Article

Salicylic acid increases the accumulation of macro and micronutrients in habanero pepper

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Abstract

The results of the effect of salicylic acid (AS) on the nutritional absorption of *Capsicum chinense* are presented. 1 μM of AS was sprayed on the canopy of habanero pepper seedlings and distilled water as control. The results obtained show that aspersions of 1μM of salicylic acid (SA) significantly increase the length, weight, weight and dry weight of roots, stems, leaves and fruits of this species, as well as the levels of nitrogen (N), phosphorus (P)) and potassium (K) in the different organs of the plants at the time of harvest. The accumulation of N, P and K was higher in fruits (116, 110 and 97%), leaves (45.5, 39.4 and 29.1%), root (52.6, 17.0 and 29.4%) and in stem (5, 39.4 and 28.3%) on the values of the control plant. The levels of copper, zinc, manganese, iron, boron, calcium and magnesium were also increased in most tissues by the effect of AS. It is proposed that the positive effect of the AS of increasing the size of the roots favors the absorption and accumulation of macro and micronutrients in the tissues of the plant.

Keywords: foliar spray, habanero pepper, leaves and fruits, macro and micronutrients, root, salicylic acid, stem.

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Introduction

Salicylic acid (AS) is a phenolic compound whose endogenous levels in plants are increased in response to biotic stress (He *et al*., 2007) and abiotic (Miura and Tada, 2014). However, foliar applications of this compound to plants induces physiological and biochemical responses that favor growth, development and yield (Hayat *et al*., 2010; Miura and Tada, 2014).

In some crops of agricultural importance such as grapes, tobacco, corn and wheat, the application of AS regulates photosynthesis (Wang *et al*., 2010); electron transport of photosystem II (Wang *et al*., 2010; Janda *et al*., 2012), transpiration and stomatal conductance (Fahad and Bano, 2012). In other crops it has also been reported that it increases plant height, stem diameter, leaf area, fresh and dry biomass, number of fruits or grains and shortens the days to flowering (Villanueva-Couoh *et al*., 2009; Martín-Mex *et al*., 2012; Tavares *et al*., 2014), favoring greater bioproductivity (Larqué-Saavedra and Martín-Mex, 2007; Martín-Mex *et al*., 2013).

On the other hand, several authors point out that AS favors the accumulation of nutrients in plant tissues under conditions of salt stress (Gunes *et al*., 2007; Khan *et al*., 2010; Fahad and Bano, 2012) or heavy metals (Chen *et al*., 2007; Fatima *et al*., 2014; Singh *et al*., 2015).

In wheat roots the AS favors the accumulation of abscisic acid (ABA) and indoleacetic acid (IAA), favoring the increase of the cellular division of the apical meristem (Shakirova *et al*., 2003), while in transformed roots of *Catharanthus*, the AS increases the size of the cap and the production of lateral roots (Echevarría-Machado *et al*., 2007). The stimulation of the growth of the root by the application of low concentrations of AS was, reported since 1998 in soy (Gutiérrez-Coronado *et al*., 1998) in corn and wheat (Tucuch *et al*., 2015; Tucuch-Haas *et al*., 2016) as well as the area, volume and perimeter in tomato roots (Larqué-Saavedra *et al*., 2010).

Habanero pepper is a crop that in recent years, in the Yucatan Peninsula, has gained great importance, due to its demand both nationally and internationally, for the various uses it is given; however, the national production, the demand is not covered, which is why they are looking for production strategies that favor the yield of this crop.

Given the effects that have been reported in the exogenous application of AS, such as the fact that it favors radical development and that it increases the area of soil exploration, it has been proposed that this effect could favor a greater absorption of nutrients in the cultivation of habanero pepper, which would result in a higher yield of the fruit, hypothesis that was tested in the present investigation.

Materials and methods

The present work was carried out in a tunnel type greenhouse of the Scientific Research Center of Yucatán (CICY), located in the city of Mérida, Yucatán. Habanero pepper seeds (*Capsicum chinense* Jacq.), an orange variety from Geneseeds, were grown in a mixture of Peat moss and agrolita in a ratio of 2:1 (v/v) in polystyrene trays. Developed seedlings were sprayed, until drip point, in the morning (8:00 am) with a solution of 1μM of AS, as treatment or distilled water as a control at 17, 22, 25 and 30 days after sowing.

Fifty days after sowing, each seedling was transplanted into a plastic pot with a capacity of 5 L, which contained a mixture of soil and peat moss in a ratio of 2:1 (v/v), under greenhouse conditions, arranged in a design of complete blocks at random, with five repetitions, where they were allowed to grow until the time of harvest. The salicylic acid (AS) solution was prepared following the methodology defined by Gutiérrez-Coronado *et al*. (1998), which consists of starting from the molecular weight, which is 138.12 g mol^{-1}. A 10^{-2} M stock solution was prepared and by rule of three the concentration of 1 μM was obtained. The product was weighed on an analytical balance and subsequently dissolved in distilled water.

At the end of the experiment (218 days after the last application), plant height data were collected, measured with a millimeter rule from the base of the stem to the terminal apex, stem diameter, taken at 5 cm from the soil with a vernier digital and fresh and dry weight of fruits, stem and root, quantified by means of an analytical balance (Sartorius, BL3100). To determine the nutritional content of the fruits, leaves, stems and roots, the tissue samples were placed in an oven (Binder, FED720) at 70 °C until constant weight was reached and ground for laboratory analysis. The concentration of nitrogen (N) was determined by the micro-Kjeldahl method and the rest of the elements by means of readings of extracts from diacid wet digestion according to the technique described by Alcantar and Sandoval (1999), using a spectroscopy equipment of Atomic emission of plasma induction (ICP-OES, Agilent 725-OES, Australia).

Once the concentrations of each element in tissue and fruit were obtained, we considered these and the aerial dry biomass weights, for the estimation of the total contents. The results of the estimated variables were analyzed by means of an analysis of variance and the comparison of means by the Tukey method ($p\leq 0.05$), using statistical package Statistical Analysis System (SAS, 2004).

Results and discussion

In the Table 1 reports the values of plant height and stem diameter. The results reflect that the AS (1 μM) significantly increased the height of the plant, 24.3% corresponding to 16.9 cm, not so for the diameter of the stem that was not significant. In the same Table 1, it can be seen that the AS significantly affected the fresh and dry weights of the root, stem, leaf and fruits, showing increments of 36.6, 23.3, 35.8 and 117% in fresh weight and 36.6, 45.3, 21.3 and 122% in dry weight, respectively in root, stem, leaf and fruit. The increase in fruit weight suggests a higher yield.

Table 1. Effect of 1 μM of salicylic acid (AS), sprinkled on the canopy of seedlings, in different development and growth variables at the time of harvest (218 days after the last application) in habanero pepper plants.

Trat	AP	DT	PFR	PSR	PFT	PST	PFH	PSH	PFF	PSF
	(cm)		($g\ plant^{-1}$)							
Control	69.4 b	1.04 a	18.7 b	66.6 b	85.5 b	31.8 b	15.9 b	56.2 b	44.8 b	6.26 b
1 μM	86.3 a	1.12 a	25.5 a	90.8 a	105.4a	46.2 a	21.6 a	68.2 a	97.4 a	13.9 a

AP= plant height; DT= Diameter of the stem; PFR= fresh weight of the root; PSR= fresh weight of the root; PFT= fresh weight of the stem; PFH= fresh weight of the leaf; PS = dry weight of the leaf; PFF= fresh weight of the fruit and PSF= dry weight of the fruit. Values with the same letter within columns are equal according to the Tukey test at $p= 0.05$. Each value is the average of 5 individuals.

Similar effects for these variables have been reported in other species of the same genus of *Capsicum* (pepper and jalapeño) (Elwan and El-Hamahmy, 2009, Sánchez-Chávez *et al*., 2011), as well as in *Lycopersicum esculentum* (Larqué-Saavedra *et al*., 2010), *Crysanthemum morifolium* (Villanueva-Couoh *et al*., 2009); *Carica papaya* (Martin-Mex *et al*., 2012); *Oryza sativa* (Anwar *et al*., 2013); *Triticum aestivum* (Hayat *et al*., 2005; Tucuch *et al*., 2015); *Zea mays* (Tucuch-Haas *et al*., 2016).

The accumulation of macroelements in the different plant organs (root, stem, leaf and fruit), by action of 1 µM of AS, are presented in Table 2. The obtained results indicate that the AS significantly increased the nitrogen, phosphorus and potassium contents in all the organics studied in comparison with the control. The effect of AS on the N content was 116% in fruits, 52.6% in roots, 45.5% in leaves and 5% in stems compared to the control. For P, the increase was 110.5% in fruits, 39.4% in leaves, 39.4% in stems and 17% in roots, in relation to control values.

Table 2. Content of macronutrients in different organs of habanero pepper plants, sprinkled to the canopy of seedlings with 1 µM of salicylic acid (AS). Estimated at 128 days after the last application.

Tissue	Treatment	N	P	K	Ca	Mg
		(mg planta^{-1})				
Fruit	Control	150.18 b	12.41 b	101.12 b	33.81 a	11.22 b
	1 µM of AS	325.72 a	26.13 a	199.37 a	34.83 a	19.85 a
Leaf	Control	440.93 b	26.01 b	108.79 b	417.1 b	78.44 b
	1 µM of AS	641.94 a	36.27 a	140.46 a	616.33 a	131.27 a
Stem	Control	512.14 b	16.09 b	142.62 b	311.55 b	111.95 a
	1 µM of AS	561.04 a	20.97 a	183.12 a	437.62 a	191.36 b
Stem	Control	1353.53 b	90.04 b	475.03 b	1819.98 a	303.65 a
	1 µM of AS	2065.7 a	105.88 a	615.12 a	1861.48 a	382.28 a

Values with the same letter in each tissue within columns are equal according to the Tukey test at $p= 0.05$. Each value is the average of 5 individuals.

The quantification of microelements by the effect of AS was also analyzed. The data of Iron (Fe) accumulation in the different organs is presented in Figure 1. It is appreciated that this element accumulated significantly in the aerial part of the plant. The highest positive effect is reported for the stem with more than 100%, followed by the fruit with 99.5% and the leaf with 55.5%. No increments of this element were found in the root.

And for K the values surpassed the control in 97.1% in the fruit, 29.4 in the root, 29.1% in the leaves and in 28.3% in the stem. This behavior coincides with that reported by Tucuch-Haas *et al*. (2017) for the cultivation of corn, where an increase of N, P and K in tissue and grain was observed. On the other hand, the aspersions of the AS significantly increased the calcium (Ca) content in 47.7% in the leaves and 40% in the stems, compared to the levels found in the control. The contents of this element were also superior to root and fruit control, although these differences were not statistically significant.

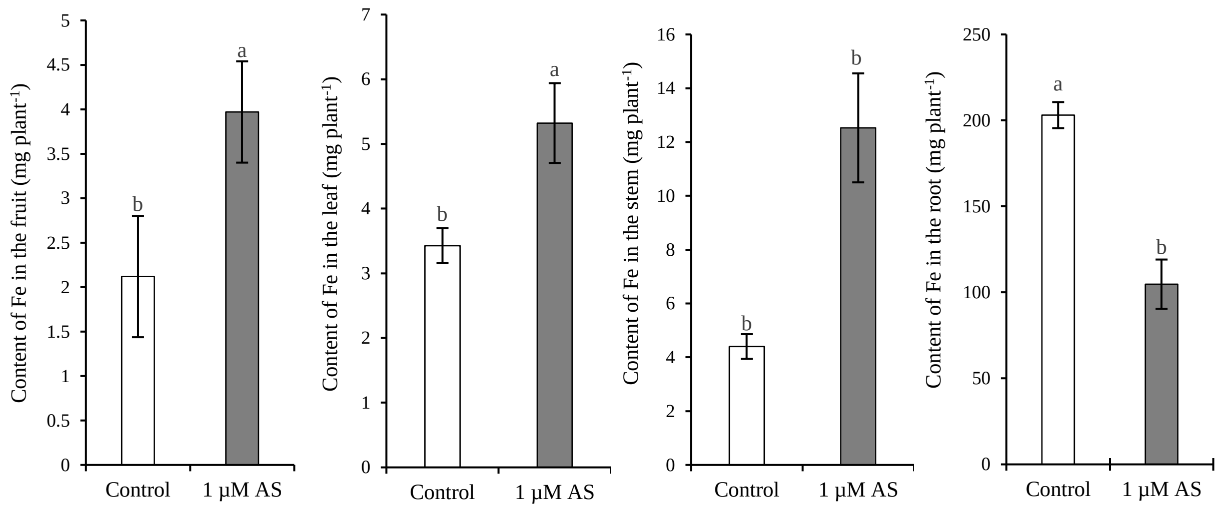

Figure 1. Effect of aspersions of 1 µM salicylic acid on the content of Fe in root, leaf, stem and fruit, in habanero pepper plants. Bars with the same letter are equal according to the Tukey test at p= 0.05. Each value is the average of 5 individuals.

The effect of AS also significantly favored the accumulation of magnesium (Mg) in 76.9% of the fruits, 67.3% in the leaves and 67.3% in the stems compared with the control treatment. At the root, although the Mg content for the plats sprayed with AS was not significant, they also exceeded the 9% control.

Calcium (Ca) was also found in significantly higher levels in stems and leaves of plants treated with AS compared to control, but not in fruits and roots. The low effect of AS on the accumulation of Ca in the fruits, could be explained as a consequence of its low mobility and tendency to accumulate in the older tissues, which also supports the significant effect on stems and leaves (Monge *et al*., 1994).

In the Figure 2 shows the results of the levels of copper (Cu), zinc (Zn), manganese (Mn) and boron (B) present in the tissues of plants by the effect of AS. The contents of these four elements, in fruits and leaves, significantly outperformed the control, except for Cu, which was not significant. In the stem of the plants treated with AS, although the contents of Mn and B exceeded the control, these values were not significant, like Cu and Zn, in which values similar to the control were obtained. At the root, the AS significantly increased the content of B, while the Cu and Mn levels were lower in the treated plants compared to the control. The levels of Zn in the roots were not affected by the sprinkling of the AS.

The lower accumulation in the roots of Fe, Cu and Mn, with respect to the control, could be due to a greater demand of these elements in the aerial part, since the AS increases the photosynthetic activity (Ghansemzadhe and Jaafar, 2013) and accumulation of chlorophylls (Vazirimehr and Rigi, 2014), where these elements participate (Alcántar and Trejo, 2007), the Fe to increase efficiency in the electron transport chain (Wang *et al*., 2010; Janda *et al*., 2012), Mn in the photolysis of water and Cu bound to plastocyanin (Alcántar and Trejo, 2007).

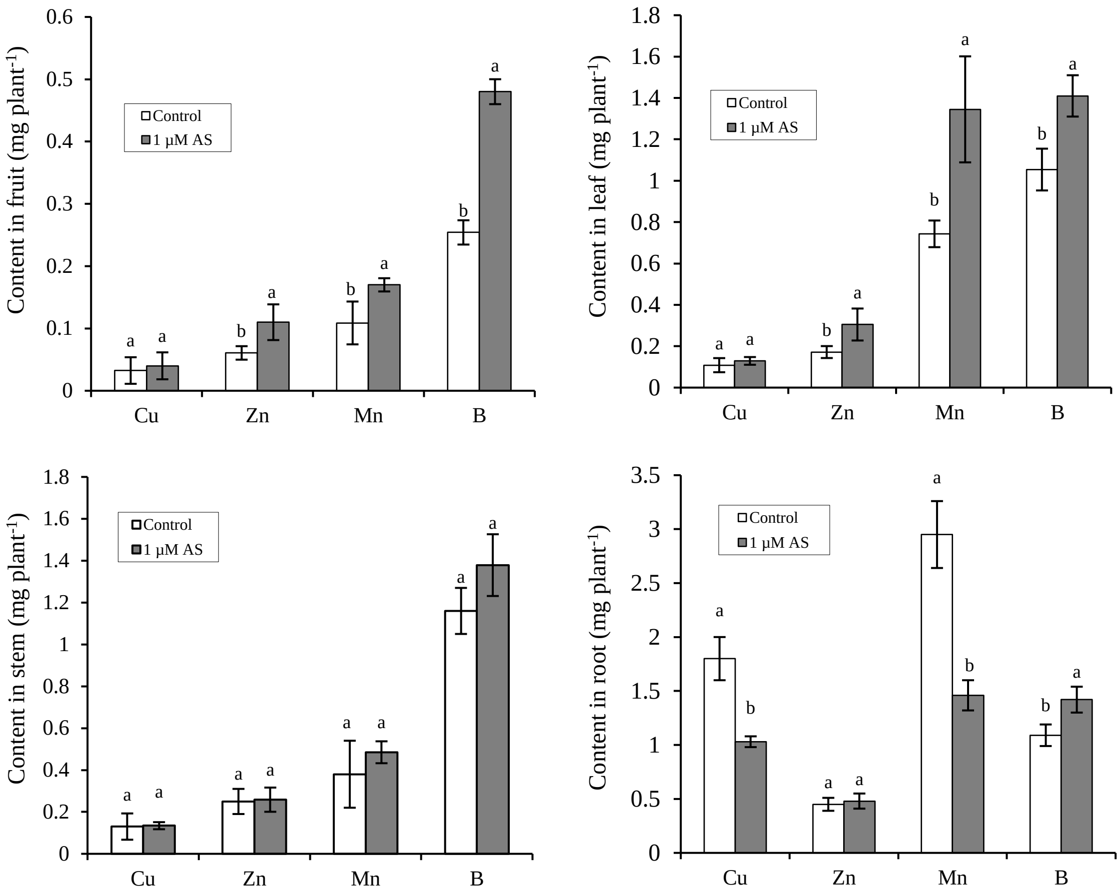

Figure 2. Content of micronutrients (Cu, Zn, Mn, B) in roots, leaves, stems and fruits of Habanero pepper plants sprinkled with 1 µM of salicylic acid. Bars with the same letter within each graph are equal according to the Tukey test at $p= 0.05$. Each value is the average of 5 individuals.

The results obtained in the present investigation prove that the AS favors the nutritional status of habanero pepper plants and supports the results obtained by Guzmán-Antonio *et al*. (2012), who reported a greater accumulation of N, P, K Ca, Mg, Mn, Fe, in seedlings of this same species, when AS is supplied together with fertilization. They also coincide with the work of Villanueva-Couoh *et al*. (2009); Khan *et al*. (2010) who found a higher content of N, P and K, in chrysanthemum and beans.

Possibly the effect of the AS of increasing the content of macros and microelements in fruits, is a fundamental component to explain the positive effect of increasing fruit yield, as has been reported by Martín *et al*. (2004, 2005) who point to increases of up to 23% in fruits of this crop when sprinkling 1 µM of AS; while in other crops such as tomato (Javaheri *et al.*, 2012), cucumber (Martín-Mex *et al*., 2013) and pepper (Elwan and El-Hamahmy, 2009) were found increases of 32, 33 and 82% respectively, with the sprinkling of the same concentration.

It is also possible to consider that this increase in macro and micronutrient is part of the answer to why the quality of the fruit is increased, measured by the increase in color, firmness, total soluble solids, vitamins C, lycopene and brix degrees in different fruits and vegetables (Elwan and El-Hamahmy, 2009; Karlidag *et al*., 2009; Javaheri *et al*., 2012). The results obtained in habanero pepper culture confirm the ability of this molecule to act as a regulator of plant growth (Rivas-San Vicente and Plascencia, 2011) and suggests that the spraying of 1 μM of AS is sufficient to trigger favorable responses has been published for other families and plant species of agricultural importance (Larqué-Saavedra and Martín-Mex, 2007; Martín-Mex *et al*., 2013).

Conclusion

Foliar sprays of 1 μM of salicylic acid (AS) to the canopy of habanero pepper (*Capsicum chinense*) seedlings significantly increases the length, weight, and dry weight of roots, stems, leaves and fruits of this species, as well as favoring the accumulation of macro and micronutrients that benefit their growth and development.

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