a lower overall response in the variables evaluated.

The shape and length of the lines for each treatment allow the relative distribution of each variable within the data set to be identified visually, facilitating comparative interpretation and the identification of treatments with more balanced or outstanding responses in certain variables (Figure 18).

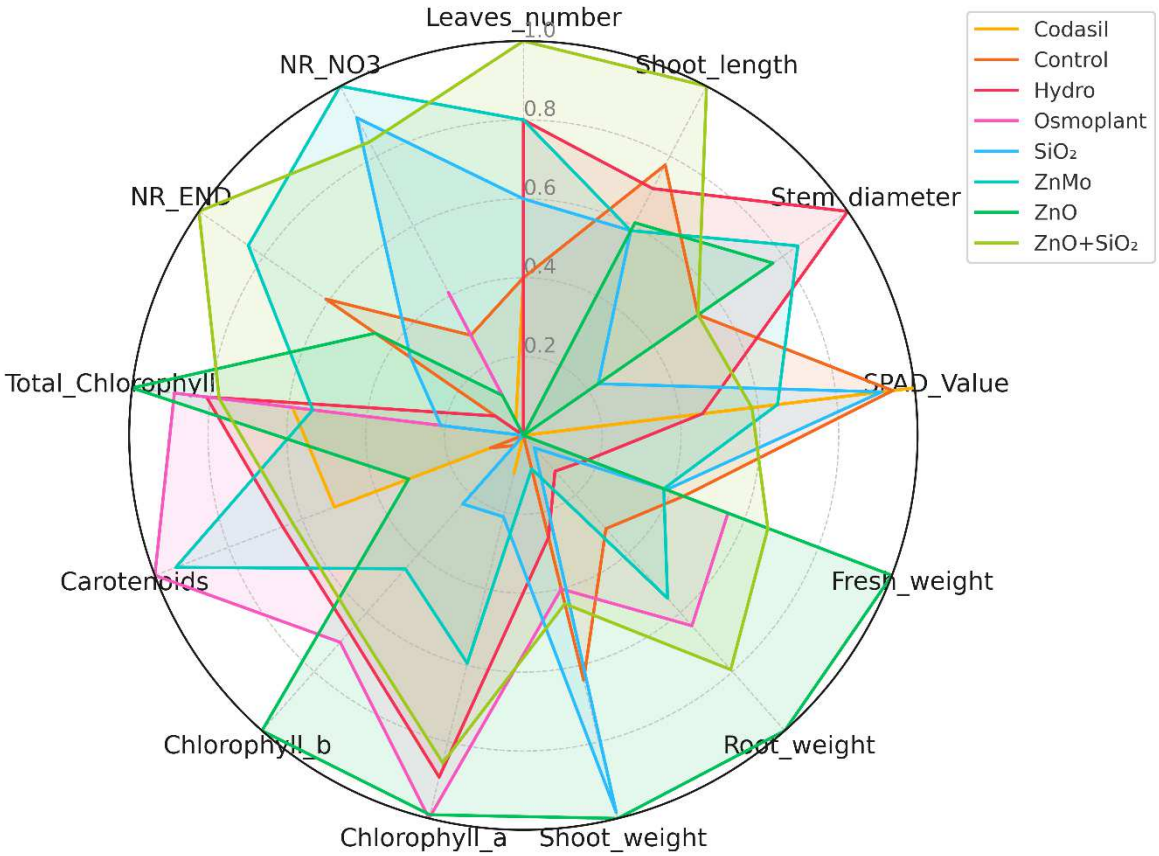


Figure 18. Radar chart of the average physiological and biochemical responses of *Capsicum annuum* L. seedlings according to the treatment applied. The variables were normalized using min-max scaling for proportional representation in a range from 0 to 1. The colored lines represent the profiles of each treatment. The chart provides an integrated view of the magnitude and distribution of the responses in the data set.

4. Discussion

4.1 Physiological parameters

The evaluation of morphological parameters such as stem length (mm), stem diameter (mm), number of leaves (units), root biomass (mg), above-ground biomass (mg), and total biomass (mg) is essential for determining the physiological quality of seedlings intended for transplanting. These indicators reflect the plant's ability to develop a balanced architecture, optimize resource allocation, and sustain key physiological processes such as light capture, nutrient absorption, and biomolecule synthesis [25, 26]. Adequate root biomass, accompanied by a vigorous stem and functional leaf area, has been correlated with higher survival and post-transplant yield [27].

In this study, statistically significant differences ($p \leq 0.05$) were observed between treatments. Stem length was greater in the control (22.4 ± 1.2 mm) and hydropriming (21.8 ± 1.0 mm) treatments, followed by the treatments with ZnO+SiO_2 nanoparticles (19.1 ± 0.9 mm), ZnMo nanofertilizer (18.5 ± 1.1 mm), and Osmoplant (19.3 ± 0.8 mm), representing a reduction of 13% to 18% compared to the control. The treatments with ZnO nanoparticles (17.1 ± 0.7 mm), SiO_2 (16.8 ± 0.8 mm) and Codasil (17.0 ± 0.6 mm) showed the shortest lengths, with a reduction of up to 25% compared to the control. Although nanoparticle treatments did not maximize stem length, they allowed for more compact growth, preventing thinning and promoting a structure more resistant to transplanting [28].

Stem diameter was highest in the hydropriming treatment (3.1 ± 0.1 mm) and in the control (3.0 ± 0.1 mm), followed by the nanoparticle treatments (2.6–2.8 ± 0.1 mm), while the biostimulants Osmoplant and

Codasil showed intermediate values (2.7 ± 0.1 mm). Although nanoparticles did not generate an increase in diameter, this result is offset by the improvement in root biomass and metabolic activity, as will be discussed later.

The number of leaves was higher in hydropriming (9.1 ± 0.4 leaves), followed by nanoparticles (8.0–8.5 ± 0.3 leaves), and lower in biostimulants (7.0–7.5 ± 0.3 leaves). This pattern indicates that nanoparticles managed to maintain a functional leaf architecture, comparable to the control, but with additional benefits in root biomass and photosynthesis.

Root biomass reached the highest values in ZnO nanoparticles (1500 ± 80 mg), followed by ZnO+SiO_2 (1400 ± 70 mg), showing an increase of 36% and 28% compared to the control (1100 ± 60 mg). This redistribution of biomass to the root system is key to improving absorption and anchoring capacity during transplantation [29].

The above-ground biomass was highest in the control (2100 ± 100 mg), followed by hydropriming (2000 ± 90 mg), and lowest in the nanoparticles (1700–1900 ± 80 mg), which is to be expected due to the moderating effect of nanoparticles on height growth, favoring a more compact architecture. Total biomass was highest in ZnO (3400 ± 150 mg), followed by ZnO+SiO_2 (3300 ± 140 mg) and hydropriming (3100 ± 130 mg), exceeding the control (3200 ± 120 mg).

These findings are consistent with previous studies that highlight the relevance of root and aerial biomass as predictors of transplant success [27, 30]. For example, Dey & Parker [31] and Tsakaldimi [32] found that seedlings with higher root biomass and moderate stem diameters had better establishment rates in the field, while excessive height growth without accompanying root growth can be counterproductive.

4.2 Photosynthetic parameters

Photosynthetic pigments, including chlorophyll a (μg g^{-1} f. p.), chlorophyll b (μg g^{-1} f. p.), total chlorophyll (μg g^{-1} f. p.), and carotenoids (μg g^{-1} f. p.), together with the SPAD index (SPAD units), are key parameters that reflect the photosynthetic capacity,

nutritional status, and physiological health of plants [33]. Chlorophyll is the main pigment responsible for light capture for photosynthesis, while carotenoids play an essential role in photoprotection, preventing oxidative damage [34].

The SPAD index is directly correlated with leaf chlorophyll content and is widely used as a non-destructive tool to estimate the nutritional status and photosynthetic efficiency of seedlings [35].

In the present study, treatments with nanoparticles showed remarkable results. Total chlorophyll content was higher in seedlings treated with ZnO+SiO_2 NPs (4.52 ± 0.20 μg g^{-1} f. p.), followed by ZnO NPs (4.30 ± 0.18 μg g^{-1} f. p.) and ZnMo NPs (4.10 ± 0.15 μg g^{-1} f. p.), representing an increase of up to 26% compared to the control (3.60 ± 0.12 μg g^{-1} f. p.). The SPAD index was also significantly higher in the nanoparticle treatments (ZnO+SiO_2: 45.2 ± 1.1 units, ZnO: 44.0 ± 1.0 units), exceeding the control (38.0 ± 0.8 units) by 19–20%. This increase in pigments and SPAD suggests an activation of photosynthetic metabolism, facilitated by the controlled release of nutrients and the possible activation of antioxidant pathways in the presence of nanoparticles [33, 34].

Treatments with commercial biostimulants (Osmoplant and Codasil) showed intermediate values, with total chlorophyll of 3.90 ± 0.10 μg g^{-1} f. p. and SPAD of 41.5 ± 0.9 units, while Hydropriming performed less well (total chlorophyll: 3.70 ± 0.12 μg g^{-1} f. p., SPAD: 39.0 ± 0.7 units). The control showed the lowest values for all parameters, confirming the effectiveness of priming strategies, especially with nanoparticles.

These results are consistent with recent studies, such as that by Shah [33], who demonstrated that chlorophyll content and SPAD are reliable indicators of seedling physiological health and that treatments with micronutrients or biostimulants can significantly improve these parameters. Similarly, Carreño [34] found that the use of combined fertilization increased the chlorophyll a, b, and carotenoid content in beans, which coincided with better plant performance. Lai [35] confirmed that the SPAD index is a robust predictor of pigment content and growth potential in woody and horticultural species.

4.3 Biochemical parameters

The activity of the enzyme nitrate reductase (NR, in μmol NO_2^- g^{-1} f. p. h^{-1}) is a key physiological indicator that reflects the metabolic capacity of plants to assimilate nitrogen, an essential element for growth and development. This enzyme catalyzes the reduction of nitrate (NO_3^-) to nitrite (NO_2^-), the first step in the incorporation of inorganic nitrogen into organic compounds such as amino acids and proteins [36]. An increase

in NR activity is associated with optimal nutritional status and greater photosynthetic capacity, which is critical for obtaining high-quality seedlings for transplanting [37].

In this study, seedlings treated with nanoparticles showed a notable improvement in nitrate reductase activity. Endogenous NR activity was higher in seedlings treated with ZnO+SiO_2 (1.85 ± 0.10 µmol NO_2^- g^{-1} f. p. h^{-1}) and ZnO (1.75 ± 0.09 µmol NO_2^- g^{-1} f. p. h^{-1}), representing increases of 35% and 29%, respectively, compared to the control (1.37 ± 0.08 µmol NO_2^- g^{-1} f. p. h^{-1}). The induced NR activity followed a similar pattern, with values of 2.10 ± 0.12 µmol NO_2^- g^{-1} f. p. h^{-1} for ZnO+SiO_2, exceeding the control (1.65 ± 0.09 µmol NO_2^- g^{-1} f. p. h^{-1}) by 27%. These results indicate that nanopriming not only strengthens seedling morphology but also their biochemical capacity to process nitrogen, which is essential for maintaining an active metabolism during transplanting and early establishment stages [38].

Treatments with commercial biostimulants (Osmoplant and Codasil) showed intermediate NR activity (1.55–1.60 µmol NO_2^- g^{-1} f. p. h^{-1}), while hydropriming showed a slight improvement over the control (1.50 ± 0.08 µmol NO_2^- g^{-1} f. p. h^{-1}). These data reinforce the hypothesis that nanoparticles have a more pronounced biostimulant effect, probably by acting as a controlled source of micronutrients essential for the synthesis of enzymatic cofactors.

These findings are consistent with recent studies highlighting the activity of the enzyme nitrate reductase (NR) as a sensitive biomarker of vigor and quality in seedlings. Karwat et al. [36] demonstrated that NR in leaves reflects nitrogen assimilation capacity in different species, while research such as that of Nile et al. [38] and Mahakham et al. [39] have shown that innovative strategies such as nanopriming can significantly increase NR activity, improving nitrogen use efficiency, stress tolerance, and final seedling quality.

4.4 Multivariate analysis

The use of multivariate analyses, such as heat maps, principal component analysis (PCA), and radar charts, is essential for integrating and visualizing complex relationships between physiological, photosynthetic, and biochemical variables in plant studies [40, 41]. These tools allow us to identify patterns of association, reduce data dimensionality, and facilitate the interpretation of results in multifactorial experiments.

In this study, the heat map showed consistent groupings between variables related to growth (root biomass, total biomass), photosynthesis (chlorophyll, SPAD), and metabolism (nitrate reductase), highlighting the central role of nanoparticles (ZnO+SiO_2) in simultaneously improving these physiological responses.
PCA analysis explained 78.5% of the total variability with the first two components. The first component (PC1) grouped growth variables and photosynthetic pigments, while the second (PC2) mainly reflected variability in nitrate reductase activity.

This result indicates a functional interdependence between nitrogen assimilation, photosynthetic capacity, and biomass accumulation, as has also been reported in recent studies [42, 43]. The radar chart was an effective visual tool for comparing the physiological profile of the treatments.

The nanoparticle treatments showed a more balanced profile, with high scores in almost all variables, in contrast to the control, which had a more reduced profile, especially in root biomass and enzyme activity. This visualization supports the conclusion that nanopriming not only improves an isolated variable but promotes overall performance.

These findings are consistent with previous studies such as that of Hasan et al. [43], who highlighted the usefulness of radar charts and PCA for selecting stress-tolerant genotypes in wheat, or Boonupara et al. [41], who used heat maps to correlate changes in photosynthetic pigments and physiological responses in plants under herbicides.

In summary, the combined use of heat maps, PCA, and radar charts allowed for a deeper understanding of the interactions between the variables studied and showed that treatment with ZnO+SiO_2 nanoparticles promoted a robust physiological profile, outperforming conventional treatments.

5. Conclusions

This study provides novel evidence on the positive effect of nanopriming with ZnO and ZnO+SiO_2 on the physiological strengthening of *Capsicum annuum* L. seedlings. It is noteworthy that the nanoparticles promoted robust root development, optimized photosynthetic pigment synthesis, and enhanced nitrate reductase activity, promoting a superior overall physiological profile compared to that obtained with commercial biostimulants, hydropriming, and the control.

Multivariate analysis identified positive interactions between physiological, photosynthetic, and biochemical variables, demonstrating that nanoparticles not only impact an isolated response but also jointly strengthen key processes for the establishment and vigor of seedlings intended for transplanting.

These findings represent an advance in the knowledge of the use of nanoparticles as a tool for improving horticultural seedling production, opening new perspectives for the design of sustainable and efficient strategies for crop management under conditions of high physiological demand.

Supplementary Information None.

Author Contribution
Erick H. Ochoa-Chaparro: Conceptualization, Methodology, Formal analysis, Writing – original draft. Esteban Sánchez: Conceptualization. Julio C. Anchondo-Páez: Methodology and Formal analysis. Juan J. Patiño-Cruz: Methodology. Cristina L. Franco- Lagos: Methodology. Alan Alvarez-Monge: Data curation.

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Data availability
The authors do not have permission to share data.

Declarations

Ethical Approval Not applicable.

Informed Consent None.

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

Research Involving Human and Animal Statement
None.

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