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EFFECTS OF ZINC OXIDE NANOPARTICLES ON GROWTH, MICROMORPHOLOGY AND HISTOLOGY OF GRAFTED EGGPLANT (*SOLANUM MELONGENA*)

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Abstract

Grafting is a technique that allows plants to maximize water and nutrient absorption, while the use of nanoscale materials as fertilizers takes advantage of their unique physical and chemical properties. This study examined the effects of different zinc oxide nanoparticle (ZnONPs) concentrations (0, 20, 40, 60 mg L^{-1}) on the micromorphology, histology, and growth of grafted eggplants. The stomatic density and index, stoma length and width, as well as the number and area of xylem vessels were evaluated. The plant height, stem diameter, leaf number, root length, root dry weight, and foliar dry weight were also recorded. The results show that grafting primarily promotes plant growth and micromorphology, increasing the number of xylem vessels but reducing their area. The zinc oxide nanoparticles at a dose of 20 mg L^{-1} had favorable effects on leaf number, while at 40 mg L^{-1}, there were positive changes in the stomatic density and index, as well as an increase in the number of xylem vessels along with a concurrent reduction in their area in grafted plants.

Key words: Graft, ZnONPs, Xylem, Stoma, *Solanum melongena.*

Introduction

Eggplant is one of the most important crops in the world due to its nutritional properties and wide-spread cultivation. Presently, there are about 2.5 million hectares in cultivation, with yields of around 28.14 Ton ha^{-1} (Anon., 2017) . Grafting is a horticultural technique of great economic and environmental interest used in crop production by various countries across Asia, Europe, and the Americas. This technique involves joining two distinct plants in such a way so that they grow and develop as one. This combination between the rootstock and scion affects the growth and productivity of the plant, and the quality of the resulting fruits(Gaion *et al.*, 2017).

Some of the benefits of grafting include an improved uptake of nutrients by the radicular system, and better responses to water deficits and osmotic stress in high salinity soils (Lee *et al.*, 2010). In recent years, this technique has also proven to be effective at controlling diseases and improving crop yields (Moncada *et al.*, 2013; Xu *et al.*, 2014). Grafting has been found to affect various fruit characteristics such as shape, pericarp color and texture, total soluble solids concentration, titratable acidity, pH, lycopene content, average size, and average weight (Flores *et al.*, 2010; Turhan *et al.*, 2011) .The grafting of eggplant increase the size of the fruit, quality characteristics may vary as function of the interaction grafting and cultivar (Moncada *et al.*, 2013).

Zinc oxide nanoparticles (ZnONPs) are widely used in the cosmetics and textiles industries, as well as in medicine, electronics, and food production. In agriculture, ZnONPs have been found to possess antifungal activity, reducing the incidence of disease from *Penicillium expansum*, *Botrytis cinerea*, *Arpergillus flavus*, *Aspergillus niger*, *Fusarium culmorum*, and *Fusarium oxysporum* (He *et al.*, 2011; Rajiv *et al.*, 2013). The full range of effects of nanoparticle application depend on the plant species, the developmental stage at application time, growth medium, and the particle coating material (Yang *et al.*, 2017). At high concentrations ZnONPs have been found to reduce root growth and length, as well as the chlorophyll concentration in eggplant seedlings (100, 250, 500 and 1000 mg L^{-1}) (Baskar *et al.*, 2018). At lower concentrations 10 and 20 mg L^{-1}, ZnONPs promote plant growth (Mahajan *et al.*, 2011; Raliya & Tarafdar, 2013).

An ever–growing world population demands that food production increase at a proportional rate, which means that the greatest challenge facing agricultural researchers is the development of innovative technologies to improve crop quality and quantity without degrading agroecosystems. The grafting of eggplant is considered a viable means for increasing productivity and improving crop quality. Similarly, the use of nanoscale fertilizers in agriculture is considered a sustainable practice as it involves using less material in a more efficient manner. For these reasons, the aim of the present study was to evaluate the effects that grafting and ZnONPs application had on the growth, micromorphology, and histology of eggplant plants.

Materials and Methods

Geographic location: The experimental work was performed in polycarbonate-covered greenhouses belonging Antonio Narro Agrarian Autonomous University Department of Plant Breeding, located in Buenavista, Saltillo, Coahuila, Mexico (25°22” N, 101°00” W) at 1760 m.a.s.l.

Vegetal material and cultivation conditions: Eggplant, variety Black Beauty, was grown from seed for this experiment. This variety's fruit is pear-shaped (round, elongated oval), with glossy, dark purple skin and compact, slightly bitter pulp with few seeds (Gisbert *et al.*, 2016). The rootstock chosen for this experiment was Colosus RZ F1 (61-071) tomato, a hybrid variety with very high vigor and production.

Eggplant seeds were sown on January 7, 2019 in 200-cavity polystyrene trays, planting one seed per cavity. The rootstock seeds were sown 21 days later, because of their greater vegetative vigor compared to the scion, to ensure that both the rootstock and scion seedlings had a similar diameter at grafting. The tray growth medium used was a 70:30 mix of peat moss and perlite.

Nanoparticles synthesis: The zinc nanoparticles used in the experiment were synthesized in the Center for Applied Chemistry Research (CIQA) pilot plant. Their 52nm size was determined by x-rays and they were covered in palmitic acid. Dilutions of 20, 40, and 60 mg L^{-1} were prepared from a master stock solution at 2000 mg L^{-1}.

Grafting and nanoparticle application: The cleft grafting method was chosen for joining the eggplant scions to their rootstocks. The grafting was carried out on February 22, 2019. The grafted seedlings were placed in 200-cavity polystyrene trays and kept for 15 days in an acclimatization chamber. They were kept in total darkness for the first three days, and photoperiod 12:12 h (light-dark) for the remaining days. Relative humidity was kept constant at 95%, and the temperature oscillated between 25 and 35°C, in order to promote graft healing, the plants were kept at room temperature for seven days for acclimatization.

Once the grafted seedlings had formed their callus at the site of union, they were transplanted to black, 15-L polyethylene (40 x 60) bags, 23 days after grafting. The growth medium used was a mix of peat moss and perlite (70:30 v/v).

Zinc oxide nanoparticles, at concentrations of 0, 20, 40, and 60 mg L^{-1}, were applied three times during the growing cycle: at the start, during vegetative growth, and during fruiting.

Measurement of plant growth variables: Plant height, leaf number, stem diameter, root length, dry foliage weight, and dry root weight were evaluated as indicators of plant growth. All growth parameters were measured 120 days after transplantation. The plant height, leaf number, and stem diameter were measured weekly, starting eight days after transplanting. The root was extracted by separating the substrate from the pot manually and the excess substrate was removed with water, the main root was measured.

Measurement of micromorphological variables: The stomatic density, stomatic index, stoma length and width, xylem vessel number, and xylem vessel area of both petioles and leaves were chosen as micromorphological parameters for evaluation.

Measurement of stomatic density and stomatic index: For the evaluation of stomata, samples were taken from four plants per treatment, 50 days after transplantation. A mature, fully expanded leaf was taken from each plant. An epidermal impression of the middle of each leaf's adaxial side, in the same orientation, was taken using liquid polystyrene-xylol, which was applied to the leaf surface with a brush. After the film was dried, it was removed with a piece of transparent adhesive tape and mounted on a glass slide (Weyers & Johansen, 1985). For each sample, three fields were chosen at random and viewed microscopically at 40X. A micrograph was taken of each chosen field and was used to count the stomata and epidermal cells, as well as to measure the length and width (in µm) of the stomata guard cells. The stomatic density (SD, stomata per mm^2) was calculated from by counting the number of stomata in 0.02479 mm^2 (the area of the photographed region). The stomatic index was calculated according to the following formula (Wilkinson, 1979):

$$SI = (\text{num. stomata} / \text{epidermal cells} + \text{num. stomata}) \times 100$$

An optical microscope with a digital camera (Pixera Winder Pro) and Axion Vision software (Rel. 4.8) was used to image the leaf impressions.

Evaluation of histological parameters: Samples were taken 15 days after grafting, when the seedlings were finishing their acclimatization stage. Pieces of petioles and leaves were removed, and the tissues were processed according to the following paraffin histological technique:

The tissue samples were deposited in 25-mL, glass jars containing formalin-acetic acid (FAA) fixative solution (5 mL formaldehyde, 5 mL glacial acetic acid, and 90 mL 70% ethyl alcohol). The samples in FAA solution were kept at room temperature in a cool, dry place for 30 days. Afterwards, they were dehydrated by successively passing through solutions of 50%, 60%, 70%, 85%, and 96% ethyl alcohol containing eosin dye. The samples were then passed through absolute ethyl alcohol I, absolute ethyl alcohol II, absolute ethyl alcohol with xylol (3:1), absolute ethyl alcohol with xylol (1:1), absolute ethyl alcohol with xylol (1:3), pure xylol I, and pure xylol II solutions. They were kept in each solution for 2 hours before passing to the next one. Afterwards, the samples were embedded in paraffin at 58°C.

Sample sectioning was performed with a manual microtome (Leica RM2125RTS) adjusted to cut 20 µm thick sections. The paraffin blocks containing the samples were secured in place, perpendicular to the microtome blade, and 5 cuts were made. The sample sections were placed in a flotation solution (prepared by dissolving 0.2 g potassium bichromate and 0.2 g gelatin in 1000 mL of warm, distilled water then filtering the solution). The sample sections were then placed on glass slides. The slides were left at an angle to drain off excess flotation solution and left to dry at room temperature until the solution joined the sample sections to the slides.

Coplin jars with capacity for 8 slides were used during the staining process. The sample slides were immersed in xylol I solution for 10–15 minutes, until all the paraffin was eliminated. Afterwards, the slides were

submerged for 2–5 minutes in the following sequence of solutions: absolute ethyl alcohol I, ethyl alcohol 96%, ethyl alcohol 85%, ethyl alcohol 70%, ethyl alcohol 60%, and distilled water. After the distilled water bath, the samples were left to rest in aqueous 1% safranine solution. After staining, the slides were rinsed, first with running water, then distilled water, 60% ethyl alcohol, 70% ethyl alcohol, 85% ethyl alcohol, 96% ethyl alcohol, and ending with a rapid immersion in 96% ethyl alcohol for 5-30 seconds. Another rinse with absolute ethyl alcohol I was followed by submerging the slides in carbol-xylol for 10 minutes, then in xylol I, II for 10 minutes. Finally, the slides were sealed by placing a drop of Canada balsam and a cover slip over the tissue samples. The prepared slides were left to dry in a stove at 30°C for a week.

Following drying, the tissue samples were analyzed under a microscope (Carl Zeiss) with integrated digital camera and Pixera Viewfinder software. Images of the sample cross-sections were taken at 10x magnification, focusing on the xylem vessels. Axion Vision software (Rel. 4.8) was used to evaluate the number of xylem vessels present and their areas.

Statistical data analysis

Six replicates per treatment were considered for each of the evaluated variables, the experimental design was randomized. The treatments were defined by a factorial arrangement of two graft states (grafted or not grafted plants) and four different concentrations of ZnONPs application (0, 20, 40, 60 mg L^{-1}), thus, there were a total of eight treatment, the control consisted of non-graft plants and 0 mg L^{-1} of ZnONPs.The results from the various experimental treatments were analyzed using the Infostat statistical software package (2016 version) were analyzed using ANOVA. were subjected to Fisher's LSD test ($p \leq 0.05$) in order to determine if there were any significant changes associated with treatment.

Results and Discussion

Plant growth analysis

Effects of grafting: Grafted plants demonstrated significant differences in height, stem diameter, leaf number, root length, dry root weight, and dry weight of the aerial plant parts (Table 1). The average plant height of grafted plants had a 46% increase , while for the root length, root dry weight and air dry weight an increase of 110, 93 and 146% respectively was recorded in relation to control ,like previous results where eggplant plants grafted onto hybrid tomato rootstocks displayed greater vigor (Sabatino *et al.*, 2018). Similarly, the average stem diameter of grafted plants increased 21%, also coinciding with a previous study that reported greater vigor in grafted tomato plants considering the significant increases observed in stem diameter and plant height (Al-Harbi *et al.*, 2017). The average number of leaves in grafted plants also increased by 70%. A similar study noted increased average plant height, leaf number and flower number in grafted bean plants. The authors argue that the grafted plants develop better thanks to their improved capacity to obtain water and nutrients from soil (Bernal-Alzate *et al.*, 2016).

Effects of zinc oxide nanoparticles: The average number of leaves was significantly affected by the application of ZnONPs. A ZnONPs dose of 20 mg L^{-1} was found to increase the average number of leaves by 19%, compared to the control, for the root length a better response was found with the dose of 40 mg L^{-1} On the other hand, the dry weight of the root showed a decrease with the dose of 60 mg L^{-1}, for the height, stem diameter and dry air weight there were no significant differences (Table 2). Foliar application of ZnONPs (50 mg L^{-1}) has been observed to improve the growth and biomass production of pepper plants (Méndez-Argüello *et al.*, 2016). Other studies have also reported results like those seen in the present study. The growth and dry biomass weight of tomato plants (*Solanum lycopersicum*) were improved after foliar application of 20 mg L^{-1} ZnONPs. A concentration of zinc was found in the leaves, confirming its absorption through the stoma and translocation via the phloem(Panwar, 2012). The same effects greater plant height and dry weight were seen in chickpea seedlings (*Cicer arietinum*) after foliar application (1.5 and 10 mg L^{-1}) of ZnONPs. That report highlighted that the application of zinc as a nano-fertilizer promotes plant growth (Bernal-Alzate *et al.*, 2016). It has been suggested that zinc promotes plant growth because it is a known co-factor for enzymes involved in photosynthesis, as well as its role in the preservation of the plant cell membrane integrity and maintenance. Although, ZnONPs have also been known to exert phytotoxic effects, especially at higher concentrations (Burman *et al.*, 2013). Foliar application of high concentrations of ZnONPs (50 mg L^{-1}) induced the production of H_2O_2 in the leaves of pepper plants(Méndez-Argüello *et al.*, 2016).

Interaction between grafting and ZnO nanoparticles: The present study found an increase in plant height, stem diameter, number of leaves and air dry weight in grafted plants and an application of ZnONPs (20 mg L^{-1}) in 43, 41, 100 and 159% respectively in relation to control , as far as the length is concerned, the root is an increase of 130% in grafted plants and a dose of 40 mg L^{-1} in relation to the control, The dry root weight showed the highest values in grafted plants without any application of ZnONPs (Table 3), similarly an experiment performed by González *et al*., (2017), watermelon plants that were grafted and treated with varying doses of copper nanoparticles in chitosan hydrogels resulted in longer average stem lengths. Foliar application of zinc oxide nanoparticles is considered more effective than soil application, as the former led to better nanoparticle absorption in tomato plants, resulting in significant increases in plant biomass and number of shoots and roots (Raliya *et al.*, 2015). Other studies have shown that NP absorption in different plant species leads to their accumulation in subcellular locations (Schwab *et al.*, 2016) and alterations to diverse physiological processes that promote plant growth and development(García-Sánchez *et al.*, 2015).

Table 1. Effects of grafting on growth variables.

Factor	T	PH (cm)	SD (mm)	LN	RL (cm)	RDW (g)	ADW (g)
Graft	G	195.42 a	17.4 a	156.75 a	62.38 a	38.21 a	331.17 a
	NG	133.5 b	13.72 b	92.08 b	29.67 b	19.71b	134.65 b
Significance		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

T: Treatments; G, Grafted; NG, Non-grafted; PH , Plant height; SD, Stem diameter; LN, Leaf number; RL, Root length; RDW, Root dry weight; ADW, Aerial dry weight. Means with the same letter were not significantly different ($p \leq 0.05$), Fisher's LSD test)

Table 2. Effects ZnONPs application on growth variables.

Factor	T	PH (cm)	SD (mm)	LN	RL (cm)	RDW (g)	ADW (g)
ZnONPs dose (mg L^{-1})	0	166.17 a	15.25 a	117 c	46.58 b	30.58 a	228.56 a
	20	165.58 a	15.9 a	139.08 a	44.83 b	27.92ab	237.65 a
	40	159.75 a	15.68 a	113.75 c	52.92 a	30.83 a	238.79 a
	60	166.33 a	15.41 a	127.83 b	39.75 c	26.5 b	226.65 a
Significance		0.1693	0.3831	0.0001	0.0001	0.0194	0.1566

T: Treatments; PH, Plant height; SD, Stem diameter; LN, Leaf number; RL, Root length; RDW, Root dry weight; ADW, Aerial dry weight. Means with the same letter were not significantly different ($p \leq 0.05$), Fisher's LSD test). ZnONPs, zinc oxide nanoparticles

Table 3. Effects of grafting and ZnONP application on growth variables.

Factor	T	PH (cm)	SD (mm)	LN	RL (cm)	RDW (g)	ADW (g)
Interactions	G-0	191.33 b	17.61 a	131.83 d	64.33 ab	43.5 a	327.55 a
	G-20	202.33 a	18.12 a	184.50 a	57.17 c	37.17 b	335.73 a
	G-40	191.33 b	17.43 ab	149.33 c	68.67 a	36.83 b	335.08 a
	G-60	196.67 ab	16.43 b	161.33 b	59.33 bc	35.33 b	326.35 a
	NG-0	141.00 c	12.88 d	92.17 e	28.83 e	17.67 d	129.58 b
	NG-20	128.83 d	13.68 cd	93.67 e	32.50 de	18.67 d	139.58 b
	NG-40	128.17 d	13.92 cd	98.17 e	37.17 d	24.83 c	142.51 b
	NG-60	136.00 cd	14.38 b	94.33 e	20.17 f	17.67 d	126.95 b
Significance		0.0138	0.008	0.0001	0.0035	0.0009	0.9572
	C.V (%)	5	6.29	7.93	10.12	13.1	6.81

T: Treatments; G, Grafted; NG, Non-grafted; PH, Plant height; SD, Stem diameter; LN, Leaf number; RL, Root length; RDW, Root dry weight; ADW, Aerial dry weight. Means with the same letter were not significantly different ($p \leq 0.05$), Fisher's LSD test). C.V= coefficient of variation. ZnONPs, zinc oxide nanoparticles

Table 4. Effects of grafting on the micromorphology of eggplant plants.

Factor	T	ASD (num*mm^{-2})	ASI (%)	ASL (µm)	ASW (µm)
Graft	G	115.12 a	20.25 a	23.69 a	12.82 a
	NG	88.23 b	16.14 b	21.16 b	10.96 b
Significance		0.0075	0.0055	0.0001	0.0001

T; Treatments, G, Grafted; NG, Non-grafted; ASD, Adaxial stomatic density; ASI, Adaxial stomatic index; ASL, Adaxial stomata length; ASW, Adaxial stomata width. Means with the same letter were not significantly different ($p \leq 0.05$), Fisher's LSD test)

Micromorphological variables

Grafting: Induced significant differences in stomatic density and index (30% and 25%, respectively) (Table 4), as well as the length and width of the stomata on the leaf surface (12% and 17%, respectively) (Fig. 1). Similar results have been previously reported for grafted pepper plants (Camposeco Montejo *et al.*, 2018), citrus (Cañizares *et al.*, 2003), and avocado (Ayala-Arreola *et al.*, 2010). In contrast, in grafted cucumber, reductions in stomatic density on the leaf surface and backside, as well as in the stomatic index of the leaf surface, were observed (Peralta Manjarrez *et al.*, 2016). Increases in the stomatic density and index are probably a result of the improved plant vigor conferred by the grafted rootstock, since those changes are correlated with the net CO_2 assimilation rate, respiration rate, and stomatic conductance, while stomatic resistance is reduced (Ayala-Arreola *et al.*, 2010). This influences the physiological efficiency of CO_2 assimilation and transformation in leaves into photoassimilates that can be transported to other sites. Greater CO_2 assimilation efficiency confers greater plant productivity.

Effects of zinc oxide nanoparticles: In Table 5, the effects of zinc oxide nanoparticles application on the micromorphology of eggplant plants are presented, on their own there were no significant differences observed.

Table 5. Effects of various ZnONPs doses on the micromorphology of eggplant plants.

Factor	T	ASD (num*mm^{-2})	ASI (%)	ASL (µm)	ASW (µm)
ZnONPs Dose (mg L^{-1})	0	99.16 a	16.32 a	21.93 a	12.35 a
	20	99.16 a	18.60 a	22.24 a	11.44 a
	40	109.24 a	20.1 a	22.13 a	11.93 a
	60	99.16 a	17.75 a	23.41 a	11.82 a
Significance		0.825	0.2734	0.2099	0.4308

T; Treatments, ASD, Adaxial stomatic density; ASI, Adaxial stomatic index; ASL, Adaxial stomata length; ASW, Adaxial stomata. Means with the same letter were not significantly different (p≤0.05), Fisher's LSD test). ZnONPs, zinc oxide nanoparticles

Table 6 Effects of grafting and various ZnONPs doses on the micromorphology of eggplant plants.

Factor	T	ASD (num*mm^{-2})	ASI (%)	ASL (µm)	ASW (µm)
Interactions	G-0	114.28 ab	19.43 ab	22.4 bcd	13.12 a
	G-20	117.64 ab	21.24 ab	24.07 ab	12.24 b
	G-40	134.45 a	23.52 a	23.43 abc	12.94 a
	G-60	104.2 b	16.81 bc	24.87 a	12.97 a
	NG-0	84.03 b	13.22 c	21.47 cd	11.58 abc
	NG-20	80.67 b	15.97 bc	20.40 d	10.65 c
	NG-40	84.03 b	16.69 abc	20.82 d	10.92 bc
	NG-60	94.12 ab	16.81 bc	21.94 bcd	10.68 bc
Significance		0.1409	0.108	0.3178	0.8819
	C.V(%)	25.61	20.94	6.58	9.06

T; Treatments, G, Grafted; NG, Non-grafted; ASD, Adaxial stomatic density; ASI, Adaxial stomatic index; ASL, Adaxial stomata length; ASW, Adaxial stomata width. Means with the same letter were not significantly different (p≤0.05), Fisher's LSD test). C.V= coefficient of variation. ZnONPs, zinc oxide nanoparticles

Interaction between grafting and ZnO nanoparticles: The effects of grafting in combination with ZnONPs application on the plant micromorphological variables are summarized in Table 6. The grafted plants and the dose of ZnONPs (40 mg L^{-1}) had the highest values for stomatic density and stomatic index in 60 and 78% respectively in relation to the control. The grafted plants treated with 60 mg L^{-1} of ZnONPs had the greatest stoma length, while the stoma width showed no differences. These results stand in contrast to those reported for watermelon, where copper nanoparticles in chitosan hydrogels had greater effect on nongrafted plants. Although, it should be noted that in that case, the nanoparticles were applied to the substrate (González Gómez *et al.*, 2017). The foliar nanoparticle application used in the present study could account for the differing results. Nanoparticles are known to be absorbed through stomatic openings (Jiao *et al.*, 2016), which could have caused an increase in stomatic index and density. The results show the effect exerted by the interaction of the graft and a dose of 40 mg L^{-1} ZnONPs that induces modifications in the foliar micromorphological characteristics of the eggplant, what may be due to the vigor that the rootstock gives to the variety, due to the greater efficiency of the root system, which impacts on a greater ASD and ASI and thus allow a greater absorption of the ZnONPs by the stomata, in turn, promoted the improvements to crop growth (Mahajan *et al.*, 2011). Diverse physiological processes, such as the development of roots, flowers, and fruits, as well as the biochemical processes involved in the production of chlorophyll, are all affected by the application of nanoparticles (Chen, 2014).

Histological plant variables

Effects of grafting: Plants subjected to grafting had a positive response in all the histological variables assessed. The number and area of xylem vessels in the petioles and leaves of the eggplant plants changed in response to grafting. In the petioles, the number of xylem vessels increased with a concurrent reduction in vessel area (Table 7). The same response was observed in the xylem vessels of the leaves (Fig. 2). A previous study had reported the opposite: an increase in the xylem vessel area in the petioles of grafted watermelon(González Gómez, 2017) . It is thought that a greater number of xylem vessels increases the quantity of water that can be transported through the stem. Grafting appears to favorably affect xylem vessel area (Sory Toure *et al.*, 2010; Santarosa *et al.*, 2015). Shorter, numerous vessels with a narrower area are more advantageous, as they help avoid the formation of embolisms which could block water transport (Laskowski, 2000). The types of plant transport tissues, the percentage and size of xylem vessels present, and the relationship between the xylem and phloem are anatomical characteristics that define the water transport capacity in plants, such that as the percentage of vascular tissues increases and their area decreases, the quantity of water that can be transported increases. Those changes in vascular tissues could be considered an indicator of plant adaptation (Reyes-Santamaría *et al.*, 2002).

Table 7. Effects of grafting on the histological characteristics of eggplant plants.

Factor	T	NPXV	APXV (μm^2)	NLXV	ALXV (μm^2)
Graft	G	88.94 a	384.55 b	73.38 a	292.45 b
	NG	34.38 b	516.09 a	34.66 b	388.16 a
Significance		0.0001	0.0001	0.0001	0.0001

T; Treatments, G, Grafted; NG, Non-grafted; NPXV, Number of petiole xylem vessels; APXV, Area of petiole xylem vessels; NLXV, Number of leaf xylem vessels; ALXV, Area of leaf xylem vessels. Means with the same letter were not significantly different ($p \leq 0.05$), Fisher's LSD test)

Table 8. Effects of various ZnONPs doses on the histological characteristics of eggplant.

Factor	T	NPXV	APXV (μm^2)	NLXV	ALXV (μm^2)
ZnONPs Dose (mg L^{-1})	0	60.88 c	465.89 a	51.50 a	353.83 ab
	20	64.25 b	458.57 ab	55.75 a	319.1 b
	40	68.13 a	463.27 a	56.13 a	300.17 b
	60	53.38 d	413.56 b	51.50 a	388.12 a
Significance		0.0001	0.0936	0.1477	0.0163

T; Treatments, NPXV, Number of petiole xylem vessels; APXV, Area of petiole xylem vessels; NLXV, Number of leaf xylem vessels; ALXV, Area of leaf xylem vessels. Means with the same letter were not significantly different ($p \leq 0.05$), Fisher's LSD test). ZnONPs, zinc oxide nanoparticles

Table 9. Effects of grafting and various ZnONPs doses on the histological characteristics of eggplant plants.

Factor	T	NPXV	APXV (μm^2)	NLXV	ALXV (μm^2)
Interactions	G-0	90 b	379.72 c	68.75 b	324.88 bcd
	G-20	91.75 b	404.17 c	74.25 ab	284.05 cd
	G-40	97 a	400.51c	76.25 a	270.41 d
	G-60	77 c	354.21 c	74.25 ab	290.45 cd
	NG-0	31.75 e	552.47 a	34.25 c	382.77 b
	NG-20	36.75 d	512.97 ab	36 c	354.15 bc
	NG-40	39.25 d	526.03 ab	37.25 c	329.93 bcd
	NG-60	29.75 e	472.90 b	31 c	485.79 a
Significance		0.0001	0.5060	0.2798	0.047
C.V (%)		5.21	10.03	8.57	15.81

T; Treatments, G, Grafted; NG, Non-grafted; NPXV, Number of petiole xylem vessels; APXV, Area of petiole xylem vessels; NLXV, Number of leaf xylem vessels; ALXV, Area of leaf xylem vessels. Means with the same letter were not significantly different ($p \leq 0.05$), Fisher's LSD test). C.V, coefficient of variation. ZnONPs, zinc oxide nanoparticles

Effects of zinc oxide nanoparticles: The application of ZnO nanoparticles also affected the plant vascular tissue. At a dose of 40 mg/L, the number of xylem vessels increased by 12%. At higher doses (60 mg L^{-1}), an 11% reduction in area was observed for the petiole xylem (Fig. 3). There were no significant differences in the number of leaf xylem vessels, although following application of 40 mg L^{-1} ZnONPs, the vessel area was reduced by 15% (Table 8).

Interaction between grafting and ZnO nanoparticles: Following grafting and foliar application of ZnONPs (40 mg L^{-1}), an increase in the number of xylem vessels in petioles and leaves was observed. Xylem vessel area in petioles was reduced in grafted plants treated with 60 mg L^{-1} ZnONPs, while in leaves, the greatest reduction in vessel area was observed after application of ZnONPs at 40 mg L^{-1} (Table 9). After entering the leaves via stomata, nanoparticles applied to the foliage are translocated throughout the plant through the phloem. The phloem is a living, vascular tissue through which photosynthetic products, proteins, and some mineral ions required for plant growth are distributed. Nanoparticles flow through the phloem, driven by osmotic pressure, until they reach the plant roots (Wang *et al.*, 2013). Xylem is a plant tissue closely related to phloem, forming vascular bonds with it. Zinc oxide nanoparticles are known to have a positive effect on the plant reactions to phytohormones, such as cytokinins (zeatin), auxins (indoleacetic acid), and salicylic acid, compounds which promote cellular division, elongation, and growth in plants (Vankova *et al.*, 2017).

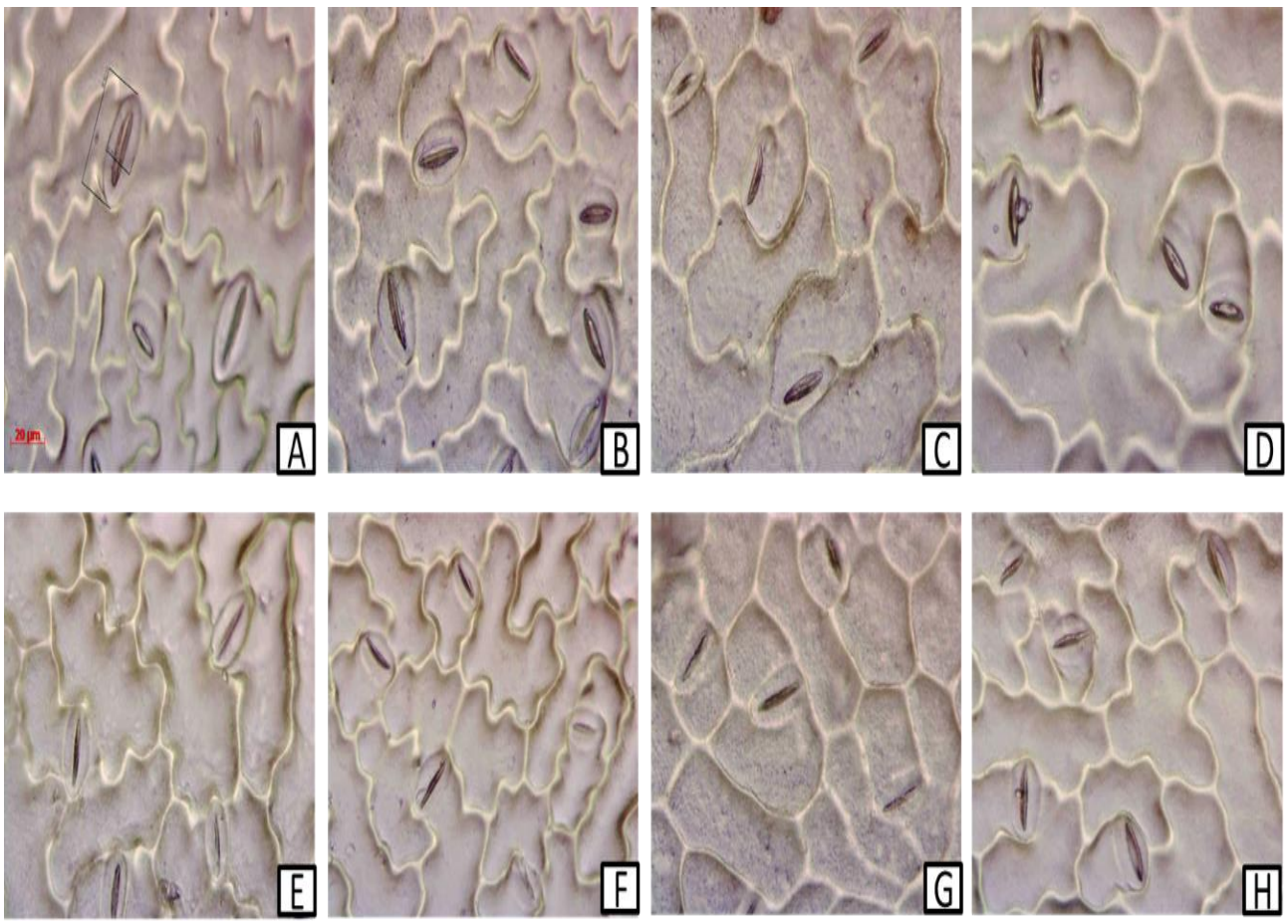

Fig. 1. Stomata distribution on the adaxial side of eggplant leaves. A) G-0, B) G-20, C) G-40, D) G-60, E) NG-0, F) NG-20, G) NG-40, H) NG-60 mg L^{-1}. 40x.

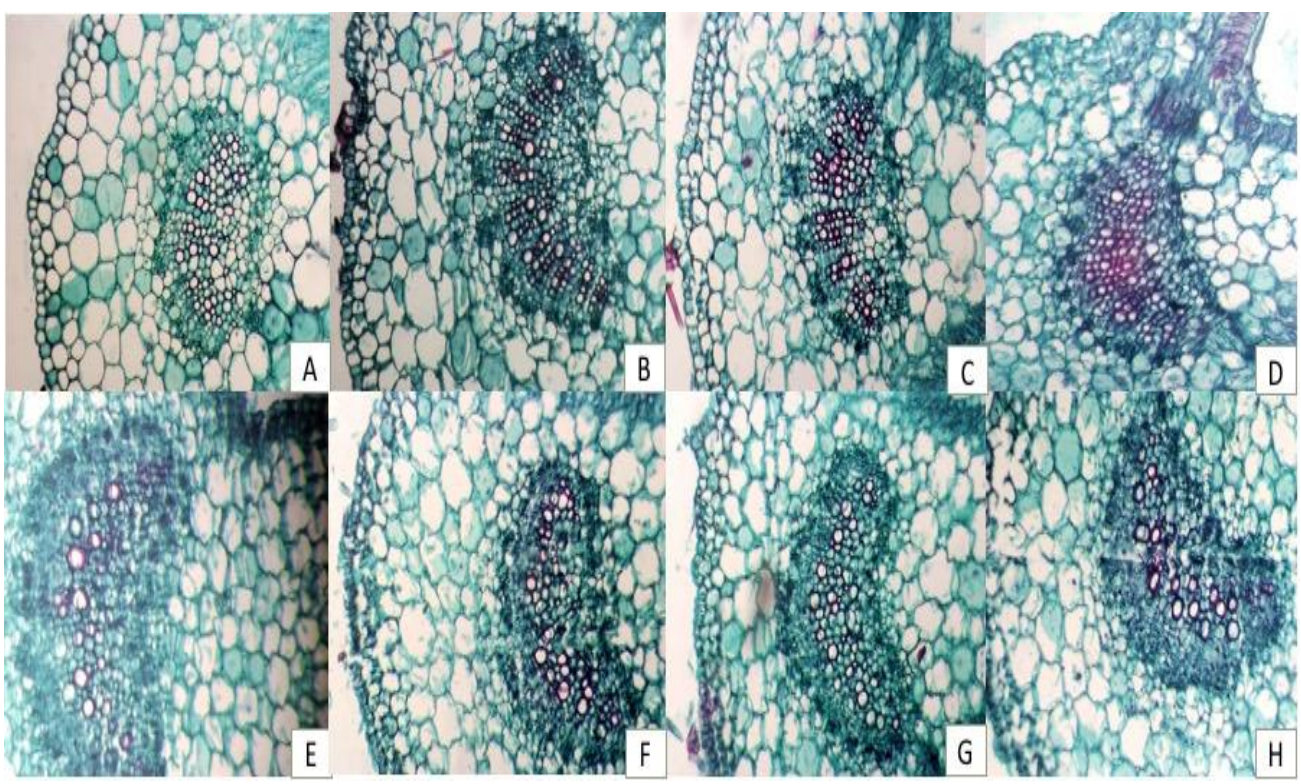

Fig. 2. Micrographs of leaf xylem vessels from eggplant plants subjected to grafted and various doses of ZnONPs. A) G-0, B) G-20, C) G-40, D) G-60, E) NG-0, F) NG-20, G) NG-40, H) NG-60 mg L^{-1}.

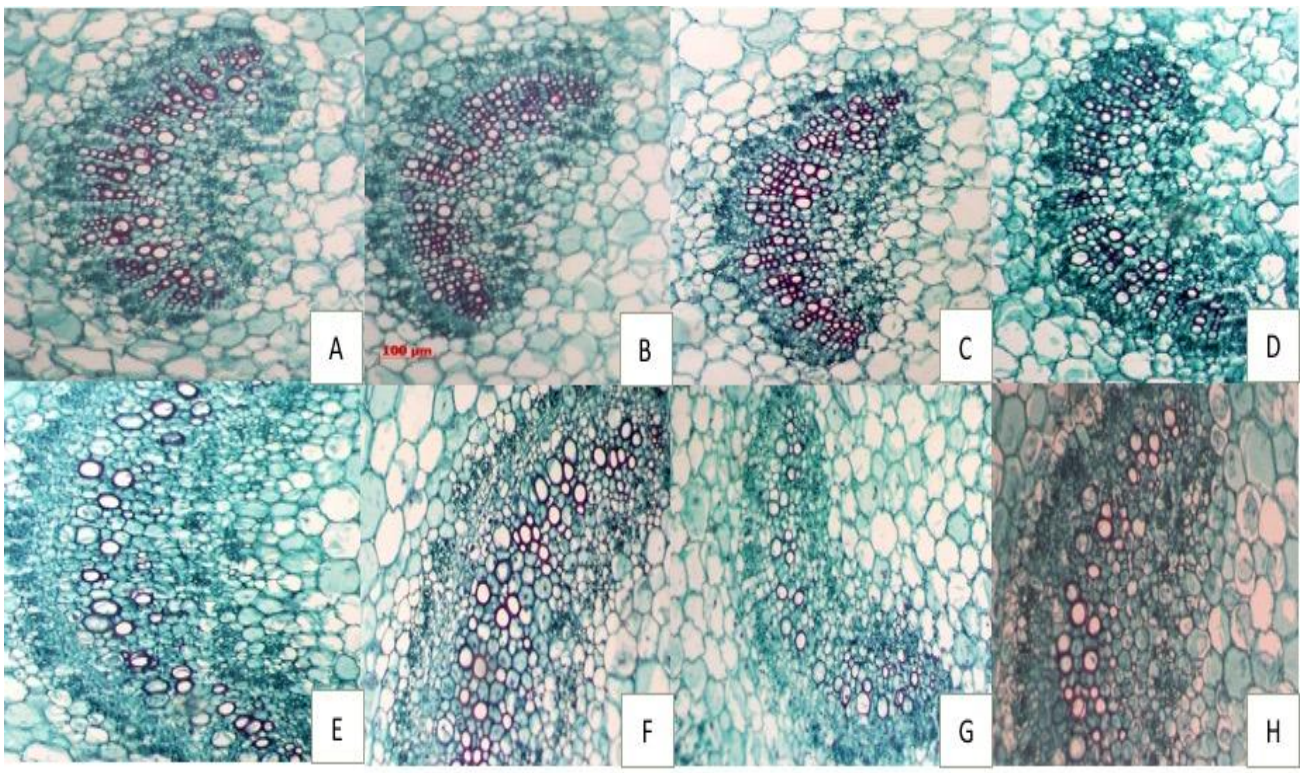

Fig. 3. Micrographs of petiole xylem vessels from eggplant plants subjected to grafting and various doses of ZnONPs. A) G-0, B) G-20, C) G-40, D) G-60, E) NG-0, F) NG-20, G) NG-40, H) NG-60 mg L^{-1}.

Conclusions

This study found that parameters associated with crop growth improved after grafting eggplant. The leaf micromorphology changed after grafting, with increases in stomatic density and index, as well as the number and area of xylem vessels. These changes promoted plant growth. Application of ZnONPs at a concentration of 20 mg L^{-1} stimulated the production of leaves, while at a dose of 40 mg L^{-1}, changes in plant histology of grafted eggplants are induced. Together, these two techniques promote improved growth and development of eggplant plants.

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