



SYNERGISTIC POTENTIAL OF GRAFTING AND COPPER NANOPARTICLES IN TOMATO (*Solanum lycopersicum* L.) HYBRIDS WITH DEFICIT IRRIGATION †

[POTENCIAL SINÉRGICO DE INJERTOS Y NANOPARTÍCULAS DE COBRE EN HÍBRIDOS DE TOMATE (*Solanum lycopersicum* L.) CON RIEGO DEFICITARIO]

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SUMMARY

Background: Tomatoes, as a nutraceutical food abundant in vitamins and antioxidants, play a significant role in reducing the risk of various diseases. Integrating advanced agricultural practices, such as using nanoparticles instead of traditional fertilizers, alongside techniques like grafting and deficit irrigation, offers a promising approach to improving their overall quality. Nevertheless, addressing the challenge of water scarcity remains a critical concern in modern agriculture. **Objective:** To evaluate the growth, yield, and fruit quality of grafted tomato hybrids under deficit irrigation conditions, along with the application of copper nanoparticles. **Methodology:** This research analyzes the effect of grafting, grafting + 100 ppm CuNPs (copper nanoparticles), and 100 ppm CuNPs on the growth, yield, and quality of Saladette-type tomato hybrids (*Solanum lycopersicum* L.) Aquiles, Cuauhtémoc, Mesías, and Moctezuma under deficit irrigation (DI) conditions, DI75, and DI50. The variables included plant height (PH), stem diameter (SD), leaf area (LA), average fruit weight (AFW), total fruit weight (TFW), and water use efficiency (WUE). The general fruit quality parameters were polar diameter (PD), equatorial diameter (ED), fruit firmness (FF), total soluble solids (TSS) content, β -carotene, and lycopene content. **Results:** The Aquiles hybrid achieved a remarkable AFW. The Cuauhtémoc hybrid showed higher β -carotene and lycopene contents. Grafted plants promoted higher AFW, TFW, WUE, and FF. The use of CuNPs induced a higher TSS content, β -carotene, and lycopene. The DI50 affected PH, but WUE was higher without TFW changes, increasing TSS and lycopene content. **Implications:** The loss of fruit quality in grafted tomato plants is compensated using CuNPs and deficit irrigation. **Conclusion:** Grafting is a highly effective method for increasing tomato yield, while the application of CuNPs significantly enhances the fruit's internal quality. Furthermore, employing deficit irrigation at 50% (DI50) maximizes water use efficiency, improving specific quality attributes without negatively impacting overall yield.

Key words: agronomic management; carotenoids; water scarcity; nanotechnology; tomato quality.

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RESUMEN

Antecedentes: Los tomates, al ser un alimento nutraceutico rico en vitaminas y antioxidantes, reducen el riesgo de enfermedades. La incorporación de nanopartículas en lugar de fertilizantes en prácticas agrícolas como el injerto y el riego deficitario puede mejorar integralmente su calidad. Sin embargo, es importante considerar el desafío de la escasez de agua en el panorama agrícola actual. **Objetivo:** Evaluar el crecimiento, rendimiento y calidad del fruto de híbridos de tomate injertados en condiciones de riego deficitario, junto con la aplicación de nanopartículas de cobre. **Metodología:** Esta investigación analiza el efecto del injerto, injerto + 100 ppm de CuNPs (nanopartículas de cobre) y 100 ppm de CuNPs en el crecimiento, rendimiento y calidad de híbridos de tomate tipo Saladette (*Solanum lycopersicum* L.) Aquiles, Cuauhtémoc, Mesías, Moctezuma en condiciones de riego deficitario (RD), RD75 y RD50. Las variables incluyeron altura de planta (AP), diámetro de tallo (DT), área foliar (AF), peso promedio de fruto (PPF), peso total de fruto (PTF) y eficiencia en el uso de agua (EUA). Los parámetros generales de calidad del fruto fueron diámetro polar (DP), diámetro ecuatorial (DE), firmeza de fruto (FF), contenido de sólidos solubles totales (SST), β -caroteno y licopeno. **Resultados:** El híbrido Aquiles obtuvo un PPF notable. El híbrido Cuauhtémoc presentó mayores contenidos de β -caroteno y licopeno. Las plantas injertadas promovieron mayor PPF, PTF, EUA y FF. El uso de CuNPs indujo un mayor contenido de SST, β -caroteno y licopeno. El DI50 afectó la AP, pero la EUA fue mayor sin cambios en el PTF, también aumentando el contenido de SST y licopeno. **Implicaciones:** La aplicación de CuNPs y el riego deficitario compensan la pérdida de calidad de fruto en plantas de tomate injertadas. **Conclusión:** Los injertos pueden ser utilizados para aumentar el rendimiento, y el uso de CuNPs promueve el aumento de la calidad interna del fruto. El uso de DI50 potencialmente mejora algunos aspectos de calidad con un uso eficiente del agua sin afectar el rendimiento.

Palabras clave: calidad de tomate; carotenoides; escasez hídrica; manejo agronómico; nanotecnología.

INTRODUCTION

The tomato is a key vegetable crop in terms of cultivated area, covering 4.8 million hectares with an annual production of 182 million tons (Anwar, Fatima, and Mattoo, 2019). Classified as a nutraceutical vegetable due to its high content of vitamins and antioxidants (Waliszewski and Blasco, 2010), the tomato has earned the designation of a "functional food." This status stems from its association with a reduced risk of developing certain types of cancer, inflammatory processes, and cardiovascular diseases (Campbell *et al.*, 2004; Giovannetti *et al.*, 2012). However, the quality of tomatoes is influenced by crop management practices, including grafting, the application of fertilizer nanoparticles, and irrigation strategies (Campbell *et al.*, 2004; Guo *et al.*, 2021; Wu *et al.*, 2021).

Grafting is a plant growth modification technique that increases yield (Melnik *et al.*, 2015; Ramírez-Jiménez, Ribeiro Marchiori and Córdoba-Gaona, 2021). In horticulture, cucurbits and solanaceous species are commonly grafted (Gaion, Braz, and Carvalho, 2018), grafting allows tolerance/resistance to abiotic factors such as salinity, temperature, and water stress (Singh *et al.*, 2020), as well as biotic factors like pathogens and viruses, depending on the graft-scion combination, other characteristics of the grafted plant can influence excessive vegetative growth, delayed fruit harvest, and physiological disorders.

Grafting techniques are often combined with strategies like deficit irrigation to optimize resource use and enhance crop performance. However, for successful outcomes under water stress conditions, the rootstock must exhibit high vigor to maintain its effectiveness and support plant growth (Al-Harbi, Hejazi, and Al-Omran, 2017), additionally, certain rootstocks are more suitable for tolerance to water deficit conditions (Al-Harbi *et al.*, 2018; Al-Harbi, Al-Omran and Alharbi, 2018).

Deficit irrigation is an alternative that arises due to water scarcity and aims to maximize water productivity, resulting in water savings (Tahi *et al.*, 2007; Lu *et al.*, 2019), in geographic areas with limited water resources, it becomes an applicable alternative due to the reduction in the actual volume of irrigation required (Patanè, Tringali and Sortino, 2011). This technique induces changes in stomatal activity, leading to increased efficiency in water use for irrigation. Although yield losses may occur due to water deficit, they are often compensated by an increase in the content of total soluble solids, vitamin C, and antioxidant compounds, which are highly valued for commercial purposes (Castel Sánchez and González Altozano, 2003; Agbna *et al.*, 2017; Khapte *et al.*, 2019).

In addition, nanoparticles (NPs), particularly metallic NPs, have emerged as a technological alternative in agriculture, offering potential

benefits surpassing those of organic and synthetic fertilization management (Lira-Saldivar *et al.*, 2018; Raliya *et al.*, 2018). Previous studies have documented the viability and effects of applying NPs at different doses, including their impact on quality, yield, increased aerial biomass, and changes in photosynthetic capacity in tomato crops (León-silva *et al.*, 2018; López-Vargas *et al.*, 2018; Morales-Espinoza *et al.*, 2019). Specifically, the foliar application of copper NPs is aimed at enhancing antioxidant activity in fruits (Rajput *et al.*, 2018; Hernández-Hernández *et al.*, 2019), and improving overall fruit quality (Hernández-Hernández *et al.*, 2018; López-Vargas *et al.*, 2018), either alone or in combination with other elements. It has also been shown to reduce pathogen severity and promote tolerance in crops (Cumplido-Nájera *et al.*, 2019; Lopez-Lima *et al.*, 2021). Therefore, the objective of this research was to evaluate the growth, yield, and fruit quality of grafted tomato hybrids under deficit irrigation conditions, along with the application of copper nanoparticles.

MATERIALS AND METHODS

Location, plant material, and seedling

The experiment was conducted in a 60 m² greenhouse located at the Facultad de Ingeniería y Ciencias, Universidad Autónoma de Tamaulipas, in Ciudad Victoria, Tamaulipas, at coordinates 23°42'57.35" N and 99°9'7.96" W. The evaluation period spanned from November 2019 to April 2020. The greenhouse, of the sawtooth type, was oriented north to south and equipped with manually operated side blinds. During the experiment, the environmental conditions inside the greenhouse averaged a temperature of 37 °C, with extremes ranging from a maximum of 47 °C to a minimum of 21.8 °C. The average relative humidity was 77.1%, reaching a maximum of 94.2% and a minimum of 55.6%.

The plant material used in the experiment consisted of tomato hybrids: Aquiles, Cuauhtémoc, Mesías, and Moctezuma (Harris Moran, Davis, CA, USA). These hybrids had indeterminate growth and produced Roma-type fruits. The seedlings of the hybrids were grafted onto the Colosus RZ F1 hybrid (Rijk Zwaan, The Netherlands). To obtain the seedlings, germination trays were used, which were filled with pre-hydrated Peatmoss as the substrate. The rootstock seeds were sown five days after the hybrid seeds. The resulting seedlings were transplanted into black polyethylene bags with a

capacity of 14 L, filled with sandy-loam soil texture. The soil had a pH of 7.8 and an electrical conductivity of 1.8 mS cm⁻¹.

Management and application of copper nanoparticles

The CuNPs used in the experiment had an average diameter of 25 nm, a chemical purity of 99.8%, and a spherical shape (SkySpring Nanomaterials). An atomizer was used to apply two doses of a solution of CuNPs with a concentration of 100 ppm, and they were distributed in two applications. The first foliar application (10 mL) was performed 30 days after transplantation (DAT), and the second foliar application (10 mL) was conducted 46 DAT, at which point most of the plants had started fruit development. The CuNPs were applied as a foliar from a zenith angle, and each experimental unit was temporarily isolated with polyethylene.

Establishment of irrigation levels

Daily irrigation was performed until the conclusion of the experiment using a drip irrigation system. The water volume applied was determined based on the specific treatment requirements, accounting for row spacing (0.80 m), plant spacing (0.50 m), cultivation coefficients (0.45, 0.75, 1.15), and evapotranspiration (ETP). The ETP was calculated daily using the EVAPO application, developed by Maldonado, Valeriano, and de Souza Rolum (2019). This application is based on the reference ETP equation, Penman-Monteith FAO 56 (Allen *et al.*, 2006), and utilizes real-time data obtained from the database of "The POWER Project" at NASA Langley Research Center (LaRC). The applied irrigation levels were 50% and 75% of the theoretical estimates of the crop's water requirements. In this context, the 50% (DI50) represents the minimum threshold within the moderate range of water deficit, while the 75% (DI75) is set 5% above the lower limit of the percentage considered appropriate for optimal crop development (Chai *et al.*, 2016; Takács *et al.*, 2020). The Steiner nutrient solution (Steiner, 1961) was used to supply nutrients to the plants.

Plant height, stem diameter, and foliar area in the productive stage

The measurement of these variables was conducted 132 days after the initiation of all treatments, with measurements taken across all experimental units. Plant height was determined

using a measuring tape graduated in centimeters, measuring from the plant base to the apical meristem. Stem diameters were measured 10 cm above the plant base to avoid variations caused by root emergence on the stem and the increased thickness at the graft union zone. Digital vernier calipers graduated in millimeters, were used for precise stem diameter measurements.

The leaf area was assessed by photographing mature leaves located below the second cluster using a Nikon D5100 digital camera. The captured images were analyzed with the ImageJ software, employing a 30 cm graduated ruler as a reference tool. The leaf area was expressed in square centimeters (cm²).

Average fruit weight, yield, and water use efficiency

The average fruit weight was recorded in grams (g). Harvested fruits were placed in labeled paper bags corresponding to the respective treatments and weighed using a digital scale with a precision of 0.01 g. The yield was calculated by summing the total weight of fruits produced per plant, encompassing fruits from the first to the sixth bunch. The yield was expressed in kilograms per plant (kg/plant).

Water use efficiency was determined using the water productivity index, which represents the quantity of fruit produced per cubic meter of irrigation water applied. This index was expressed in kilograms of fruit per cubic meter (kg/m³) of irrigation water.

Polar, equatorial diameter, and fruit firmness

The size or caliber of the fruit was determined by measuring the polar and equatorial diameters. Measurements were taken using a vernier caliper graduated in millimeters (mm), and the measurements were represented in mm to determine the fruit firmness, a manual penetrometer was used. An 8 mm diameter strut was applied to the fruit, and the firmness was measured in units of kilogram per square centimeter (kg cm⁻²). The sampled fruits were representative of each bunch, healthy, and ripe, following standard parameters (USDA, 2005).

Total solid soluble content, lycopene, and beta-carotene

The concentration of soluble solids was determined using the same fruit samples analyzed

for firmness. Measurements were taken with a SperScientific model 300054 digital refractometer, and the results were expressed in degrees Brix (°Brix). For pigment analysis, red fruits from clusters 2, 3, and 4 were collected from all experimental units across treatments. Each fruit was homogenized in 200 mL beakers, which were kept on ice in a cooler to prevent pigment degradation. Pigment extraction and quantification were then performed in triplicate, following the methodology outlined by Nagata and Yamashita (1992). Absorbance readings were taken using a spectrophotometer BioMate 3. The units for beta-carotene and lycopene were represented in micrograms per 100 grams of fresh fruit weight (µg 100g⁻¹ FFW).

Statistical analysis

The experiment was designed as a completely randomized trial with four replications, following a factorial arrangement of 4 × 4 × 2. The first factor was the tomato hybrids: Aquiles, Cuauhtémoc, Mesías, and Moctezuma. The second factor consisted of four agricultural management techniques: foliar application of CuNPs per plant, grafted plants, grafted plants with foliar application of CuNPs per plant, and a control. The third factor included two levels of deficit irrigation (DI): DI75 and DI50. Statistical analyses were performed using SAS 9.0 software. Analysis of variance (ANOVA) was applied to evaluate the overall significance of the treatments. Tukey's test (p = 0.05) was used for multiple comparisons of means for the hybrids and agronomic treatments, while the Least Significant Difference (LSD) test (p = 0.05) was applied to the deficit irrigation treatments due to the absence of additional comparisons. Additionally, principal component analysis (PCA) was employed to visualize the relationships between the hybrids, agronomic management techniques, and levels of deficit irrigation. PCA was instrumental in correlating variables and evaluating the contribution of the combined factors to the observed outcomes.

RESULTS AND DISCUSSION

Crop growth and yield

The plant height variable showed no interaction between factors (Supplementary table 1); however, the comparison of means separately between factors (Figure 2) illustrates the contribution of the factors to the variable. Thus, the hybrid AQ exhibited a significant difference

with greater plant height compared to the other evaluated hybrids. On the other hand, the control plants and those with the application of CuNPs as agronomic management showed similar heights, which distinguished them from the grafted plants and the combination of grafted plants + CuNPs (Table 1). Copper nanoparticles, as well as other metals, can promote plant height (Priyanka *et al.*, 2019), however, in this experimental case, the difference was determined using grafted plants rather than the foliar application of 100 ppm of CuNPs, a dosage that did not interfere with stem elongation, a fact confirmed by the combination of agronomic management practices. On the other hand, DI75 showed greater height compared to DI50. In addition to the advantages associated with growth and photosynthesis in tomato crops, copper in other forms based on nanometric size reduces the progression of fungal diseases such as *Fusarium oxysporum*, which gives it an additional advantage as an effective fungicide (Deng *et al.*, 2023).

The total fruit weight, analyzed as a yield variable, did not exhibit any type of interaction among the studied factors (Supplementary Table 1). However, a significant difference was observed for the agronomic management factor, with the highest total weight accumulation (Figure 2). Specifically, grafted plants demonstrated the best performance in this variable, indicating that the applied dose of CuNPs did not contribute significantly to total fruit weight. In contrast, other studies have shown that the application of different copper doses can positively influence fruit yield and plant growth. However, these studies utilized copper in forms combined with chitosan and hydrogels applied to the soil, rather than isolated nanoparticles (Hernández *et al.*, 2016). The remaining factors in this study

displayed similar behavior, showing no significant differences in their effects on total fruit weight.

Water use efficiency showed a significant difference in agronomic management and deficit irrigation level aspects (Figure 3). In the first factor, grafted plants exhibited a higher index of the WUE variable. However, the control treatment, as well as plants with CuNPs application, showed a similar performance, except for the grafts. In the case of DI50, it had a superior response compared to DI75.

The irrigation volume for the DI75 and DI50 treatments remained below the daily evapotranspiration rate (Figure 4). However, on January 27, 46 days after transplantation and the onset of fruit filling, the irrigation volume for the DI75 treatment slightly exceeded the evapotranspiration values, while the DI50 treatment consistently remained below them. Despite this, the soil in the DI75 treatment retained a stable moisture level throughout the evaluation period. In contrast, the soil in the DI50 treatment exhibited signs of dehydration on days with elevated temperatures, suggesting a potential stress effect due to insufficient water availability.

Leaf area was significantly influenced by the interaction between hybrids and agronomic management, as well as the interaction between agronomic management and deficit irrigation levels (Supplementary Table 1). Furthermore, stem diameter and average fruit weight demonstrated a three-way interaction involving hybrids, agronomic management techniques, and deficit irrigation levels (Supplementary Table 1), highlighting the complex interplay of these factors in determining plant performance and yield characteristics.

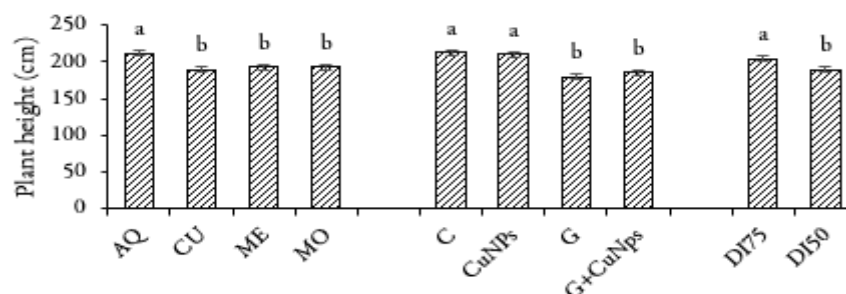


Figure 2. Contribution of factors to the plant height variable, Tukey ($p=0.05$) for the first eight treatments. LSD test for the last two treatments. Hybrids; AQ: Aquiles, CU: Cuauhtémoc, ME: Mesías, MO: Moctezuma. Agronomic treatments; T: control, CuNPs: copper nanoparticles (100 ppm), G: grafted, G+CuNPs: grafted + copper nanoparticles (100 ppm). DI50: 50% deficit irrigation, DI75: 75% deficit irrigation.

Table 1. Mean of morphological and yield variables of grafted tomato plants (G), application of copper nanoparticles (CuNPs), grafting combined with nanoparticles (G+CuNPs) under deficit irrigation (DI) conditions.

DI	T	H	PH (cm)		FA (cm ²)		SD (mm)		AWF (g)		TWF (kg plant ⁻¹)		WUE (kg m ³)	
DI75	C	AQ	240.25	±5.07	162.65	±6.01	10.05	±0.77	87.41	±3.19	2.62	±0.18	19.53	±1.36
		CU	208.25	±10.18	216.79	±9.24	7.18	±0.21	71.55	±4.26	2.08	±0.21	15.50	±1.55
		ME	208.75	±18.78	291.31	±21.59	8.18	±0.32	85.40	±9.10	2.64	±0.23	19.71	±1.75
		MO	220.75	±22.83	339.81	±2.64	8.25	±0.56	70.60	±1.73	2.25	±0.16	16.80	±1.17
	CuNPs	AQ	245.75	±5.81	332.19	±44.23	7.83	±0.21	88.88	±6.03	2.70	±0.49	20.13	±3.65
		CU	200.00	±13.64	241.03	±20.77	7.80	±0.33	74.79	±2.41	2.28	±0.16	16.99	±1.22
		ME	208.25	±4.84	345.87	±30.04	7.20	±0.33	73.02	±3.36	2.28	±0.31	16.98	±2.28
		MO	222.75	±9.41	346.49	±34.83	8.13	±0.54	57.85	±12.30	2.00	±0.39	14.94	±2.91
	G	AQ	200.00	±5.48	609.43	±32.36	9.50	±0.77	77.83	±2.99	2.85	±0.19	21.24	±1.43
		CU	170.25	±11.45	693.70	±35.22	9.48	±0.77	69.75	±4.29	2.73	±0.22	20.34	±1.62
		ME	196.00	±11.15	631.27	±25.44	9.93	±0.48	78.72	±2.70	3.02	±0.17	22.50	±1.27
		MO	188.50	±4.86	757.22	±23.85	9.58	±0.26	88.27	±6.00	2.61	±0.18	19.49	±1.31
	G+CuNPs	AQ	193.50	±8.91	452.53	±24.72	9.90	±0.60	90.84	±6.01	2.50	±0.21	18.64	±1.53
		CU	196.75	±7.76	635.88	±22.87	9.58	±0.23	68.71	±5.82	2.39	±0.09	17.80	±0.70
		ME	196.00	±11.15	571.81	±32.40	9.23	±0.24	76.18	±2.74	2.69	±0.25	20.07	±1.87
		MO	188.50	±4.86	618.08	±25.98	10.03	±0.25	78.79	±4.58	2.55	±0.20	19.00	±1.52
DI50	C	AQ	219.50	±8.07	131.87	±4.34	10.00	±0.22	86.32	±5.34	2.30	±0.10	25.64	±1.08
		CU	210.75	±13.38	145.20	±8.66	8.90	±0.64	61.30	±13.21	2.03	±0.38	22.59	±4.20
		ME	208.75	±4.89	170.89	±10.70	9.05	±0.49	86.75	±3.44	2.66	±0.11	29.69	±1.21
		MO	195.50	±14.52	153.54	±5.38	7.78	±0.38	76.45	±3.28	2.26	±0.20	25.16	±2.28
	CuNPs	AQ	211.75	±1.89	179.72	±4.05	9.85	±0.21	87.90	±5.83	2.44	±0.06	27.20	±0.62
		CU	195.50	±5.33	192.51	±16.08	8.50	±0.36	75.91	±4.65	2.26	±0.19	25.21	±2.11
		ME	193.50	±1.19	209.82	±21.06	8.53	±0.35	80.53	±3.90	2.66	±0.10	29.67	±1.11
		MO	203.00	±0.58	251.94	±20.77	8.65	±0.35	83.20	±2.55	2.48	±0.06	27.65	±0.64
	G	AQ	186.75	±12.98	457.50	±18.60	8.48	±0.39	87.54	±3.22	2.58	±0.15	28.73	±1.69
		CU	155.00	±6.63	521.01	±26.25	10.60	±0.58	76.60	±3.30	2.55	±0.04	28.46	±0.46
		ME	171.75	±8.41	410.57	±32.26	9.73	±0.36	75.49	±7.12	2.62	±0.01	29.27	±0.09
		MO	170.00	±10.40	550.72	±51.63	9.88	±0.19	78.41	±4.35	2.89	±0.12	32.25	±1.38
	G+CuNPs	AQ	199.25	±5.25	333.75	±27.93	9.05	±0.34	93.77	±3.76	2.48	±0.18	27.71	±2.01
		CU	176.00	±4.64	499.53	±33.29	8.23	±0.13	86.65	±6.09	2.80	±0.12	31.25	±1.34
		ME	181.50	±6.89	482.31	±33.67	8.95	±0.17	67.96	±6.56	2.36	±0.05	26.38	±0.59
		MO	158.00	±5.76	494.28	±30.01	10.48	±0.40	72.21	±2.48	2.32	±0.06	25.87	±0.63

PH: plant height, FA: foliar area, SD: stem diameter, AWF: average fruit weight, TWF: total weight fruit, WUE: water use efficiency. ±: standard error of the mean.

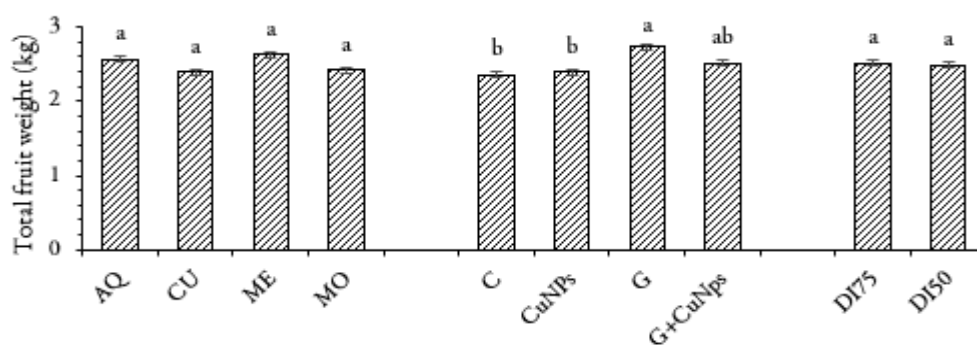


Figure 2. Contribution of factors to the total fruit weight variable. Tukey ($p=0.05$) for the first eight treatments. LSD test for the last two treatments. Hybrids; AQ: Aquiles, CU: Cuauhtémoc, ME: Mesías, MO: Moctezuma. Agronomic treatments; T: control, CuNPs: copper nanoparticles (100 ppm), G: grafted, G+CuNPs: grafted + copper nanoparticles (100 ppm). DI50: 50% deficit irrigation, DI75: 75% deficit irrigation.

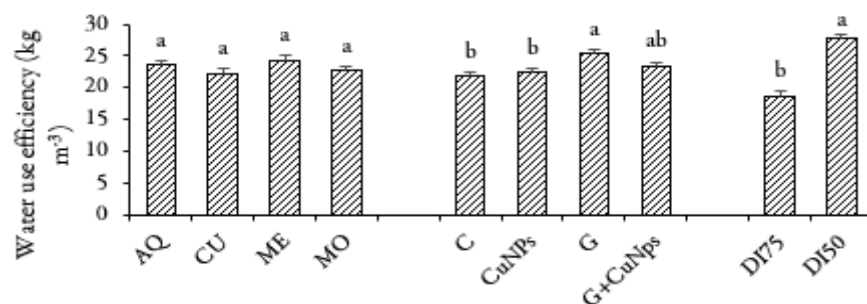


Figure 3. Isolated comparison of the contribution of factors for the water use efficiency variable. Tukey ($p=0.05$) for the first eight treatments. LSD test for the last two treatments. Hybrids; AQ: Aquiles, CU: Cuauhtémoc, ME: Mesías, MO: Moctezuma. Agronomic treatments; T: control, CuNPs: copper nanoparticles (100 ppm), G: grafted, G+CuNPs: grafted + copper nanoparticles (100 ppm). DI50: 50% deficit irrigation, DI75: 75% deficit irrigation.

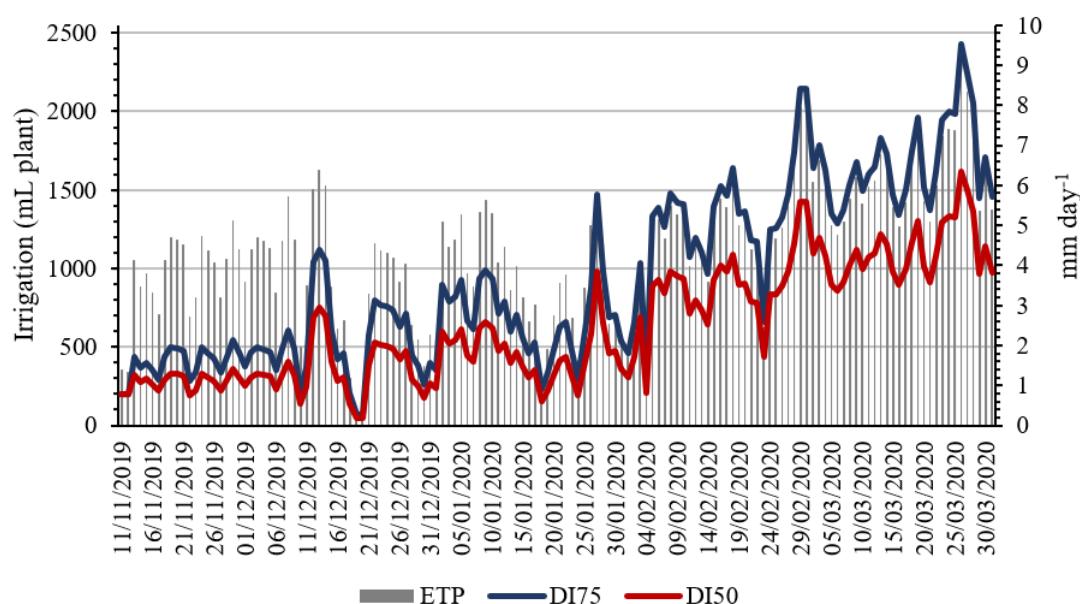


Figure 4. Dynamics of crop evapotranspiration and applied irrigation. The left y-axis represents the irrigation of the applied levels. The right y-axis represents crop evapotranspiration.

External and internal fruit quality

The average fruit calibre determined by polar diameter (Figure 5) and equatorial diameter (Figure 6), was not influenced by interactions between factors (Supplementary Table 2). However, agronomic management led to changes in the polar diameter of the fruit (Table 2), while the equatorial diameter was modified by the hybrid factor and agronomic management (Supplementary Table 2). Fruit firmness was influenced by the interaction between the factors: hybrid and deficit irrigation. Total soluble solids were influenced by treatments and deficit irrigation. Regarding the content of lycopene and

beta-carotene in the fruits, a three-way interaction of the factors: hybrid, treatment, and deficit irrigation was observed (Supplementary Table 2). The application of nanoparticles has been associated with quality variables, which has allowed the increase of firmness, vitamin C content, and antioxidant capacity, as well as bioactive compounds such as flavonoids and lycopene (Hernández *et al.*, 2016, López-Vargas *et al.*, 2018). However, the dynamics of lycopene and beta-carotene content are influenced by the deficit of applied irrigation, as it contributes significantly to the nutritional quality of the crop from the increase of total soluble solids as well as carotenoids (He *et al.*, 2024).

Table 2. Mean of internal and external quality and major carotenoids of grafted tomato plants (G), application of copper nanoparticles (CuNPs), grafting combined with nanoparticles (G+CuNPs) under deficit irrigation levels (DI75, DI50).

DI	T	H	ED (mm)		PD (mm)		FF		TSS (°Brix)		LY (ug 100 ffw ⁻¹)		BE (ug 100 ffw ⁻¹)	
DI75	C	AQ	55.51	±1.52	66.06	±3.44	2.31	±0.17	3.71	±0.10	97.34	±2.75	72.35	±3.87
		CU	54.14	±1.02	63.35	±1.06	2.28	±0.06	3.44	±0.14	84.60	±2.50	76.62	±2.87
		ME	56.40	±1.64	64.95	±1.95	2.43	±0.09	4.18	±0.08	101.87	±6.39	72.63	±3.42
		MO	54.26	±1.87	65.37	±1.19	2.23	±0.06	3.55	±0.25	104.05	±7.10	71.94	±4.70
	CuNPs	AQ	59.58	±1.54	68.43	±0.69	2.32	±0.11	3.74	±0.16	81.70	±2.40	78.01	±0.28
		CU	54.29	±1.25	64.71	±1.95	2.04	±0.11	3.71	±0.15	117.63	±6.17	82.08	±2.71
		ME	53.86	±2.01	62.85	±1.84	2.27	±0.08	3.76	±0.23	88.59	±6.00	71.39	±4.69
		MO	51.46	±3.11	57.05	±5.87	2.40	±0.17	3.91	±0.22	97.98	±4.62	83.01	±3.26
	G	AQ	60.91	±1.48	68.63	±1.82	2.89	±0.19	3.26	±0.18	85.70	±2.01	60.31	±2.51
		CU	56.57	±0.74	65.92	±0.66	2.61	±0.12	3.21	±0.07	92.20	±1.99	57.31	±1.40
		ME	59.66	±1.31	66.70	±1.81	2.60	±0.11	3.28	±0.21	84.32	±1.16	61.37	±2.93
		MO	60.39	±1.70	67.52	±1.25	2.75	±0.09	3.15	±0.07	68.85	±2.55	63.52	±1.51
	G+CuNPs	AQ	61.69	±0.77	70.17	±1.33	2.33	±0.21	3.44	±0.08	92.15	±1.29	70.78	±2.12
		CU	58.59	±0.74	65.58	±0.56	2.51	±0.12	3.05	±0.10	89.26	±1.87	67.21	±0.98
		ME	59.30	±0.27	66.06	±0.71	2.68	±0.12	3.60	±0.25	66.36	±1.85	57.37	±1.31
		MO	56.97	±1.67	61.91	±3.80	2.36	±0.09	3.23	±0.21	89.06	±5.34	61.97	±0.95
DI50	C	AQ	55.51	±1.52	63.17	±2.22	2.22	±0.12	4.21	±0.09	87.17	±8.47	73.86	±5.88
		CU	54.14	±1.02	56.29	±6.17	2.21	±0.17	4.71	±0.13	117.88	±6.30	73.66	±1.30
		ME	56.40	±1.64	62.01	±1.18	2.35	±0.04	5.03	±0.27	82.87	±1.77	62.01	±0.53
		MO	54.26	±1.87	61.67	±1.58	2.23	±0.08	4.74	±0.25	117.82	±9.19	67.27	±1.92
	CuNPs	AQ	59.58	±1.54	58.04	±4.75	2.19	±0.05	4.18	±0.14	114.65	±4.83	73.37	±1.34
		CU	54.29	±1.25	62.30	±1.76	2.23	±0.04	4.55	±0.17	148.03	±5.06	82.27	±0.98
		ME	53.86	±2.01	64.03	±0.86	2.43	±0.11	4.75	±0.14	93.59	±2.53	64.91	±2.52
		MO	51.46	±3.11	67.21	±0.88	2.04	±0.08	4.24	±0.09	121.12	±7.28	74.38	±3.17
	G	AQ	60.91	±1.48	64.70	±0.50	2.64	±0.14	4.07	±0.04	85.36	±5.78	78.37	±3.72
		CU	56.57	±0.74	64.51	±1.19	2.54	±0.14	4.30	±0.20	137.60	±8.52	81.49	±1.87
		ME	59.66	±1.31	64.38	±2.72	3.19	±0.18	4.51	±0.28	76.53	±5.65	50.55	±2.06
		MO	60.39	±1.70	62.83	±2.03	2.65	±0.11	4.44	±0.27	122.77	±2.00	77.06	±3.72
	G+CuNPs	AQ	61.69	±0.77	66.10	±0.79	2.49	±0.12	4.26	±0.19	105.74	±5.48	70.29	±2.16
		CU	58.59	±0.74	67.89	±1.30	2.60	±0.19	3.94	±0.18	87.13	±6.99	57.72	±1.49
		ME	59.30	±0.27	67.24	±5.00	3.16	±0.11	4.01	±0.19	61.05	±1.58	47.16	±0.43
		MO	56.97	±1.67	64.73	±1.22	2.48	±0.17	3.71	±0.30	70.35	±3.56	55.40	±1.74

ED: ecuatorial diameter, PD: polar diameter, FF: fruit firmness, TSS: total soluble solids, LY: lycopene concentration, BE: beta carotene concentration, ±: standard error of the mean.

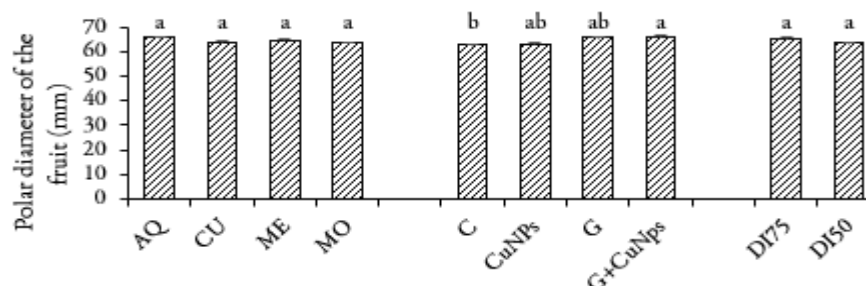


Figure 5. Isolated comparison of the contribution of factors for the polar fruit diameter variable. Tukey ($p=0.05$) for the first eight treatments. LSD test for the last two treatments. Hybrids; AQ: Aquiles, CU: Cuauhtémoc, ME: Mesías, MO: Moctezuma. Agronomic treatments; T: control, CuNPs: copper nanoparticles (100 ppm), G: grafted, G+CuNPs: grafted + copper nanoparticles (100 ppm). DI50: 50% deficit irrigation, DI75: 75% deficit irrigation.

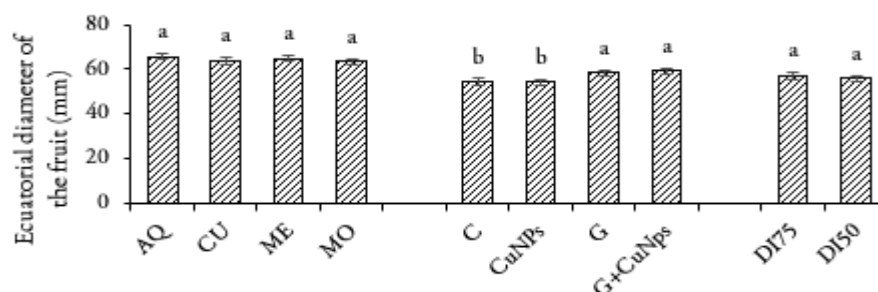


Figure 6. Isolated comparison of the contribution of factors for the polar fruit diameter variable. Tukey ($p=0.05$) for the first eight treatments. LSD test for the last two treatments. Hybrids; AQ: Aquiles, CU: Cuauhtémoc, ME: Mesías, MO: Moctezuma. Agronomic treatments; T: control, CuNPs: copper nanoparticles (100 ppm), G: grafted, G+CuNPs: grafted + copper nanoparticles (100 ppm). DI50: 50% deficit irrigation, DI75: 75% deficit irrigation.

The behavior of the variables and treatments under both irrigation conditions

The Biplot (Figure 7) clusters the 32 treatments resulting from the experimental design. Component 1 explains 39.52 % of the data

variability, and Component 2 explains 19.01% of the variability. The variables are visualized, along with the hybrids, with their respective agronomic management; application of CuNPs, grafting, and grafted with the addition of CuNPs under both established irrigation conditions.

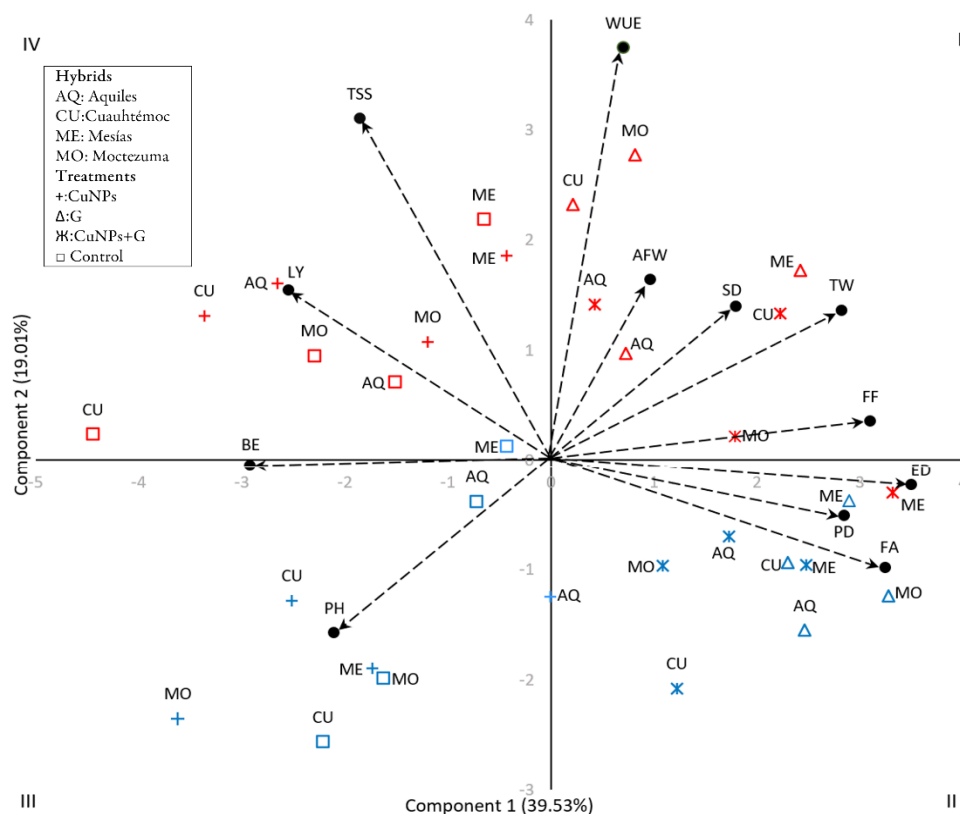


Figure 7. Dispersion of CP1 and CP2 for the analyzed variables: plant height (PH), leaf area (FA), stem diameter (SD), average fruit weight (AFW), total fruit weight (TW), and water use efficiency (WUE); polar diameter (PD), equatorial diameter (ED), fruit firmness (FF), total soluble solids (TSS), lycopene (LY), and beta-carotene (BE), depending on the hybrids, treatments (CuNPs: 100 ppm Copper nanoparticles, G: Grafted plants, CuNPs+G: 100 ppm of Copper nanoparticles and grafted plants, control: without treatment), and deficit irrigation (DI75: markers in blue, DI50: markers in red).

Plant height, stem diameter, and leaf area

Plant height was greater in treatments without restrictive irrigation, with projections aligning more closely with the quadrant IV axis. Within this quadrant, hybrids treated with CuNPs, such as MO, ME, and CU, achieved heights of 220.75 cm, 208.25 cm, and 200.00 cm, respectively (Table 1). This suggests that the foliar application of CuNPs may promote an increase in plant height, likely due to their potential role in enhancing nutrient availability and uptake (Hafeez *et al.*, 2015; Priyanka *et al.*, 2019), however, the response fluctuates depending on the concentration and the crop (Faraz *et al.*, 2022). Furthermore, its influence may be accompanied by the intrinsic characteristics of each hybrid, or in this case, the genetic load of the evaluated hybrids (Cortez and Montejo, 2020). On the other hand, the CU hybrid under control conditions had a height of 208.25 cm, unlike the case with nanoparticle application. This suggests that the material exposed to the dose of CuNPs may have generated a type of toxicity due to copper accumulation in the roots, which in turn restricted stem elongation (Broadley *et al.*, 2012).

Additionally, the CU hybrid with combined agronomic management had a plant height of 176.00 cm, followed by the ME hybrid grafted with 171.75 cm, both visualized in quadrant II. These values were conditioned by the rootstock, due to the increased energy consumption in nutrient translocation and absorption, thus limiting stem elongation. However, this is compensated for by other attributes such as yield (Palada and Wu, 2007; Bogoescu *et al.*, 2011; Soare, Dinu, and Babeanu, 2018; Sora *et al.*, 2019). In this sense, copper nanoparticles in their oxidized form, in addition to being negatively charged, promote the translocation of calcium and iron in large proportions, which is reflected in the nutrient content in tomatoes (Deng *et al.*, 2023)

In quadrant III, a high correlation was observed between the variables of equatorial diameter, as well as the polar diameter of the fruit with leaf area. In the latter variable, under DI75 conditions, the grafted hybrids; MO, CU, ME, and AQ, had leaf areas of 757.22, 693.69, 631.26, and 569.7 cm², respectively (Table 1). Under DI50, the leaf area is affected, tending to significantly reduce this variable (Pal *et al.*, 2016), as in the case of the control hybrids MO and AQ, which obtained values of 153.54 and 131.86 cm², respectively, under severe DI50 conditions. This suggests that the combination of grafting and the Colosus RZ

rootstock promoted vegetative growth of the hybrids, a dynamic similar to using other rootstocks like Beaufort, Maxifort, or Spirit (Al-Harbi *et al.*, 2018), as reported by Djidonou *et al.* (2013), and Ramírez-Jiménez, Barrera-Sánchez, and Córdoba-Gaona (2020), primarily attributed to changes in assimilate distribution (Schwarz *et al.*, 2013).

Yield, average weight, and water productivity

In parallel, in quadrant II and under DI50 conditions, there is a negative correlation between plant height and total fruit weight. The grafted hybrids ME and CU grafted + CuNPs had higher total fruit weights at 2.89 and 2.80 kg per plant, respectively (Table 1). According to the above, foliar application of CuNPs points to the potential for increasing crop yield (Hernández-Hernández *et al.*, 2019), at least for the conditions given in the experiment, albeit linked to potential tolerance to DI50 (Prakash *et al.*, 2019). However, the use of grafting is the agronomic management that contributes the most to the variable, as the hybrids CU and MO with CuNPs application showed contrasting yields of 2.27 and 2.00 kg per plant, respectively (Table 1). Therefore, the combination of both agronomic managements proved positive, as the use of grafted plants promotes not only vigor but also yield. While using grafted plants can lead to compact plants due to the limitation in stem elongation, changes in nutrient absorption dynamics are attributed, which is reflected in the productive potential of tomato cultivation (Palada and Wu, 2007; Bogoescu *et al.*, 2011). It is important to note that not all graft-rootstock combinations have advantages, it is important to validate compatibility, to obtain adequate yields (Voronkova and Rzayeva, 2024).

On the other hand, the highest average fruit weight was represented by the hybrid AQ in the grafted mode, and the same hybrid in combination with the foliar application of CuNPs, with an average of 93.76 and 87.54 g, respectively (Table 1). This could suggest tolerance of the rootstock to severe water stress. This finding is consistent with the work of Kolečka *et al.* (2018), who reported that rootstocks can enhance tolerance to abiotic stress. Additionally, the foliar application of CuNPs may have synergized with the grafted plants, further improving their performance. In contrast, hybrids located in quadrant IV under DI75 conditions exhibited lower average fruit weights. For example, the MO and CU hybrids without any agronomic management achieved average fruit weights of 70.60 g and 71.55 g,

respectively. This highlights the potential trade-off between irrigation strategies and fruit weight under specific management practices. However, the application of nanoparticles did not promote advantages in the addressed variable for the hybrids ME with 73.01 g, and the hybrid MO reflected a value of 57.85 g, indicating a lower agronomic performance, at least for the yield component. The lower fruit weight may be linked to severe water restriction; however, the higher values are attributed to the use of grafted plants, a frequent response in various studies. Soare, Dinu, and Babeanu (2018), reported an increase of 68.53% in average fruit weight compared to non-grafted plants. Nevertheless, the percentage increases in this variable can vary depending on the graft-rootstock combination (Koleška *et al.*, 2018; Jenkins *et al.*, 2022), which is regularly reflected in fruit yield.

The values found in the water productivity variable show a contrasting dynamic, visualized in quadrant II, where the grafted hybrids under DI75 conditions, MO and CU, presented 32.24 and 28.46 kg m³, respectively (Table 1), with an accumulated irrigation volume of 89.6 liters per plant in a cycle of 132 days. These values are slightly lower than those reported by Flores *et al.* (2007), who reported 35 kg m⁻³, with saladette-type tomatoes cv. Tequila, with an accumulated irrigation volume of 110 liters per plant in a cycle of 137 days. Thus, the variety plays an important role in this variable (Wu *et al.*, 2021), as well as changes in the dynamics of water productivity based on crop stages (Takács *et al.*, 2020). In this way, some rootstocks resist certain abiotic factors, from salinity, extreme temperatures, and drought, so some will present a significant improvement in survival and yield (Faisal *et al.*, 2024; Janaharshini *et al.*, 2024), thus granting a great advantage in the use of grafts in agriculture in the face of limiting growth conditions (Davis *et al.*, 2024; Reshma *et al.*, 2024).

In quadrant IV, under the DI75 mode, the same hybrids, MO, and CU, without any agronomic management, obtained 16.80 and 15.50 kg m⁻³, respectively, with an accumulated irrigation volume of 134 liters per plant. These were also accompanied by hybrids with nanoparticle application, ME, and MO, with values of 16.98 and 14.93 kg m⁻³ (Table 1). These values are like those reported in other studies, where irrigation corresponding to 100% of the water volume required by the crop is applied, with values ranging between 15.9 and 14.8 kg m⁻³ (Al-Ghobari and Dewidar, 2018). This is in line with

Takács *et al.* (2020), who indicate that water uses efficiency at 75% and 100% irrigation levels is similar, so the percentage of irrigation volume saved can allow for an increase in cultivable areas (Noreldin *et al.*, 2015; Halli *et al.*, 2021). Authors have determined that in critical stages of the crop, such as in the development of the crop, it is possible to reduce the crop yield by up to 66.5%, which corresponds to commercial yield (Gebreigziabher and Assefa, 2024), however, the method is considered an advantage for arid regions by optimizing the use of water resources, making it a sustainable practice (Alkhateeb *et al.*, 2024).

Lycopene, beta-carotene, and total soluble solids contents

In quadrant I, under DI50 conditions, nanoparticles influenced the lycopene content in fruits, a quality variable. The hybrids CU and AQ with CuNPs were grouped, showing 148.02 and 114.64 µg per 100 g of fresh fruit weight (ffw), respectively (Table 2). This influence is evident in the contrasting results observed in quadrant III, where the grafted hybrid MO achieved a concentration of 68.85 µg ffw⁻¹, and the combined use of grafting and copper nanoparticles promoted a concentration of 66.35 µg ffw⁻¹ in the hybrid ME. Therefore, the combination of grafting and rootstock may have affected the lycopene content specific to some hybrids (Jenkins *et al.*, 2022). However, the increase in lycopene concentration by establishing irrigation frameworks in deficit irrigation mode, as well as partial root-zone irrigation, has been addressed in various studies (Bogale *et al.*, 2016; Abdulaziz *et al.*, 2017; Takács *et al.*, 2020). Furthermore, this strategy can be used for a functional food context, considering the influence of other agronomic factors such as the variety used, based on the response to water deficit (Coyago-Cruz *et al.*, 2019), which should be moderated to ensure acceptable performance of the hybrids (Ripoll *et al.*, 2016).

Regarding the beta-carotene content, visualized in quadrant IV under a DI75 framework, the content of this antioxidant was influenced by the application of CuNPs in the following hybrids: MO, CU, and AQ, with contents of 83.00, 82.07, and 78.00 µg ffw⁻¹, respectively (Table 2). From this, it could be inferred that grafted plants, due to their tolerance to adverse conditions, require lower antioxidant activity compared to non-grafted plants (Koleška *et al.*, 2018).

On the other hand, in quadrant I under DI50 conditions, there was a good representation of the vector for the variable of total soluble solids content, an aspect of fruit quality. The hybrid ME in the control mode and with CuNPs application stood out with values of 5.03 and 4.75 °Brix, respectively (Table 2). Additionally, the hybrid MO had the lowest concentration at 4.24 °Brix, compared to the previous ones. At the same time, in quadrant III, the grafted CU hybrid with CuNPs application had a concentration of 3.04 °Brix. These concentrations are addressed in other studies with values of 4.1 °Brix for grafted plants and 4.6 °Brix in non-grafted plants (Soare *et al.*, 2018), with increases of over 1.25 °Brix (Pogonyi *et al.*, 2005), starting from values of 4.1 to 4.6-4.7 °Brix reported in Saladette-type fruits (Andrade *et al.*, 2014; Martínez-Damián *et al.*, 2018). The concentration of total soluble solids increased in parallel with the water deficit in the crop, which depends on the moisture content in the soil and are responsible factors for the changes that occur (Turhan *et al.*, 2016; Lovelli *et al.*, 2017; Wang, Li and Niu, 2020). Therefore, the combination of grafting and rootstock did not increase the total soluble solids, so the feasibility of using grafts must be assessed through other parameters (Sora *et al.*, 2019). This is because a decrease in the concentration of total soluble solids is common when using grafted plants, as seen in eggplant crops (Mozafarian, Ismail, and Kappel, 2020), and a similar dynamic in watermelon grafts (Turhan *et al.*, 2012; Lopez-Galarza *et al.*, 2015).

Equatorial diameter, polar diameter, and fruit firmness

The equatorial diameter of the fruit was greater for the grafted hybrid ME + CuNPs with 67.68 mm under DI50 conditions (Table 2). In contrast, the hybrids surrounding the vector, MO, and grafted ME, obtained an average of 60.38 and 59.65 mm, respectively, under DI75 conditions. These diameters indicate that the rootstock can change the characteristics of the fruits. Although these changes occur depending on the graft-rootstock combination, there is a possibility of producing rounded fruits (Ramírez-Jiménez, Barrera-Sánchez and Córdoba-Gaona, 2020). This is supported by the equatorial diameter presented by the control hybrid CU, projected in quadrant I, with 48.93 mm, which is smaller compared to the grafted ME hybrid. This is the expected response when dealing with Saladette-type fruits, according to the description from the seed company.

The polar diameter of the fruit, represented in quadrant III, was exemplified by the hybrid ME under DI50, grafted + CuNPs, with a length of 67.24 mm (Table 2). This suggests that despite being subjected to water stress, the length of the fruits was not significantly affected when the graft-rootstock combination proposed in this research was used, along with the foliar application of 100 ppm copper nanoparticles. In contrast, slight differences were observed among the grafted hybrids under DI75 conditions. The hybrids MO, ME, CU, and AQ recorded fruit lengths of 67.52 mm, 66.69 mm, 65.61 mm, and 66.38 mm, respectively. These similar values may be attributed to the homogeneity of the hybrids used, at least for this variable. It is noteworthy that grafting can influence fruit shape (Musa *et al.*, 2021). In some crops, this change might result in less visually appealing fruit (Milošević and Milošević, 2022); however, fruit shape is not the primary characteristic of interest for tomatoes. Other factors, such as shelf life and overall crop performance, play a more significant role in determining the suitability of the crop (Carrillo-Rodríguez *et al.*, 2019).

The grafted MO hybrid + CuNPs demonstrated good fruit firmness performance at 2.48 kg cm⁻² (Table 2). According to the projection, it is also grouped with the grafted ME and grafted CU + CuNPs hybrids at 3.19 and 2.60 kg cm⁻², respectively. Changes in texture and flavor aspects can occur depending on the characteristics of the rootstock (Sora *et al.*, 2019). Additionally, water restriction may have led to a reduction in fruit size, which is inversely proportional to an increase in fruit firmness (Cui *et al.*, 2020).

CONCLUSIONS

The agronomic evaluation revealed that the application of copper nanoparticles (CuNPs) to grafted plants, combined with different irrigation regimes, significantly influenced yield, fruit quality, and water use efficiency in tomato crops. The hybrids displayed distinct agronomic characteristics: the Aquiles hybrid excelled in plant height and average fruit weight, the Mesías hybrid stood out for greater fruit firmness and total soluble solids within acceptable ranges, and the Cuauhtémoc hybrid demonstrated higher concentrations of lycopene and beta-carotene.

Grafted plants and those treated with CuNPs yielded positive outcomes. Under deficit irrigation conditions, grafted plants achieved higher fruit yield, water productivity, and stem diameter.

Moreover, combining grafting with CuNPs applications enhanced internal fruit quality, increasing total soluble solids, beta-carotene, and lycopene concentrations. However, grafting alone hurt lycopene concentrations and total soluble solids, suggesting that its primary benefits are improvements in fruit firmness and size.

Deficit irrigation at 50% (DI50) enhanced water use efficiency without causing significant yield losses, while promoting acceptable increases in lycopene content and total soluble solids. This indicates that DI50 is a viable strategy for optimizing water resources in regions with limited irrigation availability. On the other hand, deficit irrigation at 75% (DI75) positively affected plant height and leaf area during the productive stage, demonstrating its potential for improving vegetative growth under certain conditions.

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Supplementary Table 1. Analysis of variance for the variables plant height (PH), leaf area (LA), stem diameter (SD), average fruit weight (AFW), total fruit weight (TFW), and water use efficiency (WUE) as a function of hybrids (H), treatments (T), deficit irrigation (DI).

FV	PH	FA	SD	AWF	TFW	WUE
H	3442.9 **	60960.82 **	2.02 *	1264.7 **	0.38 ns	26.8 ns
T	8999.51 **	1089387.52 **	12.6 **	18.1 ns	0.93 *	74.45 *
DI	6286.01 **	530923.26 **	2.91 *	184.21 ns	0.03 ns	2557.4 **
H*T	469.16 ns	16368.47 **	3.67 **	270.04 *	0.15 ns	14.6 ns
H*DI	392.84 ns	3834.34 ns	0.44 ns	35.49 ns	0.18 ns	10.34 ns
T*DI	210.78 ns	12737.83 *	3.93 *	131.17 ns	0.12 ns	7.09 ns
H*DI*T	383.17 ns	4065.67 ns	1.75 *	264.8 *	0.16 ns	16.17 ns
Error	353.36	2688.84	0.73	122.77	0.16	12.17
R ²	0.61	0.94	0.61	0.44	0.33	0.73
CV	9.55	13.35	9.51	14.09	16.26	15.04

* Significance at 5%; ** Significance at 1%; ns: non significance. C.V.: Coefficient variation

Supplementary Table 2. Analysis of variance for the variables polar diameter (PD), equatorial diameter (ED), fruit firmness (FF), total soluble solids (TSS), lycopene (LY), and beta-carotene (BE) as a function of hybrids (H), treatments (T), and deficit irrigation (DI).

	ED	PD	FF	TSS	LY	BE
H	49.14 *	29.93 ns	0.47 **	0.60 *	4157.09 **	915.17 **
T	223.50 **	95.60 *	1.81 **	2.14 **	3562.27 **	1366.36 **
DI	37.56 ns	99.28 ns	0.05 ns	22.57 **	4418.39 **	40.99 ns
H×T	9.20 ns	15.21 ns	0.07 ns	0.18 ns	923.51 **	103.88 ns
H×DI	37.53 ns	59.56 ns	0.24 *	0.20 ns	1639.16 **	293.72 **
T×DI	73.95 ns	39.45 ns	0.12 ns	0.41 *	1387.63 **	553.26 **
H×DI×T	24.93 ns	39.29 ns	0.08 ns	0.16 ns	706.40 **	98.19 *
Error	16.53	25.25	0.06	0.14	100.28	28.65
R ²	0.45	0.34	0.61	0.73	0.84	0.80
C.V.	7.19	7.8	10.19	9.36	10.43	7.79

* Significance at 5%; ** Significance at 1%; ns: non significance. C.V.: Coefficient variation