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RESEARCH ARTICLE

Influence of pre-sowing red laser irradiation of tomato seeds on the initial plant development, salinity stress tolerance, and harvest yield

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ABSTRACT

This work discusses the laser irradiation effects on tomato seeds (Solanum lycopersicum L), regarding the initial plant development, salinity stress tolerance and harvest yield. The aim of this study was to find an optimal pre-sowing laser irradiation treatment to enhance tomato crop. A diode laser with wavelength of 660 nm and power 100 mW was used, 16 treatments were tested by 4 power densities (0.2, 0.4, 2 and 4 mW cm^{-2}) applied during 4 exposure times (15, 30, 60, 120 s) and a control group without treatment. The study was divided into three stages, firstly it was selected an optimal laser treatment (04 mW cm^{-2}, 30 s), in the second stage the optimal laser treatment was evaluated under salinity stress, and finally in the third stage the optimal laser treatment was evaluated on harvest yield. The optimal laser treatment, on the initial plant development enhanced germination by 10%, radicle growth by 19%, and hypocotyl growth by 13%, in comparison to the control. On seedlings under salinity stress, optimal laser treatment promote germination up to 20%, radicle growth up to 23%, and hypocotyl growth up to 12%. According to this study, saline stress inhibits PSII activity in tomato seedlings, whereas optimal laser treatment increased PSII activity around 71%. About greenhouse crop, optimal laser treatment improved mass produced by 26%. The present study shows a path for application of pre-sowing red laser radiation treatments in tomato seeds to improve development, saline stress tolerance and final production of tomato crop.

Keywords: Chlorophyll fluorescence, Harvest yield, Red laser irradiation, Saline stress, Optimal treatment.

INTRODUCTION

The implementation of various methods to improve the vigor of the seeds is a way to ensure the crop and its final production at harvest. The application of physical methods as laser radiation is an option to improve activity and performance of the seeds (Mohammadi *et al*. 2012).

The promotion of seed germination by light was observed in the nineteenth century, Borthwick et al., (1952), report the reversible regulation of lettuce seed germination by far red and red light. With low-intensity red laser irradiation phytochrome can be signaled (Swathy et al., 2021), phytochrome can influence morphogenesis processes comprising all light-dependent processes involved in plant growth and development (Balcerowicz et al. 2021). Laser irradiation is a proven technique that improves seed germination, plant growth and development, photosynthetic system, plant productivity and can improve resistance to disease and abiotic stress (Ćwintal and Dziwulska-Hunek, 2013; Mohammadi et al., 2012; Podleśna et al., 2015; Prośba-Białczyk et al., 2013; Sacała et al., 2012). Low-intensity diode laser radiation treatments in the field of agriculture are considered environmentally friendly (Qi et al., 2000).

Laser radiation treatments permit to precisely control the dose of radiation applied, this allows to analyze the effects of different laser radiation treatments in search of a treatment that improves the development of the different crops.

The application of an optimal pre-sowing laser treatment on tomato seeds could promote the production of the tomato crop. This study aimed to analyze the effects of different pre-sowing red laser irradiation treatments on

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tomato seeds to find an optimal treatment to enhance tomato crop yield.

MATERIALS AND METHODS

Research location

The research was developed in an experimental greenhouse in Guasave, Sinaloa, Mexico (5°42'42.0''N 108°40'30.2''W), from July 2020 to July 2021.

Experimental stages

The investigation included three basic stages, the first stage of the study defined the optimal laser radiation treatment, the second stage evaluated the optimal treatment under salinity stress, and finally the optimal treatment was evaluated on harvest yield (Fig 1).

Seeds characteristics

Hybrid tomato seeds *Solanum lycopersicon* L. type Roma (E 2634770 F1) were used. The seeds were supplied by Enza Zaden Mexico company.

Laser treatments

Before the radiation process, the seeds were immersed in distilled water for 60 minutes, then the excess water was removed and dried with filter paper, then laser radiation treatments were applied, the radiation process was according to Rivera-Talamantes et al., (2022) (Fig 1-A). A laser diode with a wavelength of 660 nm and 100 mW (LNCQ28PS01WW model) made in Japan was used. To find an optimal laser radiation treatment, 16 irradiation treatments were tested, by 4 powers of density (0.2, 0.4, 2 and 4 mW cm^{-2}) applied during 4 exposure times (15, 30, 60 and 120 s).

Plant parameters

The germination test was performed in Petri dishes, with three layers of filter paper as a substrate, moistened with distilled water (Kornarzyński et al., 2018; Podda et al., 2017). Twenty-five seeds per petri dish were used, four replicates for each treatment (Rodrigues-Junior et al. 2016; Podda et al. 2017; Badran and Savin 2018). The germination temperature was 22 ± 2 ° C (mean ± standard deviation), without light for selecting the optimal laser treatment and under controlled light to evaluate saline stress. Seedlings with atrophied primary roots, short, thick or spiral hypocotyls were considered abnormal germinations (Ashrafi and Razmjoo, 2015).

Vigor index values were calculated according to Mariappan et al. (2014).

$$V = G\ (\%).\ T$$

Where, V is vigor index; G is germination (%); T is total seedling length.

On the other hand, germination rate (GR), germination index (GI), germination energy (GE) and germination vigor index (GVI), were calculated according to Li et al., (2020).

$$GR = \frac{G_{10}}{N}.100\%;$$

$$GI = \sum \frac{G_t}{T};$$

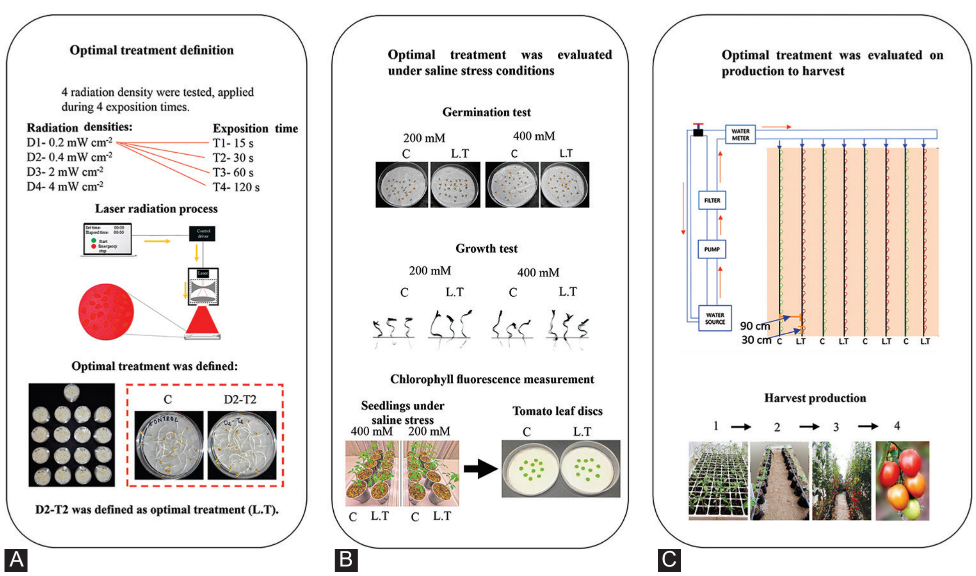

Fig 1. Basic steps of the research process. A: Definition of the optimal treatment under laser radiation, B: Evaluation of the optimal treatment under salinity stress, C: Evaluation of the optimal treatment on harvest.

$$GI = \sum \frac{G_t}{T};$$

$$GE = \frac{G_7}{N}.100\%;$$

$$GVI = \sum \frac{G_t}{T} \,.\, AFW\,;$$

T is the number of days after sowing, Gt is the total number of germinated seeds on the T^{th} day, G_7 and G_{10} are the total numbers of seeds germinated on the 7^{th} and 10^{th} days after sowing, N is the total number of seeds, and AFW is the average of fresh weight of seedlings.

Image capture and processing were implemented to calculate the length of seedlings (Tu et al. 2018). An EPSON V850 Pro scanner (manufactured in Indonesia) was used in the capture, the seedlings were placed over the scanning area accompanied by a graduated reference object, then the images were captured. The Image J software was used for image processing.

Salinity stress

Saline stress was with two solutions based on distilled water and NaCl (0.2 mol l^{-1} and 0.4 mol l^{-1}). The stress was maintained for four days, then it was removed, and the seeds were washed with abundant distilled water (Podda et al. 2017), subsequently they were sown in Petri dishes lined with three layers of filter paper moistened with distilled water.

Chlorophyll fluorescence measurement

In the measurement of chlorophyll fluorescence, a non-destructive quantitative diagnostic method was used according to photosynthetic activity parameters, which were expressed in arbitrary units and determined by the dynamic characteristics of the Kautsky curve (Borodin et al. 2008). The parameters of slow induction of chlorophyll fluorescence according to the maximal quantum yield of PSII (Fv/Fm) and the actual quantum yield of PSII (Yield), were estimated using a portable fluorometer LPT-5/CF model manufactured in Michurinsk Russia, by Michurinsk State Agrarian University into bio-photonics department (Budagovskaya and Budagovsky, 2015).

Ten seedlings were analyzed per treatment, the seedlings were affected by salinity (0.2 mol $l^{-1}$and 0.4 mol l^{-1}) for four days, afterward, ten leaf discs were prepared one per plant (Kasote et al., 2019) and placed in Petri dishes. It was used filter paper as substrate and distilled water to maintain moisture, the evaluation took place 4 and 6 days after applying the saline stress.

Greenhouse production

Seedlings were generated, in cavities of 3.6 x 3.6 cm with a capacity of 46 ml and drainage of 0.8 mm, using peat-type substrate mixed with vermiculite (PRO-MIX GTX) and watered with common tap water, the irrigation rate alternated daily. Seedlings were transplanted 33 days after planting. Twenty-one seedlings were used per group 4 replicates per treatment, they were transplanted into polyethylene bags with dimensions of 25x25x13 cm, with a mixed substrate of 50% peat sphagnum for commercial use (PRO-MOSS TBK, manufactured in Canada), 30% perlite and 20% vermiculite. Drip irrigation was used with nutrition according to the plant cycle based on Phosphorus, Calcium, Sulfur, Cobalt, Copper, Iron, Magnesium, Zinc, Molybdenum, Boron and UREA. The crop was developed entirely at the transplant site.

Statistical analysis

Data were statistically analyzed using STATISTICA software and tests of significant differences ANOVA and TUKEY HSD were applied (De Souza et al., 2022; Matsoukis et al., 2019). Significance levels were indicated: *a* ($p<0.05$) there is a statistical significance, *b* ($p<0.01$) there is strong statistical significance, *c* ($p<0.001$) there is very strong statistical significance.

RESULTS AND DISCUSSION

Optimal treatment selection by analysis of initial plant development

The radiation parameters were defined based on an analysis of the influence of different treatments on tomato seeds. The germination and growth rates were analyzed in tomato seedlings during the first days of their development. Seed germination and seedling development are essential stages in the plant growth cycle and are the most critical stages in plant life (Walck et al., 2011).

After analysis, it was evident that the treatment at 0. 4mW cm^{-2}, for 30 seconds, generated the best results respect to the control (C), improved germination (10%) and radicle (19%, $P<0.01$) and hypocotyl growth (13%; $P<0.01$), in general, the vigor index of the seeds increased 30% ($P<0.01$) (Fig. 2-D). As a result, the treatment at 0.4 mW cm^{-2} in a time of 30 s, was defined as optimal treatment. For representation in the development of the research, we will relate it to the acronym L.T.

According to Sacała et al. (2012), the effects of laser stimulation of seeds are revealed at all stages of plants' development but are especially evident in the early stage of growth. Red laser irradiation in tomato seeds, can improve the initial plants' development and increase the size of the seedling, mainly in the radicle part.

Laser stimulation on Salt stress resistance

Saline stress affect physiological and biochemical processes in plants that adversely affect their growth and development (Muchate et al., 2016). Red light can be used as a signaling mechanism for phytochromes (Kim et al., 2002). Some researchers have shown that red laser radiation treatments on seeds can improve their tolerance to salinity, this mechanism is based on the fact that a part of the phytochrome family improves tolerance to salt stress in seeds and plants. (Gavassi et al. 2017; Yang et al. 2018; Cao et al. 2018).

In seeds under saline stress, the improvement by L.T. was visually appreciable, improved the homogeneity and normality of germination, and significantly improved the kinetics of germination (Fig. 3-A, B).

Saline stress reduced the percentage of normal germinations (N), and the optimal laser treatment suppresses saline stress affects, by 20% for 0.2 mol l^{-1} and 18% for 0.4 mol l^{-1}, respect to the control (Table 1).

Normally saline stress reduces the vigor and potential of the seeds on this way affects the plant's development. The optimal laser treatment increases the vigor index 46% for 0.2 mol l^{-1} and 42% for 0.4 mol l^{-1} concerning the control (Table 1).

In seedlings affected by salinity, L.T improved radicle and hypocotyl growth, for 0.2 mol l^{-1} the improvement was 23% ($P<0.01$) for radicle and 12% ($P<0.01$) for hypocotyl, in the same way in seedlings affected by 0.4 mol l^{-1} of saline stress, the improvement was 13% ($P<0.01$) for radicle and 12% ($P<0.01$) for hypocotyl (Table 1).

According to Li et al. (2020), optimal laser treatment (L.T) exhibited large differences on germination analysis. Germination energy (GE), increased by 35% for 0.2 mol

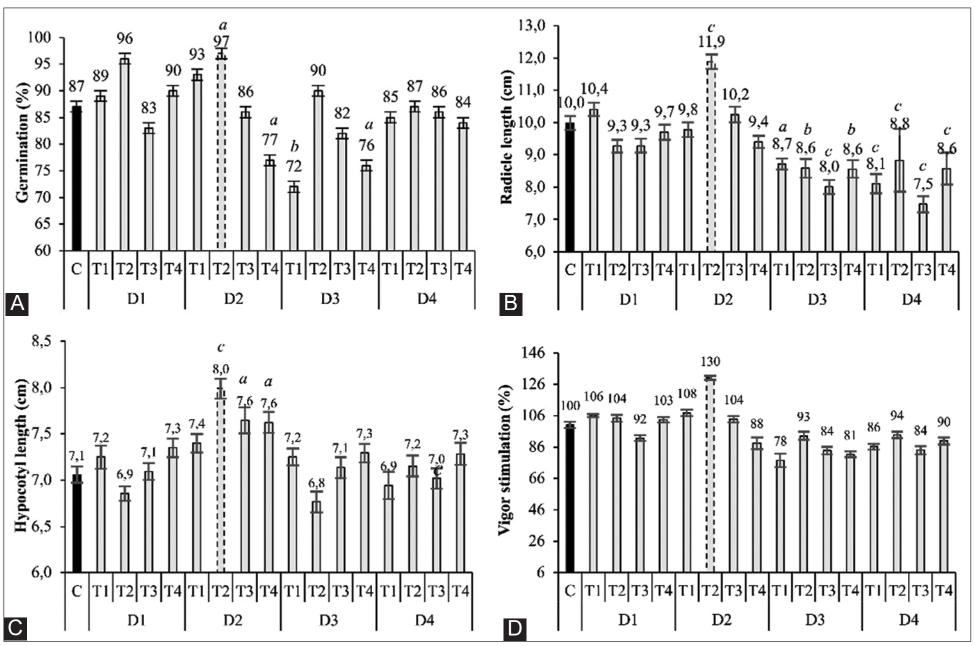

Fig 2. Effects of pre-sowing red laser treatments on the initial plants' development of tomato seedling. A) germination, B) radicle, C) hypocotyl, D) vigor stimulation. C: control, D1: 0.2 mW cm^{-2}, D2: 0.4 mW cm^{-2}, D3: 2 mW cm^{-2}, D4: 4 mW cm^{-2}; T1: 15 s, T2: 30 s, T3: 60 s, T4: 120 s, (Mean value ± SE of the mean). *a*: statistically significant differences ($P<0.05$), *b*: strong statistically significant differences ($P<0.01$), *c*: very strong statistically significant differences ($P<0.001$).

Table 1: Biometric measurements of seedlings from seeds pretreated with red laser radiation (04 mW cm^{-2}, 30 s), affected by saline stress (Mean value±SE).

Treatment	Vigor	Seedling growth		Germination				
	Index	RL	HL	N	AN	GI	GE	GVI
			0.2 mol l^{-1}					
C	340	1.3±0.05	3.3±0.06	74±1.25	13±0.47	57	59	40
L.T	498	1.6±0.03[b]	3.7±0.05[b]	94±0.02[b]	3±0.47	69	81	61
			0.4 mol l^{-1}					
C	326	1.5±0.05	3.3±0.04	68±0.60	18±0.96	43	24	33
L.T	464	1.7±0.03[b]	3.7±0.02[b]	86±0.50[a]	5±0.50	59	60	65

AN: abnormal germination, GE: germination energy, GI: germination index and GVI: germination vigor index, HL: hypocotyl length (cm), N: normal germination (%), RL: radicle length (cm). *a*: statistically significant differences ($P<0.05$), *b*: strong statistically significant differences ($P<0.01$).

Table 2: Effects of pre-sowing laser treatment (04 mW cm-2, 30 s), on Fv/Fm and *yield* in seedling under saline stress (Mean value±SE)

Treatment	0.2 mol l-1		0.4 mol l-1	
	Fv/Fm	Yield	Fv/Fm	Yield
		4 days after		
		Without saline stress		
C	0.558±0.053	0.497±0.049	0.558±0.053	0.497±0.049
L.T	0.535±0.043	0.483±0.051	0.535±0.043	0.483±0.051
		With saline stress		
C	0.509±0.022	0.475±0.014	0.518±0.031	0.483±0.037
L.T	0.515±0.014	0.465±0.014	0.494±0.026	0.454±0.027
		6 days after		
		Without saline stress		
C	0.544±0.041	0.484±0.048	0.544±0.041	0.484±0.048
L.T	0.618±0.038	0.579±0.039	0.618±0.038	0.579±0.039
		With saline stress		
C	0.285±0.040	0.263±0.026	0.297±0.022	0.258±0.004
L.T	0.485±0.015^b	0.459±0.013^b	0.321±0.016	0.254±0.021

Fv/Fm: represents the maximal quantum yield of PSII. Yield: represents the actual quantum yield of PSII. *b*: strong statistically significant differences (P<0.01).

l-1, and 150% for 0.4 mol l-1, also had a strong impact on germination index (GI), 20% for seeds affected for 0.2 mol l-1, and 37% for 0.4 mol l-1 (Table 1).

In terms of germination vigor index (GVI), laser treatments improved by 52% for the 0.2 mol l-1 group, and 95% for the 0.4 mol l-1 group (Table 1). An alternative and hypothetical explanation that requires evidence is that red light is improving the percentage of final germination (Costa et al. 2016).

In canola (Brassica napus) seeds under saline stress conditions, laser irradiation could be useful for improving rapeseed yield (Mohammadi et al., 2012).

Chlorophyll fluorescence analysis is a bioanalytical tool used to quantify stress by contaminant in plants, on this way it's an effective tool for determining the photosynthetic capacity of plants, on normal or stress conditions, including saline stress (Salim Akhter et al., 2021; Bhagooli et al., 2021). The present study showed that saline stress directly affects the photosynthetic apparatus of tomato plants, Fv/Fm and yield levels decrease, the effect magnitude will be proportional to the saline stress level applied to the plant (Table 2).

PSII levels of plants treated with red laser radiation were generally higher compared to control (Table 2), on day 6, plants affected by 0.2 mol l-1 of saline stress, showed a significant improvement in Fv/Fm (71%; P<0.01) and yield (74%; P<0.01), compared to control.

The response in PSII is related to the functioning of the photosynthetic apparatus of plants (Guidi et al. 2019), improving the response of the PSII reflects an

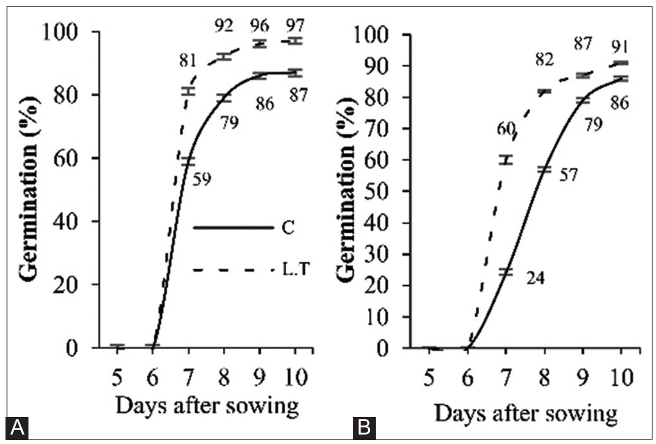

Fig 3. Germination dynamics of seeds affected by saline stress after red laser radiation treatment (04 mW cm-2, 30 s). A) 0.2mol l-1; B) 0.4 mol l-1stress.

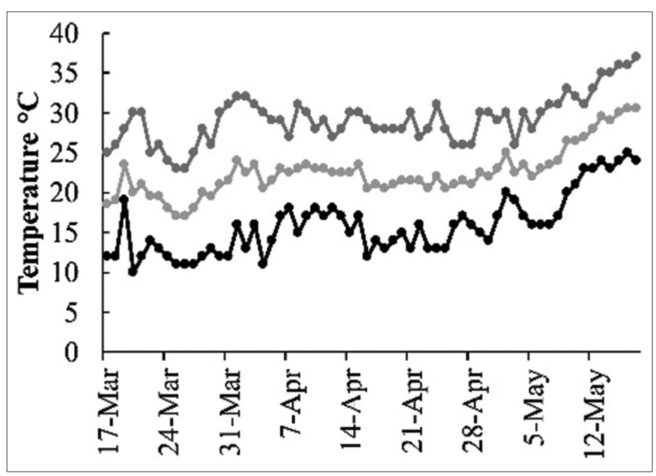

Fig 4. Temperature at the greenhouse. Daily maximum (dark grey), minimum (black), and average (grey) temperatures.

improvement in the photosynthetic apparatus, this can positively influence the development of the plant under normal or stress conditions.

For 0.4 mol l^{-1} no significant differences were found (Table 2), this may be a consequence of exceeding the tolerance levels of the tomato plants to saline stress.

Red laser radiation treatment in tomato seeds, improve tolerance to saline stress in germination rate, growth rate and increase photosynthetic activity (PSII), it is hypothesized that red laser treatment on seeds pre-sowing, could be correlated with an improvement in the final production of tomato crop under saline stress conditions.

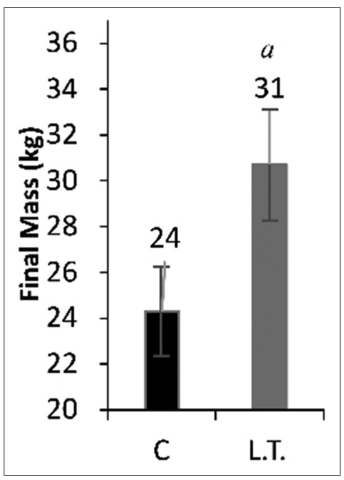

Fig 5. Mass produced on harvest production. (C, control; L.T, laser treatment). *a*: statistically significant differences (P<0.05).

Laser stimulation harvest yield

About environmental conditions, the temperature inside of greenhouse was monitored since first day of harvest, the minimum temperature was 10 ºC and maximum 36 ºC (F*i*g. 4). The temperature is an important parameter that affects production and quality of tomato (Kittas et al., 2009).

Laser radiation treatment increased the total mass-produced (Fig. 5), Consequently, final production increased significantly (29%) compared to control. Dziwulska-Hunek et al. (2020), in the cultivation of corn, found that red light pre-treatments can increase the quantity and quality of yield.

Laser radiation treatment increased the mass produced each harvest day (Fig. 6-A), this reflected a significant increase (29%, P<0.05) in the accumulated final mass (Fig. 6-B). Concerning to the number of tomatoes produced (Fig. 6-C), only on the third day significant differences were found (30%, P<0.05) compared to the control.

The tomatoes produced were evaluated by longitudinal and transverse measurements (Fig.7), the first day of harvest, L.T transversal diameter of the fruits was increase (P<0.05).

Incident light quality during the development of a plant is a crucial element, light is the main source of energy for photosynthesis, photomorphogenesis and has a direct impact on the development and performance of plants (Li et al. 2021). Literature reports indicate that pre-sowing laser radiation treatments on seeds could be generate

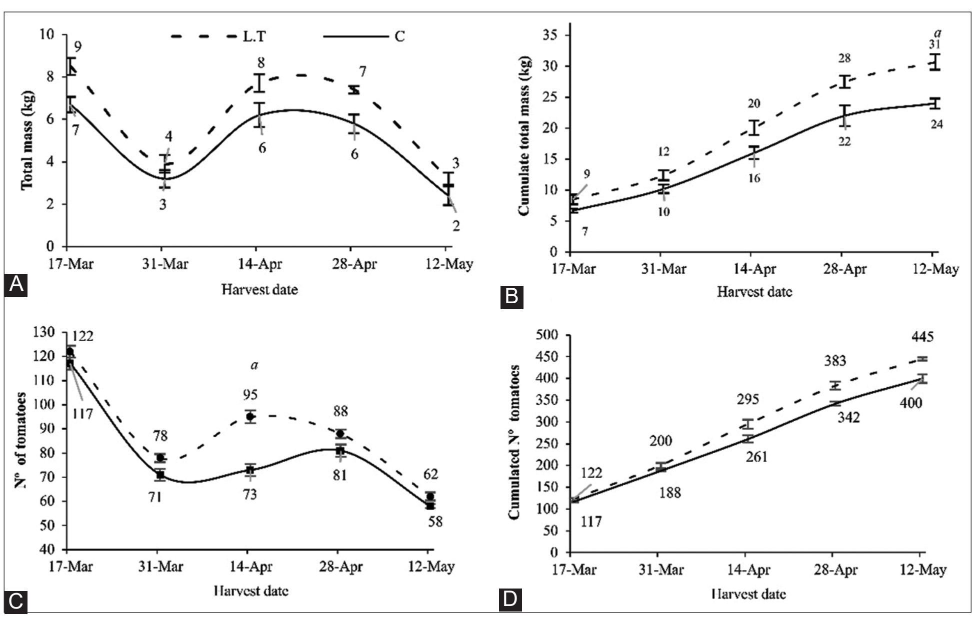

Fig 6. Production per harvest day. A) Total mass, B) Cumulate total mass, C) Number of tomatoes, D) Cumulate number of tomatoes. *a*: statistically significant differences (P<0.05).The tomatoes produced were evaluated by longitudinal and transverse measurements (Fig.7), the first day of harvest, L.T transversal diameter of the fruits was increase (P<0.05).

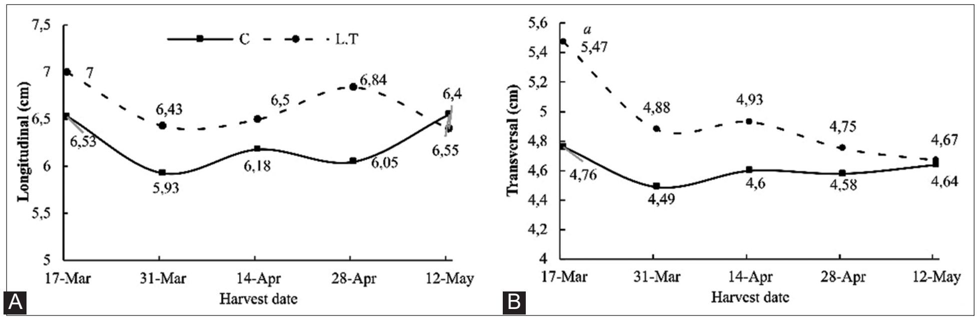

Fig 7. Tomato fruit length. A) transversal diameter and B) longitudinal diameter. *a*: statistically significant differences (P<0.05).

directly affect crop development. Dziwulska-Hunek et al. (2020), found that red light treatment on corn seeds before sowing, increase the quantity and quality of yield of corn crop, red light improved the length of corn cobs which consequently increased the weight produced. On the other hand, there is evidence that with supplementary red LED light it is possible to improve the development and final production of tomato crop, Paucek et al. (2020), reported that with supplementary red led light during the development of the tomato crop it is possible to improve the final production of their crop. In the present study the selection of optimal red laser treatment by the analysis on the initial tomato plants development, resulted in significant increase on crop yield (P<0.01), on this way, the definition of an optimal pre-sowing red laser treatment by analysis of the effects on the initial plant development, is a right way to select a red laser treatment that improves tomato crop yield, under controlled conditions in greenhouse.

The laser light treatment used in this work in the context of the radiation technique used, being a physical method is an applicable treatment to organic agriculture.

CONCLUSIONS

The study of red laser treatments at different power densities (0.2, 0.4., 2 and 4 mW cm^{-2}) and different exposure times (15, 30, 60, 120 s) on the initial plants development allows to find an optimal treatment that improve germination (10%), radicle growth (19%) and hypocotyl growth (13%). Optimal red laser treatment (0.4 mW cm^{-2} applied for 30 seconds) increases saline stress tolerance on vigor index (46%, 0.2 mol l^{-1}; 42%, 0.4 mol l^{-1}), speed and final percentage of germination (18%, 0.2 mol l^{-1}; 20%, 0.4 mol l^{-1}), radicle growth rate (23%, 0.2 mol l^{-1}; 13%, 0.4 mol l^{-1}) and hypocotyl growth rate (12%, 0.2 mol l^{-1}; 12%, 0.4 mol l^{-1}). Saline stress suppressed PSII activity, on this way optimal laser treatment improves photosynthetic activity up to 70%. An optimal treatment can enhance the number and size of the fruits produced, and harvest yield in 29%.

Conflict of interest

The authors do not declare any conflict of interest.

Acknowledgments

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Authors contribution

Alexandre Mitchenko and Carlos Rivera Talamantes conceived and designed the experiments; Carlos Rivera Talamantes, Gabriela González López carried out– experiments; Carlos Rivera Talamantes, Gabriela Gonzalez Lopez, Andrei V. Budagovsky and David Correa Coyac analyzed the data; Carlos Rivera Talamantes, Alexandre Mitchenko and Gabriela González López, Juan Acosta Castro wrote the article.

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