

Foliar application of zinc oxide nanoparticles and grafting improves the bell pepper (*Capsicum annuum* L.) productivity grown in NFT system

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Abstract

The bell pepper (*Capsicum annuum* L.) is a food vegetable with a high nutritional intake, with rich content in vitamins, minerals and antioxidants. In this study, using nutrient film technique (NFT) system, the effect of the zinc oxide nanoparticles on the micromorphology, histology, physiology and production of the grafted pepper was evaluated. The treatments used were grafted and non-grafted plants, four concentrations (0, 10, 20, 30 mg L⁻¹) of zinc oxide nanoparticles, and the experience was organized in a completely randomized design. An increase in grafted plants was observed in the weight, number and size of fruits in 18.1%, 21.8% and 9.6%, the concentration 30 mg L⁻¹ of nanoparticles statistically affected the weight, number and size 46.9%, 47.7% and 18% compared to the control. The interaction with grafted plants and the treatment of 30 mg L⁻¹ of zinc oxide nanoparticles increased fruit weight, number of fruits and size by 62.60%, 57.69% and 29.17% compared to plants without grafting and the control treatment. These results indicate that the use of grafts and zinc oxide nanoparticles could be used in bell pepper production to increase yield.

Keywords: histology; hydroponic; micromorphology; physiology; productivity

Introduction

The bell pepper is one of the vegetables with the highest production worldwide (FAOSTAT, 2019), the world production of bell pepper in 2019 was 1,990,926 harvested hectares, of which from which a production of 38,027,164 tons was obtained, with an average yield of 19.10 t ha⁻¹ (FAO, 2019). In Mexico 152,772.55

Received: 30 Mar 2021. Received in revised form: 06 May 2021. Accepted: 20 May 2021. Published online: 25 May 2021.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

hectares were planted, of which a production of 3,238,244.81 tons was obtained, with a yield of 21.65 t ha⁻¹ (SIAP, 2019). However, the pepper also has a great contribution to human health, providing for its great nutritional value, it contains a wide range of nutritional and bio functional properties related to its phenolic compounds, flavonoids, capsaicinoids, carotenoids, tocopherols, vitamin C, A, E, B, potassium, magnesium, iron, calcium and phosphorus (Ganguly *et al.*, 2017; Vera-Guzmán *et al.*, 2017). It is of great importance to know the micromorphological, histological, physiological and productive processes in the bell pepper crop, since it is one of the most consumed chili peppers both fresh, dry, and processed.

The micromorphology, histology, physiology and productivity are positively modified by the graft and the use of nanoparticles in the bell pepper culture, for which varieties of commercial interest and good adaptability to conditions of the region were used. Currently there are no works in which the effects of grafting and nanoparticles and their interaction in the production of bell pepper established and grown in the nutrient film technique (NFT) system have been recorded.

Grafting is a special type of plant propagation, in which one part of a plant (scion) joined to another plant (rootstock) so that the two parts grow together and form a new plant (Rouphael *et al.*, 2018). By selecting the appropriate rootstock and with this technology, the morphology of the scion can be manipulated, and biotic and abiotic stress can be managed (Kumar *et al.*, 2015; Kumar *et al.*, 2017). Nutrient uptake and utilization in horticultural crops is enhanced by selecting appropriate rootstocks, which play a vital role in manipulating the nutrient status of the shoots by directly affecting ion absorption and transport (Amiri *et al.*, 2014). The use of suitable rootstocks tends to simultaneously improve the environmental, economic and social aspects of agriculture (Martínez-Andújar *et al.*, 2020).

NFT is a system that uses water that contains the dissolved nutrients necessary for plant growth and can be an open or closed system in which the nutrient solution recirculates constantly being in direct contact with the root system of the plants (Cooper, 1979). This growing hydroponic technique is gaining acceptance as it provides a high return on investment, supporting the growth of the hydroponics market (Love *et al.*, 2015). The NFT has its origins in England during the sixties, it was developed to increase the productivity of the hydroponic production sector, generally it is composed of PVC pipes, collecting tanks, recirculating pumps for the nutrient solution (Triunfo *et al.*, 2018). It allows the reuse of nutrients, for a better use of the resource, favoring absorption in the root systems, it is considered a closed system since the nutrient solution recirculates as a sheet through the roots (Brenes and Jimenez, 2016), seek to reduce the use of water, and therefore reduce production costs, the use of grafts will benefit with greater vigor, improved plant development, and defense against abiotic stress (Huang *et al.*, 2015; Love *et al.*, 2015; Miglietta *et al.*, 2017).

With nanotechnology, the effectiveness of the use of micronutrients, macronutrients, and pesticides in crops can be increased (Mazzaglia *et al.*, 2017). A nanoparticle is defined as any designed particle with a dimension of 1 to 100 nano meters and has properties that are not shared by nanoscale particles with the same chemical composition (Auffan *et al.*, 2009; Hajra and Mondal, 2017). However, little is known about the effect that zinc oxide nanoparticles (ZnO NPs) have on the micromorphology, histology, physiology, and productivity of the grafted bell pepper. The use of grafts gives vigor, improves the absorption and transport of water and nutrients, influencing the micromorphology, histology and physiology of plants (Camposeco-Montejo *et al.*, 2018; García-López *et al.*, 2019; Salehi *et al.*, 2010). The application of zinc oxide nanoparticles generates oxidative stress in the plant as defense mechanisms the plant produces secondary metabolites which have an effect on the micromorphology, histology and physiology (Mantoan *et al.*, 2016; Rossi *et al.*, 2019; Zhu *et al.*, 2020). The hypothesis used in this work was that micromorphology, histology, physiology, and production are positively modified by grafting and the use of ZnO NPs in bell pepper cultivation. Therefore, in this work we report the effects produced in 'SVEN RZ F1' bell peppers grafted and cultivated in the NFT system with foliar application of zinc oxide nanoparticles.

Materials and Methods

The present work was carried out in the spring-summer 2020 cycle in a greenhouse located in the Department of Horticulture at the Antonio Narro Autonomous Agrarian University, Buenavista, Saltillo, Coahuila, Mexico, at latitude 25°21'23.4", longitude 101°02'10.6" and 1,760 meters above sea level Figure 1.



Figure 1. Established experiment; Cultivation established in greenhouse

Plant material and growing conditions

The 'SVEN RZ F1' hybrid from the Rijk Zwaan seed company is a blocky type bell pepper characterized by having short internodes, which adapts very well in greenhouses, with very good bearing and great fruit setting capacity in hot conditions. since it is a very precocious material. With fine, bright yellow fruits of very good quality, a plant with good vigor and a good generative tendency. For the rootstock, an 'ULTRON F1' pepper from the HM CLAUSE commercial seed company was chosen, which is a hybrid of indeterminate growth that has great vigor and tolerance to salinity, with a yellow blocky-type fruits.

Inside the greenhouse, an average irradiance of 4.5 kWh m⁻² day⁻¹ was reached, the average recorded temperatures were a maximum of 36 °C and a minimum of 22 °C and the average relative humidity inside the structure was 40%.

The scion was sown in a greenhouse on February 7, 2020 using a 200-cavity polystyrene tray Figure 2A, peat was used as substrate, 10 days later, on February 17, 2020, the rootstock was sown in a tray of 200 cavities, using peat as substrate and one seed per cavity. The rootstock has greater vigor and vegetative growth, which is why it was sown later, for this reason it reached the scion in size and stem width. Obtaining similarity in the thickness of the stem, which benefits the union of both plant structures.

Graft

On March 7, 2020, the grafting was performed using the splicing technique (Lee Jung Myung, 1994) when the plants had 30 days after germination Figure 2B. The splice graft was performed when both structures had a stem diameter of two millimeters. The rootstock and scion were cut at an angle of 60° downwards and upwards respectively, both plant structures were joined with a 2.0 mm size silicone clip.

The recovery of the plants began immediately after grafting, they were kept in a holding chamber with relative humidity between 80 to 85% and a temperature of 25 to 28 °C, for 10 days. On March 17, 2020 the silicone clip was completely removed as the union between scion and rootstock had occurred and healed. The

plants were supplied daily with water, 25% nutrient solution (Steiner, 1961), and commercial foliar amino acids Metamin Max[®] (Agroestimulantes Mexicanos SA de CV., Aguascalientes, México) with a composition of 64.92% glutamic acid, 5.08% thiamine and 30% inert conditioners, a dose of 1 g L⁻¹ in foliar application with a manual water spray.

Nutrient Film Technique (NFT)

The NFT system used was composed of 8 PVC pipes of 6 inches in diameter, with 16 holes of 6 centimeters wide, and with 30 cm between holes, 68-liter plastic boxes were used to collect the nutrient solution, and 25-watt submersible electric pumps for fish tanks with a circulation capacity of 1500 liters per hour were used for recirculation of the nutrient solution.

Transplant to NFT system

The transplant was carried out on April 13th and 14th, 2020 Figure 2C, the substrate was removed from the seedlings rinsing with water and leaving the root free of substrate, then the root was immersed in a solution of water with 3% hydrogen peroxide as a preventive treatment against disease-causing microorganisms. Plastic baskets for hydroponics of 3 inches were used, for the adjustment of the plant inside the basket, a polyurethane sponge was used leaving the root free to have contact with the nutrient solution.

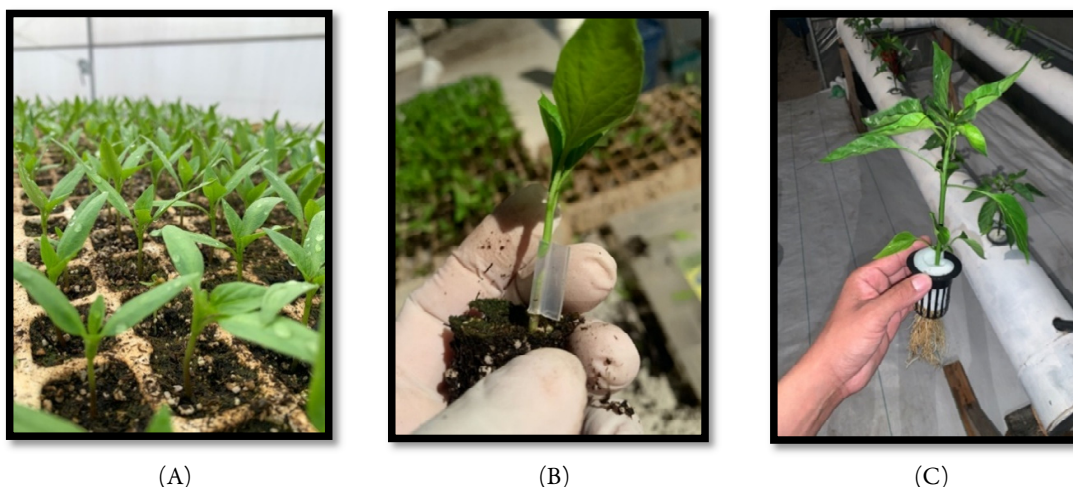


Figure 2. Establishment and work in greenhouse; (A) rootstock and scion sowing; (B) splice graft; (C) establishment in NFT system

Zinc oxide nanoparticles

The ZnO NPs used in the experiment were synthesized and characterized in the pilot plant of the Center for Research in Applied Chemistry (CIQA) Saltillo, Coahuila, Mexico Figure 3, their size of 52 nm, spherical morphology, white-yellow powder appearance clear, purity 99.8% were determined by X-ray, and covered with palmitic acid.

Application of the ZnO NPs

The application was by foliar route 10 days after the healing of the graft, on March 27th, 2020, the different doses of nanoparticles were prepared using concentrations of 0, 10, 20, and 30 mg L⁻¹. The method for preparing the different doses was using a ZnO NPs stock solution. Subsequently, the four doses of nanoparticles were prepared, using a 1-liter volumetric flask, each of the nanoparticle concentrations were poured separately and made up with distilled water. The solutions were placed in manual sprinklers with a

capacity of 1 liter, and the different nanoparticle treatments were applied homogeneously on the bundle, underside of the leaves and stems (shoots).

The first application of nanoparticles was in the growth stage, when the seedlings had 10 days after grafting. The second application of nanoparticles was made in the flowering stage 80 days after transplantation, the third application was made in the fruit filling stage at 95 days after transplantation, in plants already established in the NFT system. The applications were made in the afternoon when temperatures of 22 °C prevailed, to avoid any problem related to high temperatures and radiation.



Figure 3. Photograph of zinc oxide nanoparticles; preparation of zinc oxide nanoparticles

Measurement of variables (Micromorphological variables)

Collection of epidermal samples: to determine the number, size of stomata, and tabloid cells, fully expanded young leaves were used Figure 4A. The leaves were cut at the same plant height, with the characteristics that were mature, fully expanded and with the same orientation. The impressions were taken immediately after cutting the leaves, from both sides (top and bottom) ensuring that all were from the middle part of each leaf following the methodology described in the manual (Hernández, 1984).

The material used to print the samples was PVC glue and transparent adhesive tape, paint brush, and glass microscope slides. A light layer of glue was placed on the middle part of the leaf (upper and lower side), it was left to dry for 30 seconds, then a centimeter of adhesive tape was cut and placed on the glue, allowed to dry and the adhesive tape was peeled off with the glue already attached. It was mounted on the slides, three random samples were taken per treatment (Hernández, 1984).

For the evaluation, a compound microscope (Carl Zeiss) with an integrated camera (Pixera Winder Pro) was used to capture photographs of the samples in the top and bottom for the number, width, length of stomata and number of cells epidermal figure 4B. Measurement software was used (AxionVision Rel. 4.8; Carl Zeiss) (Hernández, 1984).

Stomatal density (SD)

Stomatal density was evaluated as follows:

$SD = \text{number of stomata} / 0.0247604 \text{ mm}^2$ (photographed image area), the result obtained are stomata per mm^2 .

$$SD = \text{Stomatal number} / \text{image area} \quad (1)$$

Stomatal index (SI)

The stomatal index was calculated by the formula suggested by (Wilkinson, 1979). A compound microscope (Carl Zeiss) with digital camera (PixeraWinder Pro) and measurement software were used (AxionVision Rel. 4.8; Carl Zeiss).

Represents the quotient between the number of stomata and the number of epidermal cells.

$$SI = \text{stomatal density} / \text{epidermal cells} + \text{number of stomata} * 100 \quad (2)$$

Histological variables: number of xylem vessels and xylem vessel area

The procedure for the histological test was as follows Figure 4C and 4D: Fixation in formaldehyde alcohol acetic acid (FAA), and dehydration with 50% and 60% alcohol for 30 minutes respectively, 70%, 80% and 96% for 3 hours, continuing with t-Butyl alcohol I, t-Butyl alcohol II, t-Butyl alcohol plus xylol in proportions of 3:1, t-Butyl alcohol plus xylol in proportions of 1:1, t-Butyl alcohol plus xylol in proportions of 1:3 and pure xylol, for 2 hours in each solution. The inclusion was in paraffin in an oven from 30 °C to 55 °C. The cuts were made in a manual microtome at 18 μm of thickness, and glued on slides with adhesive and heat, the staining was safranin-fast green. Finally, the tissues were sealed with a drop of Canada balsam and a slide the size of the tissue, and left to dry in an oven at 30 °C for a week (Hernández, 1984).

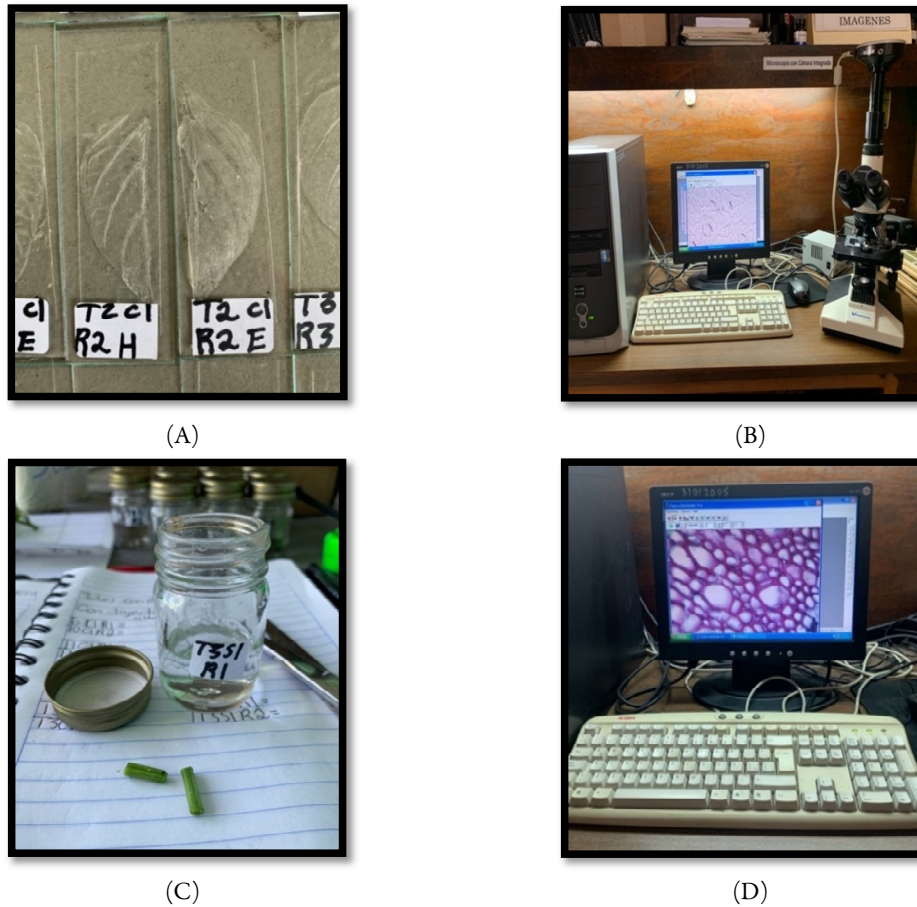


Figure 4. Sampling; (A) leaf impression on slide; (B) AxionVision Rel. 4.8 Carl Zeiss for measuring conductive tubes; (C) horizontal stem cut; (D) AxionVision Rel. 4.8 Carl Zeiss for stomata measurement

Physiological variables

For the measurement of gas exchange, a portable photosynthesis system Li-6,800 (LI-COR, Inc., Lincoln, NE, USA) was used in the phenological stage of production for being a significant stage in gas exchange, on July 22, 2020, data were taken 100 days after transplantation, taking five means per treatment, the light conditions were: light 379.20 μmol m⁻² s⁻¹; CO₂ 550 ppm; temperature 32 °C; relative humidity 65%, data collection was during the day at 12:00 pm and with environmental conditions of a totally clear sky, a single

measurement was made, with the PAR and CO₂ variables fixed and there was no variation during the measurement. Figure 5A.

Production variables: fruit weight, number of fruits, polar and equatorial diameter

For the fruit weight variable, an I-2,000 Superior mini digital portable scale (METER8, Co., Shenzhen, China) was used where 5 fruits were weighed per treatment Figure 5B. The number of fruits was counted at the time of cutting Figure 5C. For the variables of polar and equatorial diameter, a HER-411 digital vernier (Electrónica Steren S.A. de C.V., Azcapotzalco, México) was used, measuring 5 fruits per treatment Figure 5D.

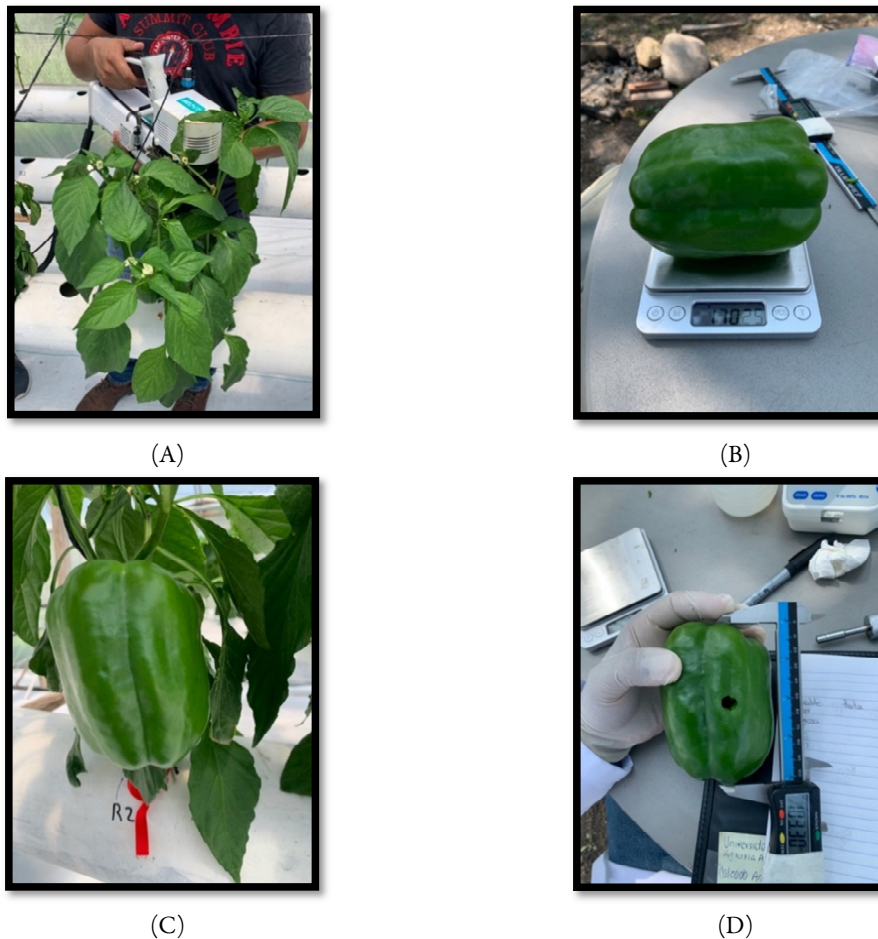


Figure 5. Production and harvest; (A) portable photosynthesis system LI-COR LI-6800; (B) fruits weight; (C) number of fruits; (D) fruits size

Statistical analysis

The experimental design used for the development of the experiment was completely random with a factorial arrangement (2*4). With the data obtained, an analysis of variance (ANOVA) was carried out; for the detection of statistical differences between treatments, the test of comparison of means by Tukey was used ($p \leq 0.05$). The factors were: with and without graft, four concentrations 0, 10, 20 and 30 mg L⁻¹ of zinc oxide nanoparticles resulting in eight treatments and four repetitions, statistical software was used for the analysis of the information (InfoStat version 2014 Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina).

Results

Micromorphological and histological results

Results of the graft effect on the micromorphological and histological variables.

The graft affected the histological variables with statistical difference in the grafted plants in the xylem vessel area (XVA) variable, which was 37.8% greater than in plants without graft, regarding the number of xylem vessels (NXV) variable the plants without graft had a greater number of vessels by 20.1% compared to grafted plants Table 1.

Results of the ZnO NPs effect on the micromorphological and histological variables.

The application of nanoparticles statistically affected the variable of stomatal index (IE), in the concentration 30 mg L⁻¹ had 3.8% higher index compared to the control, while the length of stomata on the bottom (LSB) had an increase of 12.1% compared to the concentration of 10 mg L⁻¹ and 15.1% than the control. The variables length of stomata in top (LST), width of stomata in top (WST) and width of stomata bottom (WSB) did not have differences between treatments Table 1.

Table 1. Comparison of means of graft effects, ZnO NPs, and interaction in micromorphological and histological variables

Factor	SD (mm ²)	SI (%)	SLT (mm)	LSB (mm)	SWT (mm)	WSB (mm)	AVX (mm ²)	NXV	
Grafted	287.41 a	89.23 a	24.69 a	23.98 a	14.83 a	17.60 a	233.42 b	90.11 b	
Ungrafted	272.28 a	88.57 a	24.75 a	24.53 a	13.94 a	17.59 a	375.38 a	112.83 a	
Control	216.82 a	87.11 b	24.29 a	22.64 a	14.52 a	17.03 a	312.42 a	92.61 a	
10 mg L ⁻¹	257.15 a	88.43 ab	23.30 a	23.44 b	14.68 a	17.75 a	311.86 a	107.39 a	
20 mg L ⁻¹	312.62 a	89.50 ab	25.34 a	24.26 ab	14.59 a	16.95 a	300.40 a	102.94 a	
30 mg L ⁻¹	332.79 a	90.57 a	25.95 a	26.67 a	13.75 a	18.66 a	292.93 a	102.94 a	
Grafted	Control	201.69 b	86.91 b	23.87 a	21.81 b	14.94 a	17.43 a	242.52 c	105.67 ab
	10 mg L ⁻¹	252.11 b	88.69 ab	23.45 a	23.70 ab	14.94 a	17.77 a	271.93 bc	118.44 a
	20 mg L ⁻¹	262.20 b	88.49 ab	25.75 a	23.89 ab	16.09 a	16.93 a	222.25 c	115.11 ab
	30 mg L ⁻¹	433.63 a	92.84 a	25.69 a	26.51 a	13.80 a	18.26 a	197.00 c	112.11 ab
Ungrafted	Control	262.20 b	87.32 b	24.71 a	23.47 a	14.10 a	16.62 a	382.32 a	79.56 b
	10 mg L ⁻¹	262.20 b	88.18 ab	23.14 a	23.17 a	14.88 a	17.72 a	351.78 ab	96.33 ab
	20 mg L ⁻¹	363.04 ab	90.50 ab	24.94 a	24.64 a	13.10 a	16.97 a	378.55 ab	90.78 ab
	30 mg L ⁻¹	231.94 b	88.30 ab	26.20 a	26.83 a	13.69 a	19.06 a	388.86 a	93.78 ab
Grafted	0.6423	0.4238	0.9363	0.4289	0.1752	0.9918	**	0.0003	
ZnO NPs	0.0662	0.0365	0.0569	0.0022	0.7119	0.2069	0.7722	0.2309	
Graft vs NPs	0.0019	0.0521	0.8262	0.7235	0.2742	0.8393	0.1164	0.9508	
CV %	25.48	2.58	7.88	7.95	12.75	9.59	12.41	12.00	

Note: stomatal density (SD), stomatal index (SI), stomatal length on top (SLT), length of stomata in bottom (LSB), stomatal width on top (SWT), width of stomata in bottom (WSB), area of xylem vessels (AXV), number of xylem vessels (NXV), means with different letters are significantly different, the test of comparison of means by Tukey. ns= not significant ($p > 0.05$), *= significant ($p \leq 0.05$), **= highly significant ($p \leq 0.0001$), CV= coefficient of variance

Results of graft vs ZnO NPs interaction in micromorphological and histological variables.

Table 1, the combination between grafting effects and application of nanoparticles, where it was found that the grafting combination and the concentration of 30 mg L⁻¹ had statistical difference in the variables stomatal density (SD) and stomatal index (SI). Regarding the LSB, concentrations of 30 mg L⁻¹ in combination with grafted and non-grafted plants presented the highest values. Regarding the WXV plants without graft with the interaction 30 mg L⁻¹ and control, showed significant difference to grafted plants and the concentrations of nanoparticles 10, 20, 30 mg L⁻¹ and control. The combination of graft effects and 10 mg L⁻¹ of nanoparticles had the highest NXV.

*Physiological results*Graft results on physiological variables

The effect of the graft on the variables of CO₂ assimilation (CA), stomatal conductance (SC), transpiration rate (TR), photosynthetic efficiency (PE) and efficiency in light capture (ELC) did not have statistical difference, Table 2.

Results of ZnO NPs in physiological variables

The nanoparticle effect had a negative statistical difference in the concentration of 30 mg L⁻¹ compared to the control, the results are described below Table 2. Regarding the variable CA, the concentration 30 mg L⁻¹ increased by 60.7% compared to the control, SC increased by 29.8% compared to the control. PE increased 29.2% compared to the control and ELC increased 60.7% compared to the control. In the variable of TR, there was no statistical difference between concentrations of 30 and 20 mg L⁻¹, but the 30 mg L⁻¹ concentration was 39.4% higher than the concentration of 10 mg L⁻¹ and 44.8% to the control Table 2.

Results of the graft vs ZnO NPs interaction in the physiological variables

Physiological variables were affected by the interaction, increasing the values of CA, SC, PE and ELC. In the variable TR, on the contrary, the controls showed the lowest values Table 2. The interaction between factors did not cause a difference between plants with graft and without graft, but did with the other concentrations and the control, which affected the physiological variables.

Table 2. Comparison of means of graft effects, ZnO NPs, and interaction in physiological variables

Factor	CA ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	SC ($\text{mol m}^{-2} \text{s}^{-1}$)	TR ($\text{mol m}^{-2} \text{s}^{-1}$)	PE ($\mu\text{mol}/\text{mmol}$)	ELC ($\mu\text{mol}/\text{mmol}$)	
Grafted	10.90 a	0.38 a	7.58 a	1.40 a	28.74 a	
Ungrafted	11.80 b	0.39 a	8.03 a	1.43 a	31.12 a	
Control	6.57 d	0.33 c	5.51 b	1.19 c	17.32 d	
10 mg L ⁻¹	8.20 c	0.38 bc	6.05 b	1.36 b	21.62 c	
20 mg L ⁻¹	13.91 b	0.39 b	9.68 a	1.44 b	36.68 b	
30 mg L ⁻¹	16.71 a	0.47 a	9.98 a	1.68 a	44.08 a	
Grafted	Control	6.59 d	0.33 c	5.45 b	1.21 c	17.37 d
	10 mg L ⁻¹	7.95 d	0.37 bc	5.95 b	1.34 bc	20.97 d
	20 mg L ⁻¹	12.87 c	0.41 abc	10.50 a	1.45 bc	33.92 c
	30 mg L ⁻¹	16.19 d	0.43 a	8.78 a	1.62 a	42.69 a
Ungrafted	Control	6.55 d	0.33 c	5.57 b	1.17 c	17.28 d
	10 mg L ⁻¹	8.45 d	0.38 bc	6.16 b	1.38 bc	22.28 d
	20 mg L ⁻¹	14.96 b	0.37 bc	10.50 a	1.44 bc	39.45 b
	30 mg L ⁻¹	17.24 a	0.50 a	9.89 a	1.74 a	45.46 a
Grafted	0.0057	0.4896	0.0909	0.4684	0.0572	
ZnO NPs	**	**	**	**	**	
Graft vs NPs	0.0985	0.1086	0.0802	0.5689	0.0983	
CV %	7.40	10.60	9.19	8.5	7.41	

Note: CO₂ assimilation (CA), stomatal conductance (SC), transpiration rate (TR), photosynthetic efficiency (PE) and efficiency in light capture (ELC), means with different letter are significantly different, the test of comparison of means by Tukey. ns= not significant ($p > 0.05$), *= significant ($p \leq 0.05$), **= highly significant ($p \leq 0.0001$), CV= coefficient of variance

*Production results*Results of the graft on the productive variables

The graft statistically increased the fruit weight (FW) with fruits with 21.8% increase, the number of fruits (NF) by 18.1%, and the polar diameter (PD) in fruits presented a 9.6% greater diameter compared to plants without graft Table 3.

Results of the ZnO NPs on the productive variables

The application of nanoparticles statistically influenced the variables FW, NF, PD and equatorial diameter (ED), with statistical difference between different concentrations. PF was affected with an increase in weight of 46.9% compared to the control. Regarding the NF the treatment 30 mg L⁻¹ increased 47.7% compared to the control. The PD did not have difference between the concentrations of 30, 20 and 10 mg L⁻¹, but it was compared to the control, where the difference in diameter was 18%. Regarding the ED, the concentrations of 30, 20, and 10 mg L⁻¹ did not show a difference between them, in the same way the concentration of 10 mg L⁻¹ and the control did not have statistical difference. On the other hand, the concentration of 20 mg L⁻¹ presented an increase of 16.1% and the concentration of 30 mg L⁻¹ a 20.2% greater in the equatorial diameter compared to the control Table 3.

Results of the interaction between graft vs ZnO NPs in the productive variables

The combination of effects influenced the production variables Table 3, where the highest results were obtained when using grafted plants and the application of nanoparticles at a concentration of 30 mg L⁻¹ compared to the lowest results obtained by the combination of plants without grafting and the control treatment.

Table 3. Comparison of means of the graft effects, ZnO NPs, and interaction in the productive variables

Factor		FW (g)	NF	PD (mm)	ED (mm)
Grafted		95.64 a	3.60 a	77.58 a	67.85 a
Ungrafted		74.83 b	2.95 b	70.14 b	64.85 a
Control		61.97 b	2.30 c	65.38 b	58.37 b
10 mg L ⁻¹		81.99 b	3.00 b	75.24 a	63.92 ab
20 mg L ⁻¹		80.26 b	3.40 b	75.06 a	69.56 a
30 mg L ⁻¹		116.72 a	4.40 a	79.75 a	73.13 a
Grafted	Control	69.26 bc	2.40 c	68.94 bc	63.94 bc
	10 mg L ⁻¹	83.22 b	3.20 b	76.28 b	59.32 cd
	20 mg L ⁻¹	83.87 b	3.60 b	77.82 b	70.64
	30 mg L ⁻¹	146.22 a	5.20 a	87.28 a	77.48 a
Ungrafted	Control	54.68 c	2.20 c	61.82 c	52.80 d
	10 mg L ⁻¹	80.76 b	2.80 bc	74.20 b	68.52 bc
	20 mg L ⁻¹	76.64 b	3.20 bc	72.30 bc	68.48 bc
	30 mg L ⁻¹	87.23 b	3.60 b	72.22 bc	66.78 bc
Grafted		**	0.0002	0.0003	0.0959
ZnO NPs		**	**	**	**
Graft vs NPs		**	0.0337	0.1005	0.0005
CV %		12.15	16.14	7.91	8.27

Note: fruits weight (FW), number of fruits (NF), polar diameter (PD), equatorial diameter (ED), means with different letters are significantly different, the test of comparison of means by Tukey. ns= not significant ($p > 0.05$), *= significant ($p \leq 0.05$), **= highly significant ($p \leq 0.0001$), CV= coefficient of variance

Discussion

The results obtained in this study Table 1 had a smaller area of xylem vessels and a lower number of vessels, increasing the efficiency in xylem translocation, and concur with the results obtained by Salehi *et al.* (2010) where all grafted plants tended to carry higher amounts of xylem sap than non-grafted plants. Alborno *et al.* (2020) reported that grafted plants had a higher number of xylem vessels, compared to the control (non-grafted plants). The results of this experiment concur with those found by Camposeco *et al.* (2018) where in bell pepper leaves, the length of the stomata were statistically significant, greater in the grafted bell pepper,

which exceeded the ungrafted bell pepper by 8.64 and 11.22%, in the same way it was also reported that the stomatal index and density were increased with the use of the rootstock, which increased the gas exchange and the photosynthetic efficiency of the plants. The results are similar to those reported by Alborno *et al.* (2020) where the number of vessels and their diameter, in the graft treatment, were similar in the autografted plants and the non-grafted treatment. Vascular connectivity through the xylem was closely correlated with the appearance of continuous elements of the xylem across the graft junction (Melnyk *et al.*, 2015). Orsini *et al.* (2013); Penella *et al.* (2017) reported that the use of tolerant rootstocks improves the photosynthesis performance of the scion under abiotic stress conditions. Xu *et al.* (2016); Baron *et al.* (2018) reported that the gas exchange of the leaves in grafted plants is directly affected by grafting, by modifying the vigor and productivity of the scion species. On the other hand, grafting does not influence the micromorphology of the plants compared to plants without grafting.

Physiology was not affected by the use of grafts Table 2, on the other hand, a trend was shown in the nanoparticle effect with the concentration of 30 mg L⁻¹, the results agree with those reported by García-López *et al.* (2019) in habanero pepper where foliar application with ZnO NPs at 1000 mg L⁻¹ had a greater impact on plant growth and physiology than conventional Zn salts (ZnSO₄), which was probably due to a greater capacity to be absorbed by the leaf. Rossi *et al.* (2019), Raliya *et al.* (2015) reported that ZnO NPs had a dual role of being an essential nutrient and a cofactor for nutrient mobilizing enzymes. Zhu *et al.* (2020) reported that the application of Zn increases endogenous hormones (auxins, gibberellins, and melatonin) and improves the activities of aquaporins and the antioxidant system, which in turn support photosynthetic efficiency. Regarding the interaction of factors, a trend was found in the concentrations of 30 mg L⁻¹ without being affected by the graft. The reason is probably due to the greater assimilation of Zn when applied in the form of ZnO NPs due to its greater ability to penetrate the leaf (Rossi *et al.*, 2019). Regarding the assimilation variables Mantoan *et al.* (2016) concluded in work with *Annona emarginata* which is used as a standard for Atemoya, it had a balance between perspiration and the rate of CO₂ assimilation to optimize the efficiency of water use, it presents adjustments in gas exchange and the photochemical process. Our results Table 2, concur with those obtained by Dabirian and Miles (2017) who found that there was a longer graft survival due to the reduction in stomatal conductance and the probable simultaneous reduction in perspiration. The results differ from those obtained by Ayala-Arreola (2010) the interaction of graft and nanoparticles affected the increase in the rate of perspiration, the rate of assimilation of CO₂, and stomatal conductance.

The use of grafts affected the productive variables Table 3, where the weight of fruits, number of fruits, and polar diameter increased, the results agree with those obtained by Velasco-Alvarado *et al.* (2019) where grafted plants obtained a yield of 7.4 kg per plant, the yield stood out with 19% compared to the plants without grafting 6.2 kg per plant. Fruit weight was influenced by rootstock, but it was not affected by grafting Ergun and Aktas (2018) reported that when using bell pepper grafts it produced about 12% more yield than the control plant without grafting. Soteriou and Kyriacou (2015) reported a study about watermelon that grafting increased the commercial yield by 43% on average over non-grafted controls. Riga (2015) reported grafting effects that influence most of the quality characteristics of tomato have been strongly influenced by the pattern-stem combination.

The use of ZnO NPs in the concentration of 30 mg L⁻¹ had a tendency to increase since when applying them the results of higher fruit weight, number of fruits, polar and equatorial diameter were obtained, the results agree with those reported by García-López *et al.* (2019) in habanero pepper where similarly, the maximum average weight of the fruit was obtained with ZnO NPs at 1000 mg L⁻¹, exceeding the control treatment by 7%. Du *et al.* (2019); Servin *et al.* (2015) reported that the application of ZnO NPs in all treatments increased wheat grain yield, Adhikari *et al.* (2016) reported that when applying a coating of 50 mg of ZnO NPs in corn seeds, it promoted the weight of 22.35 g dry sprout, compared to the control without NPs with 13.70 g. Elizabeth *et al.* (2017) reported in a study carried out on carrots with applications of zinc oxide fertilizers, the yield and growth of the plant were increased compared to the control treatment. Saadati *et al.* (2016) reported results of higher fruit weight with the application of Zn with an average of 3.16 kg. Zinc is a

promoter in the production of phytohormones which promote an increase in the development and production of fruits, it obtained a significant difference compared to the control treatment 3.08 kg (Elizabeth *et al.*, 2017). The application of Zn increases endogenous hormones (auxins, gibberellins, and melatonin) which will benefit growth, development and fruit production (Zhu *et al.*, 2020).

The interaction, grafting and 30 mg L⁻¹ of nanoparticles directly influenced productivity with a trend in all variables and obtaining the highest productive values, works carried out by García-López *et al.* (2019) reported the maximum average fruit weight with the application of ZnO NPs at 1000 mg L⁻¹, exceeding the control treatment by 7% and the ZnSO₄ treatment by 3.6% Penella *et al.* (2017) reported that in grafted tomato plants increased commercial yield compared to non-grafted plants (44 and 40% more, respectively). Ergun and Aktas (2018) reported that the length of the fruit was significantly influenced by the graft.

Conclusions

The graft did not affect the micromorphology, but it did affect the histology where the number of vessels and the area of xylem vessels increased. Physiology was not affected by the use of grafts; on the other hand, the productive variables increased, such as the weight of fruits, number of fruits and polar diameter. The application of ZnO NPs affected the micromorphology by increasing the stomatal density, the stomatal index, and the length of the bottom stomata. The nanoparticles did not affect the histology, while they did affect the physiology, where the assimilation of CO₂, stomatal conductance, transpiration rate, photosynthetic efficiency, efficiency in light collection, productivity showed a favorable effect, which translates into fruit weight, polar diameter and equatorial diameter. The interaction between factors affected the micromorphology by increasing the stomatal density, stomatal index, and length of stomata in the bottom, histology was affected by the interaction in the area of xylem vessels and the number of xylem vessels, physiology only had an effect in the largest applications of nanoparticles, increasing the assimilation of CO₂, stomatal conductance, transpiration rate, photosynthetic efficiency, and efficiency in light capture. Productivity increased with the interaction, having the best results in weight of fruits, number of fruits, polar and equatorial diameter. Due to the results obtained in this work, we recommend the use of grafts and the foliar application of ZnO NP, which could be used in the production of bell pepper to increase productivity.

Authors' Contributions

Contributions (JU); Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Writing - original draft; Writing - review and editing, (MC, AB, AS); Project administration; Supervision; Validation; Funding acquisition, (AZ, RC, HO); Resources; Visualization; Supervision: All authors read and approved the final manuscript.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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