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Effect of salicylic acid on pre-transplant acclimatization of native tomato populations

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ABSTRACT

The aim of this study was to evaluate the effect of salicylic acid (SA) application on pre-transplant acclimatization of native populations of tomato. SA is a growth regulator that modifies plant growth and development by inducing changes in cell processes, physiology and morphology. Five populations of native tomato were sown in polystyrene trays. Peat moss was used as substrate and plants were maintained at field capacity continuously. After emergence, seedlings were applied during three weeks with different concentration of SA (0.0, 0.01, 0.1, 0.5 and 1.0 μM). A completely random experimental design was used with five replications per treatment. The growth parameters evaluated were height, stem diameter, number of leaves, hypocotyl length, shoot fresh and dry weight (leaves and stem), and root length and fresh and dry weight. An analysis of variance was carried out, and means were compared with the Tukey test (5%) using SAS statistical software. The recorded data show that pre-transplant seedlings of each of the evaluated populations responded significantly ($P=0.01$) to the SA treatments. Also, the comparison of means of each of the factors under study showed positive changes. With the concentrations of 0.5 and 0.1 μM SA, higher values of the studied variables were obtained than with the concentrations 0.01 and 1.0 μM SA. The native tomato populations sprayed with SA at concentrations of 0.5 and 0.1 μM responded positively in terms of seedling growth and development. Based on these findings, SA treatments can help acclimatize and present better growth conditions to the seedlings before being transplanted.

Keywords: *Lycopersicon esculentum*, stress tolerance, chlorophyll, carotenoids, growth regulators.

RESUMO

Efeito do ácido salicílico na aclimatização pré-transplante de populações de tomate nativo

O objetivo deste estudo foi avaliar o efeito da aplicação do ácido salicílico (SA) na aclimatização pré-transplante de populações nativas de tomateiro. SA é um regulador de crescimento que modifica o crescimento e desenvolvimento das plantas, induzindo mudanças nos processos celulares, na fisiologia e morfologia das plantas. Cinco populações de tomate nativo foram semeadas em bandejas de poliestireno. Usou-se turfa como substrato. As plantas foram mantidas em capacidade de campo continuamente. Após a emergência, aplicou-se nas plântulas, durante três semanas, diferentes concentrações de SA (0,0; 0,01; 0,1; 0,5 e 1,0 μM). O delineamento experimental foi inteiramente casualizado, com cinco repetições por tratamento. Os parâmetros de crescimento avaliados foram altura, diâmetro do caule, número de folhas, comprimento do hipocótilo, massa fresca e seca (folhas e caule) e comprimento da raiz e peso fresco e seco. Os dados foram submetidos à análise de variância e as médias foram comparadas pelo teste de Tukey (5%) usando o software estatístico SAS. Os dados registrados mostram que plântulas pré-transplante de cada uma das populações avaliadas responderam significativamente ($P=0,01$) aos tratamentos com SA. Ainda, a comparação das médias de cada um dos fatores em estudo mostrou mudanças positivas. Com as concentrações de 0,5 e 0,1 μM SA, foram obtidos valores mais elevados das variáveis estudadas do que com as concentrações 0,01 e 1,0 μM SA. As populações de tomate nativas pulverizadas com SA em concentrações de 0,5 e 0,1 μM responderam positivamente em termos de crescimento e desenvolvimento de plântulas. Baseado nesses resultados, os tratamentos SA podem ajudar a aclimatar e apresentar melhores condições de crescimento às mudas antes de serem transplantadas.

Palavras-chave: *Lycopersicon esculentum*, tolerância ao estresse, clorofila, carotenóides, reguladores de crescimento.

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Production using transplanted seedlings of vegetable crops is an important method for vegetable growers. Healthy seedlings ensure the successful development of the plants and ultimately result into more profits for the farmer (Gupta *et al.*, 2012). The

horticultural sector is experiencing an increasingly marked trend toward early or out-of-season production, seeking market niches that allow growers to obtain better sales prices and improve their economic capacity. Advances in transplant, such as the use of special

substrates, fertilization programs for seedlings, multiple cavities plug trays, high value hybrids and the use of greenhouses have contributed to the growth of the industry, by increasing the security of crops (Samaniego *et al.*, 2002). In this sense, it is expected

that the production and consumption of certain vegetables such as tomatoes will continue to increase and, to address the sustainability of this sector, intensive research should be carried out to develop varieties with better agricultural characteristics and the maximization of seedling technology (Gerszberg & Hnatuszko-Konka, 2017).

The application of compounds known as growth promoters or bioregulators is one of the strategies increasingly used in agricultural practice to stimulate the development of plants and increase their resistance to abiotic and biotic stresses (Bulgari *et al.*, 2014) improving the quality and health of plants. Plants employ many strategies in response to abiotic stresses that ultimately enhance the plant growth and productivity in stressful environments. These phenomena include change in morphological and developmental pattern (growth plasticity) as well as physiological and biochemical processes against several stresses (Tuteja, 2007).

Plant hormones play vital roles in the ability of plants to acclimatize to varying environments by mediating growth, development and nutrient allocation (Fahad *et al.*, 2015). Salicylic acid (SA) is a plant hormone that not only mediates plant defense responses against biotic and abiotic stresses but also plays a crucial role in regulating many physiological and biochemical processes during the entire plant lifespan (Zhang *et al.*, 2017) as well as multiple aspects of plant growth and development, including seed germination, vegetative growth, flowering, fruit yield, senescence, root initiation/growth (Khan *et al.*, 2015) some of these processes are induced by SA in a concentration-dependent manner, as they are activated by treatment with a low dose of SA and inhibited by a high dose (Dempsey & Klessig, 2017).

Induction of a larger number of roots in tomato seedlings is fundamental in the pre-transplant stage since they depend on adequate uptake of water and nutrients for later development (Larqu e-Saavedra & Mart n Mex, 2007). On the other hand, Larqu e-Saavedra *et al.* (2010) reported similarly favorable effects

on the vegetative growth of tomato seedlings (*Lycopersicon esculentum*) when sprinkled with low concentrations of SA (0.0001, 0.01, 1.0 μM), also saw that the concentration of 1.0 μM improves the size of the stem and the length of the roots. So, the application of SA to native tomato seedlings could be a strategy to improve the quality and health of the seedling, improving its morphological and physiological characteristics. For this reason, the aim of this study was to evaluate the effect of SA application on seedlings native populations of tomato by measuring the growth parameters.

MATERIAL AND METHODS

The experimental work was conducted during the summer 2016 on the experimental field of the Laboratorio de Biotecnolog a Vegetal de la Facultad de Ingenier a y Ciencias at the Universidad Aut noma de Tamaulipas, located in the municipality of Victoria, Tamaulipas, Mexico (23°42'52"N, 99°9'12"W, 316 m altitude).

The treatments were distributed in an experimental design of random blocks with five replications per treatment; one seedling was the experimental unit. The treatments consisted of five concentrations of SA [0 (control), 0.01, 0.1, 0.5 and 1 μM] and five native tomato populations (LOB2, LOB4, LOB5, LOB6, LOB8), the combination of these were 25 treatments and 125 experimental units in total, a seedling was a repetition. The salicylic acid solutions (Merck) used in the present study were prepared following the methodology defined by Guti rrez-Coronado *et al.* (1998).

Seeds from five native tomato populations (25 of each populations) from the states of Puebla and Hidalgo, Mexico were sown (Table 1). These seeds were provided by Dr. Ricardo Lobato Ort z, specialist in the collection of tomato genetic material plant genetic resources and plant breeding of tomato and related species as well as the use of Mexican native tomato landraces and wild species as a source of new allele combinations for biotic and

abiotic stresses and other traits related whit yield and quality. Seeds were germinated in polystyrene trays with peat moss substrate in blocks of 5 x 5 for a total of 25 cavities for each population and a total of 125 seeds for the whole experiment. One seed was sown per cavity and moistened with 100 mL water. The tray was then covered with black polyethylene bags to simulate a germination chamber and placed on metal tables where they were maintained and supervised during the experiment. On the third day after sowing (DAS), the bag was removed from the trays once its objective of stimulating germination had been fulfilled. From the fourth day on, the seedlings were hydrated only with water for 15 days.

From day 16 on (seedlings with 10 cm height and two expanded leaves) the seedlings were sprayed with concentrations of SA (0 control, 0.01, 0.1, 0.5 and 1 μM) for 21 days, each 3 days. Time of application was between 7:00 and 8:00 hours while the seedlings were in the trays. The nozzle of the hand-held sprayer was adjusted to deliver 0.5 mL of solution per spray and two shots were done so that each plant received 1 mL of SA (for each one of the concentrations) or distilled water for control. The application was direct, being all foliage sprayed. SA spraying with an atomizer was alternated with auxiliary irrigation (100 mL water) to maintain the level of moisture of the substrate in the trays, according to the visual quality of the plants and the climatic conditions.

At 36 DAS (time required to perform a transplant), the following parameters were determined for each one of the seedlings, according to the International Plant Genetic Resources Institute (IPGRI, 1996) tomato descriptor: CH= color of hypocotyl; CIH= color intensity of the hypocotyl; HP= hypocotyl pubescence; LPL= length of the primary leaf (cm); PLWd= primary leaf width (cm); PH= plant height (cm); SD= stem diameter (cm); NL= number of leaves; LL= leaf length (cm); LWd= leaf width (cm); RL= root length (cm); RV= root volume (mL); LWg= leaf weight (mg); SWg= stem weight (mg); RW= root weight (mg); Chla= chlorophyll

a; Chlb= chlorophyll b; TotCar= total carotenoids. Plant height was measured from the base of the trunk to the first terminal flower with a graduated ruler. Stem diameter was measured with a manual Vernier at 5 cm from the stem base. Root volume was evaluated by displacement of volume in water. The photosynthetic pigments, chlorophyll a, b and total carotenoids were quantified according to the methodology reported by Lichtenthaler (1987).

Data were submitted to the analysis of variance (F test) and the Tukey test of comparison of means (5%), using the statistical program SAS.

RESULTS AND DISCUSSION

The data analysis of variance show that the seedlings from native tomato populations respond significantly to SA treatments in all growth parameters (Table 2). The differences among populations, SA application and the interaction populations x SA application are visible in morphological parameters such as seedling height, root length, root volume, root weight principally ($P \leq 0.01$) and for length, width and leaf weight, stem weight, and photosynthetic pigments as chlorophyll a, chlorophyll b and total carotenoids (5%). Thus the response to exogenous SA application is specific for each plant. Usually, SA at relatively low concentrations enhanced,

Table 1. Collection of native tomatoes from the states of Puebla and Hidalgo. Mexico, Universidad Autónoma de Tamaulipas, 2016.

Identification	Growth habit	Local name	Municipality	State
LOB2	Indeterminate	Arriñonado	Cuetzalan	Puebla
LOB4	Indeterminate	Arriñonado	Cuetzalan	Puebla
LOB5	Indeterminate	Chino criollo	Tehuacán	Puebla
LOB6	Indeterminate	Chino criollo	Tehuacán	Puebla
LOB8	Indeterminate	Ojo de venado	Zacualtipán	Hidalgo

LOB refers to Dr. Ricardo Lobato Ortiz, who provided the seed.

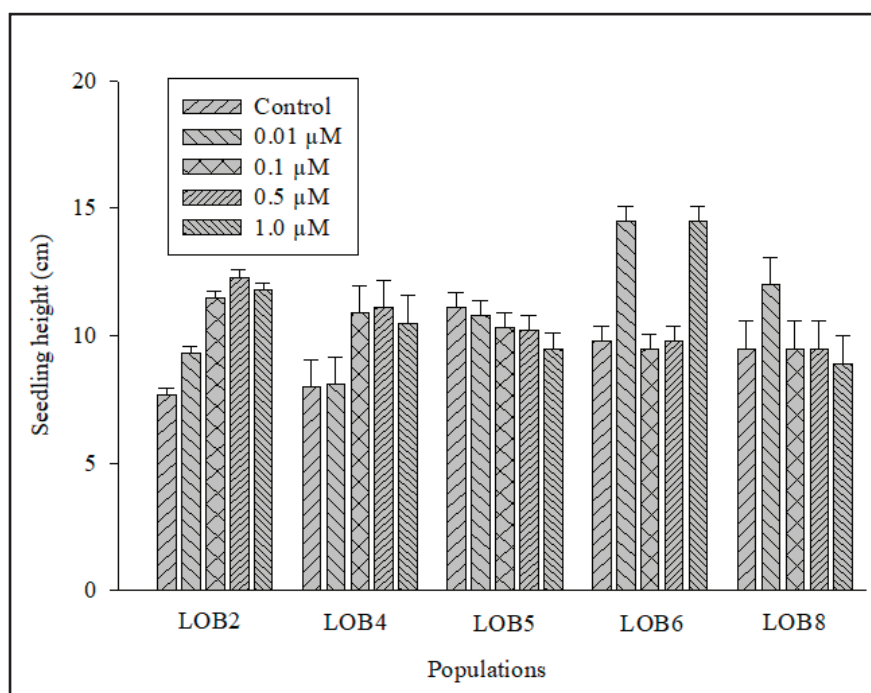


Figure 1. Height of seedlings (cm) from native tomato populations sprayed with different concentration of SA. Mexico, Universidad Autónoma de Tamaulipas, 2016.

Table 2. Analysis of variance of the growth parameters, based on the effect of salicylic acid (SA) on seedlings from native tomato populations (P). Mexico, Universidad Autónoma de Tamaulipas, 2016.

FV	CH	CIH	HP	LPL	PLWd	PH	SD	NL	LL
P	**	**	**	**	**	**	*	*	**
SA	ns	ns	ns	ns	ns	**	ns	ns	*
P*SA	ns	ns	ns	ns	ns	**	ns	ns	*
CV (%)	14	25	35	14	25	22	31	26	36
	LWd	RL	RV	LWg	SWg	RWg	Chl _a	Chl _b	TotCar
P	**	**	**	*	*	**	*	*	*
SA	*	**	**	*	*	**	*	*	*
P*SA	*	**	**	*	*	**	*	*	*
CV (%)	34	15	32	31	11	21	16	32	32

ns; *, **: Not significant and significant ($P \leq 0.5, 0.1$ respectively); CH: color of hypocotyl; CIH: color intensity of the hypocotyl; HP: hypocotyl pubescence; LPL: length of the primary leaf; PLWd: primary leaf width; PH: plant height; SD: stem diameter; NL: number of leaves; LL: leaf length; LWd: leaf width; RL: root length; RV: root volume; LWg: leaf weight; SWg: stem weight; RWg: root weight; Chl_a: chlorophyll a; Chl_b: chlorophyll b; TotCar: total carotenoids.

and at relatively high concentrations decreased growth in diverse plant species (Rivas-San Vicente & Plasencia, 2011). Moreover, the differences are due mainly to their geographic origin and type of tomato which are morphologically different (Table 1). In this sense Dempsey & Klessig (2017) mention that this type of conduct is normal, given the wide range in basal SA levels between (and even within) plant species. The effect of SA on seedlings

morphology is the result of many physiological processes that favor said expression at the level of the organism. It is perhaps not surprising that conflicting reports have been published concerning the effect of exogenously supplied SA on various plant processes (Dempsey & Klessig, 2017). It is important to conduct experiments to solve some methodological issues, such as the number of applications necessary to produce the desired effects since in our

study SA was applied seven times, while in the comparative study of Yildirim & Dursun (2009) SA was applied four times. Also, it is necessary to define the quantity of SA that penetrates the tissue and produces cascade effects that result in morphological changes that can be quantified and to define whether there is one or more receptors of SA since no studies report this in the literature.

Height was modified by both genotype and SA application (Figure 1). Within and between the native populations there were differences in seedling height. Significant differences among populations due to level of SA application were also found (Table 2). The highest seedling height was presented in LOB6 (14.5 cm) with the concentrations 0.01 μM SA and 1.0 μM SA, followed by LOB2 (12.3, 11.8, 11.5 cm) with concentrations 0.5, 1.0 and 0.1 μM SA respectively and LOB8 (12 cm) with the concentration 0.01 μM SA. In LOB2 the concentration of 0.5 μM SA led to an increase in height of up to 38% compared to the control, which received only distilled water (Figure 1). Similar results were reported by Larqu e-Savedra *et al.* (2010). Although not all treatments followed the same trend, there were exceptions such as the case of LOB8 which, with low doses (0.01 μM) of SA, presented seedlings up to 30% taller than those in high-dose (1.0 μM) of SA treatments. In this sense, Dempsey & Klessig (2017) indicate that the levels of SA vary greatly even within the same species of plant, depending on subcellular location, tissue type, developmental stage, and with respect to both time and location after an environmental stimulus and that this provides a tremendous flexibility and multiple mechanisms through which the SA can act in the plants.

Chlorophyll a, chlorophyll b and total carotenoids content was different in seedlings of tomato native populations treated with different concentrations of SA (Figure 2). A clear difference among native populations is observed; the populations with the highest content of these photosynthetic pigments were LOB2 and LOB8. The rest of the populations (LOB4, LOB5

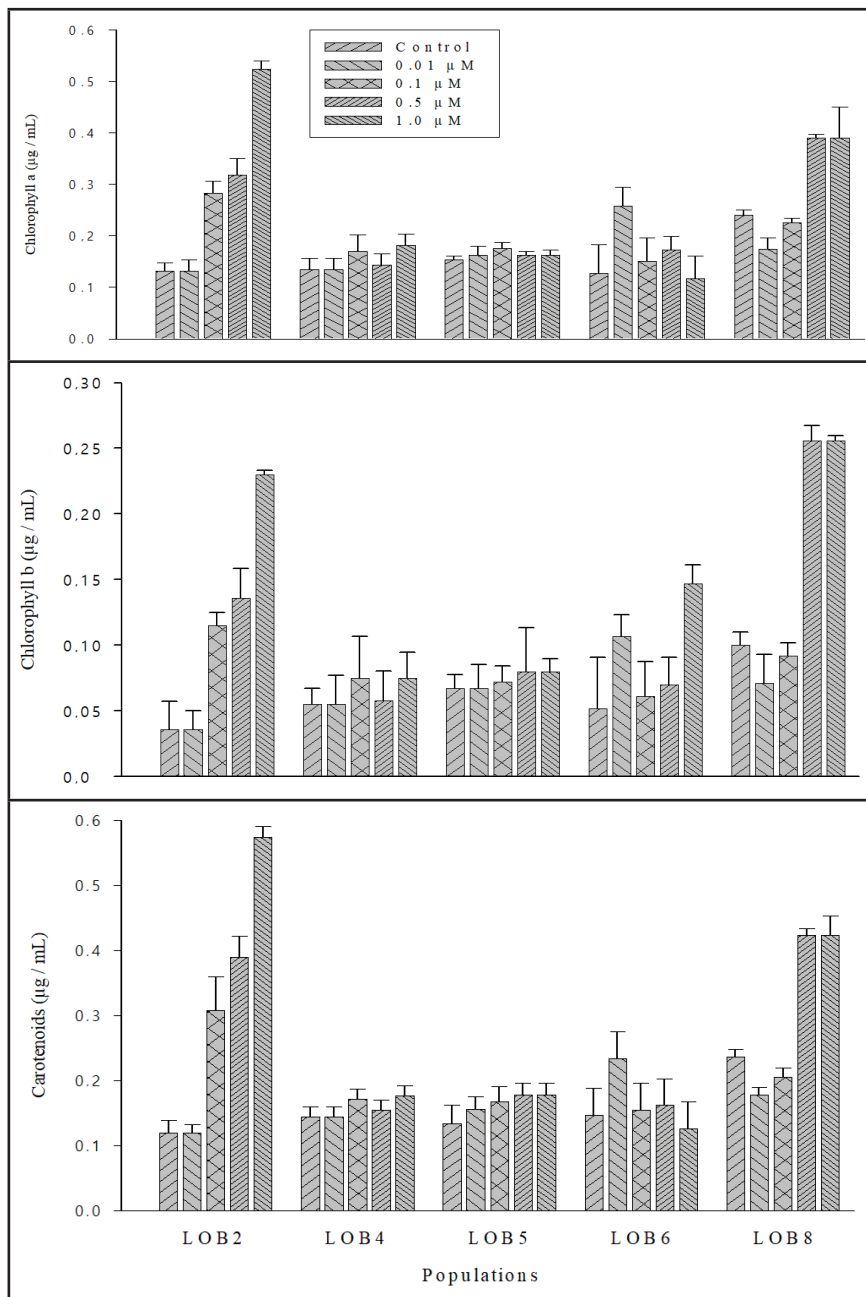


Figure 2. Content of chlorophylls a, b and total carotenoids in seedlings from native tomato populations in function of the effect of salicylic acid. Mexico, Universidad Aut noma de Tamaulipas, 2016.

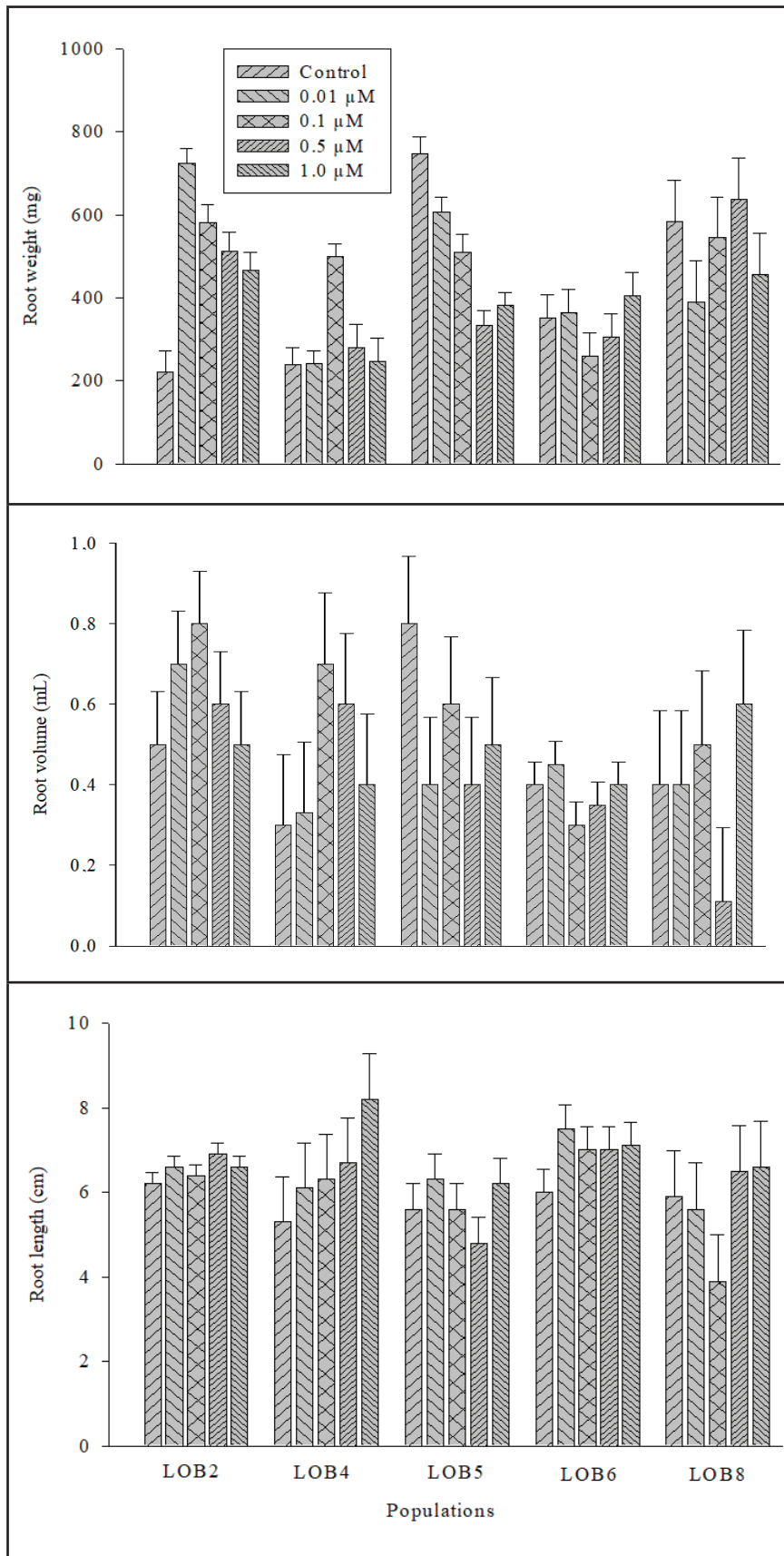


Figure 3. Root weight (mg), root volume (mL) and root length (cm) in tomato populations sprayed with different concentration of SA. Mexico, Universidad Autónoma de Tamaulipas, 2016.

and LOB6) did not have the same tendency, and therefore there were no significant changes in the content of these pigments. However, for LOB2 the concentration 1.0 μM of SA increased the content of chlorophyll a, b and total carotenoids (74%, 84% and 79% respectively) with respect to the control. Similarly, in LOB8 the two highest concentrations of SA (0.5 μM and 1.0 μM) had the same effect with respect to the content of the photosynthetic pigments (0.3900, 0.2560, 0.4230 mg/mL respectively for chlorophyll a, b and total carotenoids) increasing in a 38%, 60% and 44% respect to control; Therefore, the increase or decrease in the content of photosynthetic pigments (chlorophylls and carotenoids) after SA applications depend on the species and the cultivar (Arfan *et al.*, 2007). In this sense, Zhang *et al.* (2007) reported that the chlorophyll content in leaves was a very important parameter for evaluating the physiological state of plants. All green leaves have a higher absorption capacity in the range of 400 – 700 nm; in this range transmission of electrons between chlorophylls and carotenoids occurs. Moreover, the content of photosynthetic pigments can change as a response to stress-causing factors, depending on the photosynthetic capacity or the development stage of the plant (Ustin *et al.*, 1998). Generally, low concentrations of applied SA alleviate the sensitivity to abiotic stresses, and high concentrations of applied SA induce high levels of oxidative stress, leading to a decreased tolerance to abiotic stresses (Miura & Tada, 2014).

The effect of SA on tomato seedlings of native populations with respect to root growth presented different responses (Figure 3). The same SA concentration (0.01 μM) stimulated root weight in LOB2 (724 mg), LOB5 (606 mg) but inhibit it in LOB4 (242 mg), LOB6 (364 mg) and LOB8 (390 mg). However, Yildirim *et al.* (2008) indicate that the application of SA increases root growth. On the other hand, for root volume, LOB2 and LOB5 populations obtained the highest values, which may indicate a greater root area, but not necessarily a greater length. In

this sense, LOB4 was the population with the highest root length (8.2 cm) with the concentration of 1.0 μM SA, followed by LOB6 and LOB2 with 7.5 cm and 6.9 cm respectively. Khan *et al.* (2015) reported that SA stimulated the root formation of some crops. In this sense, Larqué-Saavedra *et al.* (2010) indicate that the tomato is a species sensitive to SA, so, as the concentration increases, root formation is favored. Therefore the productivity of plant crops cannot be considered without the existence of good root apparatus. It has been suggested that SA has great agronomic potential to improve the stress tolerance of agriculturally important crops. In commercial plantations SA can be expected to have an impact on productivity of these highly economically important plants. However, the utility of SA is dependent on the concentration of the applied SA, the mode of application, and the state of the plants (e.g., developmental stage and acclimation).

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