

THE GRAFT IN THE GROWTH AND STOMA- TIC DENSITY IN (*Lycopersicon esculentum* Mill) CULTIVATED IN SHADE MESH

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Abstract: The low yield of a crop is the result of a series of biotic and abiotic factors that intervene from the initial growth, which has an important impact during all stages of development. For this reason, the Climate Change present today has predominance over the development of the plants, for this reason it is important to generate and implement technology to face them. Vegetable production, especially tomato cultivation (*Lycopersicon esculentum* Mill.), offers a production opportunity in the southeastern region of Mexico. However, the lack of technology and the high costs of agricultural inputs have inhibited the projection of this crop. The grafting technique is presented as an innovation to improve the productive potential of the plant under protected conditions. The objective of this work was to study the growth behavior of the tomato crop with the grafting technique under shade mesh conditions. The varieties used were Multifort (Rootstock), El Cid (Graft) and Enforcer, El Cid F1 (as control); During growth, the variables height, diameter, leaf area, phytomas, and stomatal density were measured. The results show that the graft did not influence the height of the plant and the diameter of the stem; also dry phytomass and stomatal density did not show significant difference in the graft. However, the leaf area was higher in the graft in the two sampling stages. The varieties used were Multifort (Rootstock), El Cid (Graft) and Enforcer, El Cid F1 (as control); During growth, the variables height, diameter, leaf area, phytomas, and stomatal density were measured. The results show that the graft did not influence the height of the plant and the diameter of the stem; also dry phytomass and stomatal density did not show significant difference in the graft. However, the leaf area was higher in the graft in the two sampling stages. The varieties used were Multifort (Rootstock), El Cid (Graft)

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Keywords: Tomato, stomatal density, graft, shade mesh.

INTRODUCTION

Among the most demanded vegetables for consumption is the tomato (*Lycopersicon esculentum* Mill.), which is a fruit rich in protein, minerals and vitamins; nutritional value that gives it greater importance due to the energy it provides. Its importance not only focuses on those who consume it, but also on those who produce it; due to the very important foreign exchange generation, if it is offered the right conditions for profitable productivity.

It must be noted that in the southeastern region of the state of Chiapas, Mexico, there are agroecological conditions for the development of this crop, unfortunately there is no technology developed for the prevailing conditions; cultivating the horticultural system with traditional methods and techniques. Production techniques that have triggered the incidence of pests and diseases, which together with climate change and the high costs of agricultural inputs have demerited the productivity and quality of this crop.

In the last years of the 21st century, alternative technologies have been developed and implemented worldwide to increase vegetable production; Mexico has not been exempt from advances in greenhouses, shade nets, plastic mulch, tunnels, and grafts. The latter, as mentioned by Miguel et al., (2007), is a technique that allows cultivating species that are tolerant to certain pathogens on infected soils; whose most important purpose, even in predictably uninfected soil, is to obtain greater production or a more extensive production cycle, due to the vigor that the rootstock confers to the grafted plant.

The objective of this work is to study the growth of the tomato crop with the grafting technique; as well as the accumulated phytomass of leaves, stem and root and the stomatal density under shade mesh conditions.

MATERIALS AND METHODS

The present research work was carried out under shade mesh conditions in the Ejido Álvaro Obregón, municipality of Tapachula, Chiapas, Mexico; located at 14° 55' north latitude and 92° 23' west longitude, with an altitude of 40 masl. The climate is warm sub-humid with summer rains Am(w)ig(INEGI, 2004).

The seedbed stage began with the planting of the rootstock (Multifor) in polystyrene trays with 200 cavities; five days later the planting of the Graft (El Cid) was made; as well as the Enforcer and El Cid F1 varieties. Grafting was performed using the splice method (Miguel, 2007); when the rootstock and graft seedlings reached 2 mm in diameter. Once the plants healed at the graft union (10 days after grafting), the transplant was carried out in 20 x 40 cm polyethylene bags, using soil + compost as a substrate in a 2:1 ratio; The grafted seedlings were distributed according to the Completely Random experimental design inside the shaded mesh area. The

conduction of the plants was carried out in a single stem, staking by tying raffia thread to a wire located at a height of 3 m. Manual irrigation was supplied with a 1000 ml graduated cylinder, applying irrigation sheets according to the needs of the plant at each stage of development.

Four treatments were evaluated for this study: 1). Grafting (as Multifor Rootstock and as Graft the El Cid variety), 2). Planting of the Enforcer variety, 3). Sowing of El Cid and 4) Regional variety El Cid F1 (Control). The experimental design used was completely random, with six sampling units, each one composed of 10 plants; the experimental unit was made up of 16 plants and the useful plot 10 central plants. Growth variables (Table 1) and stomatal density were measured in each randomly selected sampling unit, weekly and starting 19 days after grafting.

To determine the stomatal density, 4 leaflets were randomly selected from the middle part of the plant, to which four mm² cuts were made and subsequently covered with a layer of gold-palladium of approximately 10 nm in a brand metal depositor. DENTON VACCUM model DESK II. The stomata count was determined by means of a TOPCON brand scanning electron microscope, model SM-510 under high vacuum conditions at 3 KV acceleration voltage, sampling was performed at 36 and 79 days after transplantation (DAT).

Due to strong winds that occurred in the study area at the beginning of April 2011, which affected the facilities and the existing plant material, no production results were obtained.

The database was subjected to analysis of variance ($\alpha = 0.05$). Variables with significant differences between treatments were compared with multiple range means by Tukey's method ($\alpha = 0.05$). Statistical analyzes of the database were carried out with the support of the statistical package STATGRAPHICS Century

Version XVI.

RESULTS AND DISCUSSION

PLANT HEIGHT

During the vegetative growth stage until the beginning of flowering, there was a marked difference in plant height of the El Cid F1 treatment (Figure 1) over the El Cid, Enforcer and Injerto varieties; there was a significant difference ($Tukey\alpha = 0.05$). However, from 37 DAT and up to 79 DAT, the Enforcer variety presented an intense vertical growth, obtaining a maximum height of 171.81 cm, followed by the El Cid treatment with 128.73 cm, the El Cid F1 treatment with 125.73 and the Graft treatment with 125.59 cm, these last three treatments were statistically equal, while the Enforcer variety presented a significant difference over the other treatments (Figure 1). These results contrast with those obtained by Lee (1994) and Leonardiet al., (2004) who found that grafted tomato plants in the open field are taller and more vigorous. They agree with the results of Khahet al., (2006), who state that plant height was not significantly affected by grafting under greenhouse conditions. According to the conditions of the present study, it is concluded that the grafted plants are not influenced in vertical growth.

STEM DIAMETER

The diameter of the stem of the plants of the El Cid F1 treatment offered a significant statistical difference (Table 2) with respect to the Micro Injertación, Enforcer and El Cid treatments, these last three were statistically similar from 37 DAT, but numerically the Injerto was who presented larger diameters only surpassed by the El Cid F1 treatment, who obtained 0.85 cm in diameter with 0.79 cm from the Graft, 0.78 cm from the Enforcer and 0.76 cm from the El Cid treatment.

Stem diameter is a good indicator of plant vigor, since it directly reflects the

accumulation of photosynthates, which can later be translocated to demand sites (Preciado et al., 2002; Liptayet al., 1981). Likewise, it is indicative of physiological changes in grafts, due to cellular interactions between two genotypes (Kokalis-Burelle et al., 2009); however, changes in diameter do not always correlate with a decrease in production (Cürüket al. 2009). The diameter values in this study are below those reported in the literature, since Rodríguez et al., (2001) point out that the diameter of the stem can reach 2.5 cm, values much higher than those indicated. Similarly, Went (1956; quoted by Folquer, 1976) mention that the diameter of the stem and its different tissues can be affected by environmental and management factors; thus, temperatures above 30°C favor the growth of thin stems, corroborating them with the results obtained in this work, where temperatures of 42°C were reached inside the shade mesh, a value higher than the temperature limits in mention.

LEAF AREA

In the illustration of the results for the leaf area variable at 52 and 79 DAT (Figure 2), it is clearly observed that the Graft presents a significant difference (Tukey $\alpha = 0.05$) in relation to the other varieties. At 52 DAT, the graft produced 181.30 cm² of leaf area, a value well above the results obtained by the El Cid, El Cid F1 and Enforcer treatments. Subsequently, at 79 DAT, the Graft again shows a significant difference (161.54 cm² foliar area) over the other treatments, average above the El Cid variety (102.90 cm²), El Cid F1 (94.15 cm²) and Enforcer (87.96 cm²)(Figure 2). The decrease in the leaf area between the sampling dates is due to fruiting, a phase where greater demand for water and nutrients is required,

According to Venema et al., (2008), the leaf area of tomato plants increases when they are grafted; Likewise, Godoy et al., (2009) affirm

that there is a greater foliar area in plants grafted under greenhouse conditions. Results that confirm those found in this work, where the graft surpassed the other varieties in leaf area.

DRY PHYTOMASS OF LEAVES, STEM AND ROOT

The phytomass of the leaves per plant for the Graft, Enforcer and El Cid treatments was statistically the same. However, the Enforcer treatment was the one that presented the greatest numerical difference with 21.90 g per plant, followed by the Graft with 21.49 g, the El Cid treatment with 18.87 g, the El Cid F1 treatment standing out numerically lower with 16.21 g. For stem biomass, there was also a significant difference between Enforcer (15.47 g) and Graft (10.50 g), El Cid (10.24 g) and El Cid F1 (10.00 g). However, the dry biomass of the root, all the treatments were statistically equal, but it is worth mentioning that the treatment that presented the highest root phytomass was Enforcer with 9.90 g, followed by El Cid with 7.58 g, El Cid F1 with 6.96 g and the Graft. with 6.90 g (Table 3)

In the same way, studies carried out by Khahet al., (2006) indicate that grafted plants have no effect on dry weight. According to the results, it is confirmed that both in leaves, stem and root there was no notable difference in the graft compared to the other varieties.

STOMATAL DENSITY

The results of stomatal density at 36 and 79 DAT are presented in Table 4, where it is observed that there is no significant difference between the treatments in the two samplings carried out. However, at 36 DAT, the graft presented a higher number of stomata per mm² in the bundle (102.22) (Figure 3), followed by Enforcer, El Cid F1 and El Cid, presenting 97.22, 85.55 and 74.44 stomata per mm², respectively. On the underside, the

dominant treatment in number of stomata was Enforcer (250.00) and Cid F1 resided with only 205.00 (Table 4).

In the second sampling (79 DAT) there was an increase in the number of stomata on the underside only for Enforcer (250.00 to 298.85) (Figure 6), while in the other treatments the stomatal density was affected with a decrease. Presenting the Enforcer treatment (88.67 and 298.85) the highest number of stomata per mm² (Table 4). According to Rubino et al., (1989) and Takur (1990) they point out that the decrease in stomatal density increases the closure of the leaf stomata, which limits excess transpiration. In the present study, these changes are influenced by the existing

temperature within the shade mesh (42°C), it is evident that the plant seeks a way to reduce the transpiration of water in the leaves, which is a physiological response to the stress caused by the environment.

CONCLUSION

Grafting tomato plants under shade mesh conditions did not influence height, stem diameter, dry phytomass and stomatal density. However, the leaf area was higher in grafted plants, which shows a high photosynthetic efficiency of the leaves; This method can influence the increase in crop yield under optimal environmental conditions.

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Variable	Method	Unit	Frequency
plant height	flexometer	cm	Weekly
stem diameter	electronic vernier	cm	Weekly
dry phytomass of leaves, stem and root.	drying in an oven at 80°C for 72 hours and weighing on an analytical balance.	g	80 days after transplant

Table I. Growth variables measured in the Grafted Tomato study.

DDT	Graft (cm)	Enforcer (cm)	El Cid (cm)	El Cid F1 (cm)
9	0.33c	0.28d	0.41b	0.53 to
16	0.41c	0.36d	0.48b	0.58 to
23	0.47c	0.42d	0.53b	0.62 to
30	0.54c	0.52c	0.58b	0.67 to
37	0.61b	0.59b	0.62b	0.71 to
44	0.64b	0.62b	0.64b	0.74 to
51	0.69b	0.66b	0.66b	0.77 to
58	0.71b	0.69b	0.70b	0.78 to
65	0.74b	0.71b	0.73b	0.81 to
72	0.77b	0.76b	0.75b	0.84 to
79	0.79b	0.78b	0.76b	0.85 to

DDT: days after transplant

*Means with the same letter are statistically equal (Tukey 0.05)

Table II. Stem diameter of different varieties and grafting of tomato seedlings

Treatment	Dry leave (g)	dry stem (g)	Dry root (g)	Total (g)
Graft	9:49 p.m.	10.50b	6.90 a.m.	38.89
Enforcer	9:90 p.m.	3:47 p.m.	9.90 a.m.	47.27
El Cid	6:87 p.m.	10.24b	7.58 a	36.69
El Cid F1	16.21b	10:00 a.m.	6.96 a	33.17

*: Means with the same letter are statistically equal (Tukey 0.05)

Table III. Leaf, stem and root phytomass per plant of different varieties and tomato graft.

Treatment	36 DAT		79 DAT	
	Beam (mm2)	Underside (mm2)	Beam (mm2)	Underside (mm2)
Graft	102.22 to	211.66 to	85.38 to	151.61 to
Enforcer	97.22 to	250.00 to	88.67 to	298.85 to
El Cid	74.44 to	223.33 to	76.62 to	168.03 to
El Cid F1	85.55 to	205.00 to	63.49 to	188.83 to

DDT: Days After Transplantation

*: Means with the same letter are statistically equal (Tukey 0.05)

Table IV. Stomatal density of the upper and lower sides in different varieties and tomato graft.

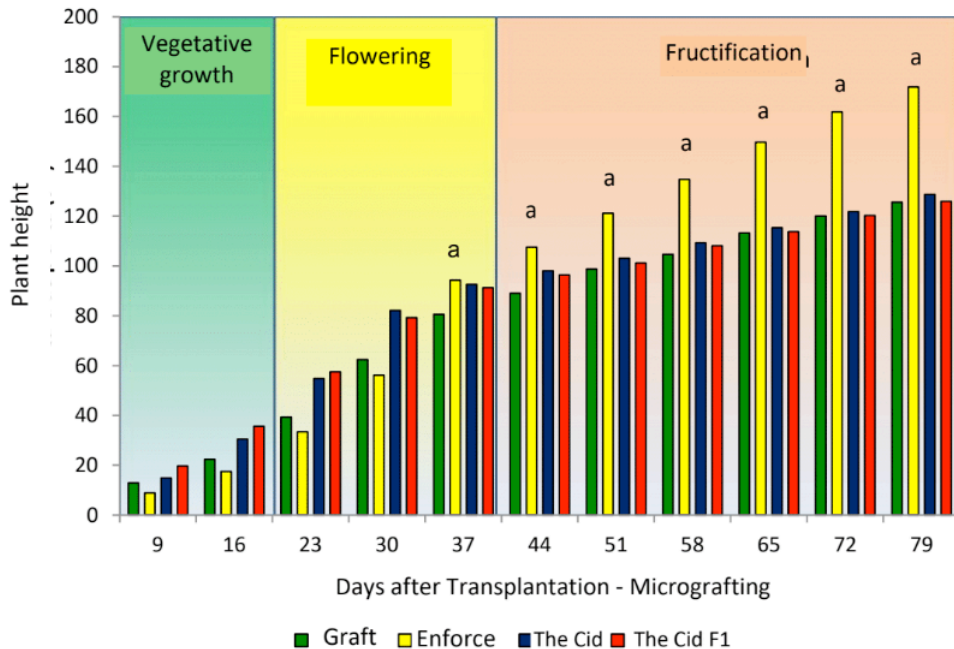


Figure 1. Plant height of different varieties and tomato graft

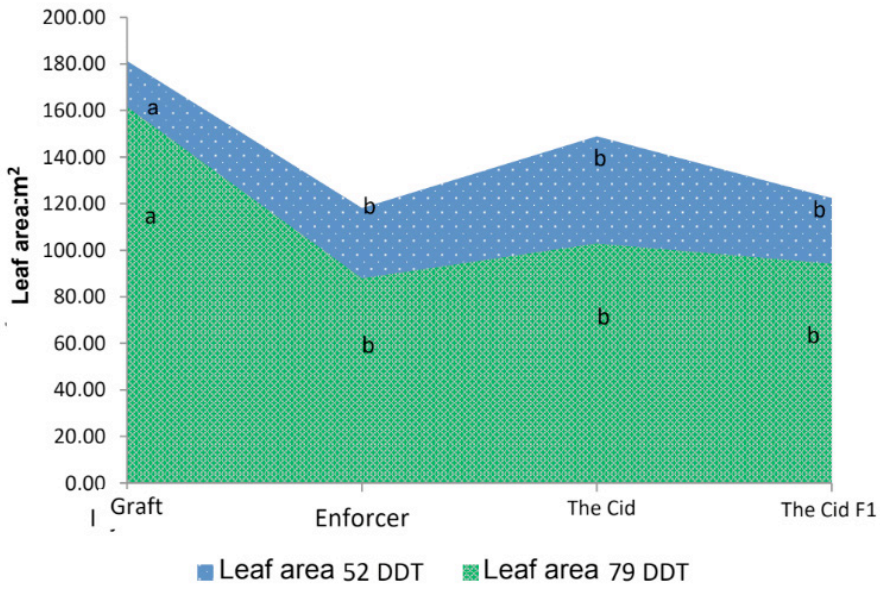


Figure 2. Leaf area of different varieties and tomato gr

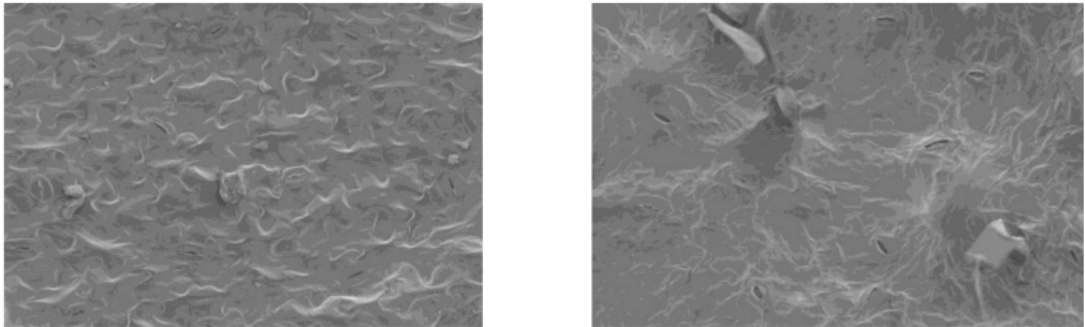


Figure 3. Stomata of the upper side (Left) and the lower side (Right) of the graft at 36 DAT

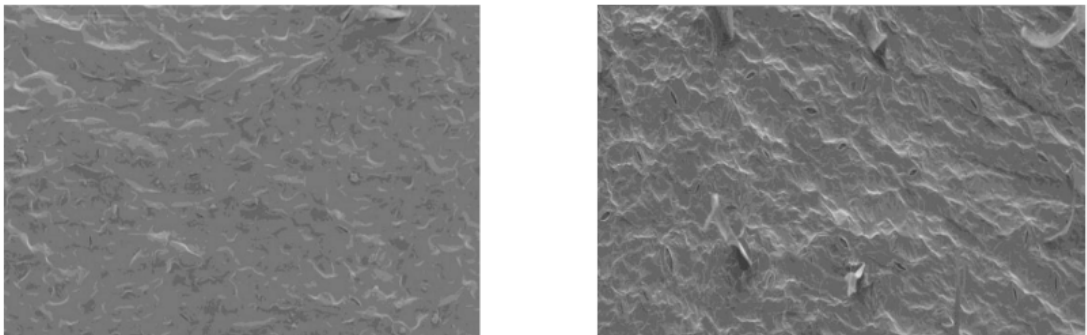


Figure 4. Upper (Left) and lower (Right) stomata of Enforcer at 36 DAT

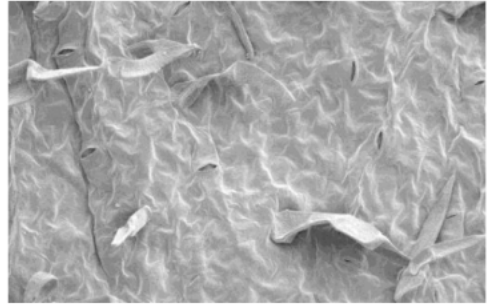
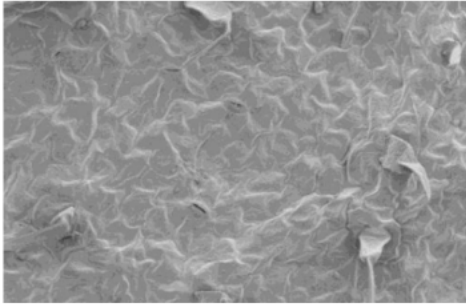


Figure 5. Stomata of the beam (Left) and the underside (Right) of the Graft at 79 DAT

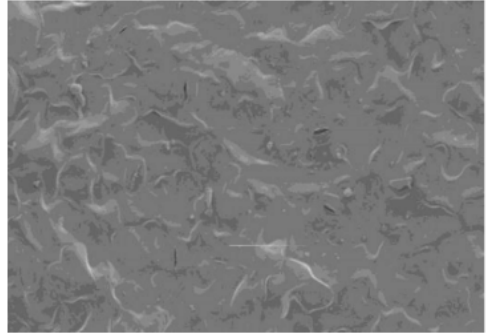
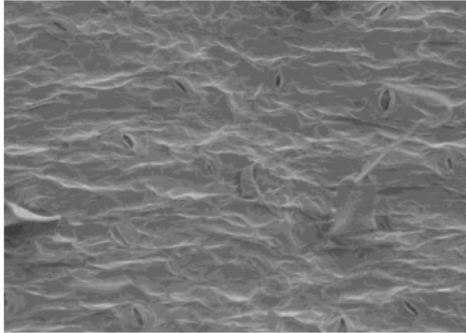


Figure 6. Upper (Left) and lower (Right) stomata of Enforcer at 79 DAT