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Prohexadione-Ca provokes positive changes in the growth and development of habanero pepper

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Abstract

In recent years, the cultivated area of habanero pepper (*Capsicum chinense* Jacq.) has grown in México as a result of increasing the culinary diet among consumers and the knowledge on its high healthy components such as antioxidants, vitamins and nutrients. The actual worldwide demand of this vegetable requires the application of new production systems in order to increase yield per hectare as well as to improve the fruit quality of this commodity. The use of growth retardants is an alternative for this challenge, therefore, the effect of prohexadione-Ca (P-Ca) was evaluated on the vegetative growth, gibberellins in the stem apex, yield and antioxidants content in ripen fruits of habanero pepper cv. 'Jaguar'. The dosages of P-Ca were: 0, 100, 175 and 250 mg L^{-1} sprayed to seedlings at one (10 days after transplanting) or two (10 and 31 days after transplanting) occasions. Results showed that P-Ca temporally reduced growth in height and diameter of main stem. This effect was related with a reduction in the synthesis of gibberellins A_1, A_4 and A_7 in the apex. The fruit number and yield per plant increased with one application of P-Ca (at 175 mg L^{-1}). The content of capsaicin and total carotenoids showed a remarkable increment in ripen fruits when plants have received one application of P-Ca at any concentration.

Key words: *Capsicum chinense* Jacq., growth retardant, capsaicin, antioxidants, gibberellins.

Introduction

Vegetables such as habanero pepper (*Capsicum chinense* Jacq.) are a rich source of vitamins, minerals, fiber and antioxidants to humans. These elements in addition to cereals, grains and animal derived products, contribute to complete a dietary need for a healthy life. In recent years, medical research has established that fruits and vegetables given to patients through controlled diet provide protection to diseases such as cancer, arteriosclerosis, diabetes and liver injury (Charles, 2013). Under this expertise, the consumption of fruits and vegetables is highly advised in order to keep a good health and life quality (Mc Cormick, 2012). Habanero pepper is a crop with an increasing demand in the national and international markets. The fruit of this vegetable is gaining importance as a result of its high minerals, flavonoids and antioxidants content, in particular capsaicin. (Materska and Perucka, 2005; Ouzounidou *et al.,* 2010). It is necessary to apply new alternative techniques which could contribute to increase both yield and fruit quality. On these bases, contemporaneous horticulture seeks technologies related to improve these referred components. Among them are: crop management systems, biotechnology and the use of bioregulators (Rademacher, 2000). Prohexadione calcium is a growth retardant used to control the excessive vegetative growth and to improve fruit quality in apple, pear and cherry trees (Costa *et al*., 2004). P-Ca inhibits the biosynthesis of the active gibberellins A_1, A_4, and A_7 (Rademacher, 2000). Little is known upon the effects of P-Ca on vegetable crops. It has been suggested that P-Ca may participate in secondary metabolite pathways linked to antioxidant status in edible fruits (Mata *et al.,* 2006; Rademacher and Kober, 2003; Roemmelt *et al.,* 2003), as well as through modifying the enzyme system activity (Forkmann and Heller, 1999), reflected quite often in an increase in anthocyanin and color intensity in ripen grapes (Giudice *et al.,* 2004). Therefore, the purpose of this work was to evaluate the effect of prohexadione-Ca on: the diameter and height of main stem; gibberellins at the apex; number of fruits and yield per plant and antioxidants content in ripen fruits of habanero pepper cv. 'Jaguar' under greenhouse conditions.

Materials and methods

Plant material and growing conditions: This research was conducted in a greenhouse facility at Universidad Autonoma Agraria Antonio Narro in Saltillo, Coahuila, Mexico. Seedlings of habanero pepper (*C. chinense*) cv. 'Jaguar' raised individually in peat moss and perlite (1:1) in black plastic boxes (Steiner, 1984), were used for this study.

Treatment application: The growth retardant prohexadione-Ca at a concentrations of 0 (water-control), 100, 175 and 250 mg L^{-1} was applied with a back pack sprayer to seedlings at one (when the plants reached eight true leaves corresponding to 10 days after transplanting) or two (10 and 31 days after transplanting). All P-Ca solutions included 0.1% v/v Regulaid® as a surfactant.

Horticultural evaluation: Height and diameter of main plant stem were evaluated every 14 days between time of P-Ca spray and the end of growing season. The number of fruits and yield per plant was recorded at each of the four harvested times.

Endogenous gibberellins: Stem tips from control and P-Ca 175 mg L^{-1} plants were collected four days after the application of prohexadione-Ca. Removed samples were kept in liquid nitrogen, frozen, freeze-dried and ground. Later, tissue samples were analyzed for gibberellins using the gas chromatography-mass spectrometry (GCMS) technique (Ramirez *et al.,* 2004). Purified extracts of tissue were dissolved in a few drops of methanol and methylated with diazomethane. A portion of a methylated extract was dissolved in pyridine and treated with trimethylchlorosilane and hexamethyl disilazane. Aliquots were examined using a pye 104 GLC coupled through a silicone membrane separator to an AEI MS30 dual beam mass spectrometer. Silanized glass columns (213 x 0.2 cm) were packed with 2% SE-33 on 80-100 Gas Chrom Q. The He-flow rate was 25 mL min^{-1}, and the column temperature was programmed from 180 to 280 °C at 20/min at 280 °C . The MS were determined at 20 eV at a source temperature of 210 °C and a separator temperature of 190 °C with a scan speed of 6.5 sec. per mass decade. The spectra were recorded by a DEC Linc 8 computer.

Capsaicin and carotenoids: The content of capsaicin in ripen fruits was determined at harvest time using the technique reported by Bennet and Kirby (1968), through which the antioxidant was extracted from fresh tissue utilizing a series of solvents and later measured in an spectrophotometer with 286 nm absorbance. Whilst total fruit carotenoids content was measured with the methodology of Tomas (1975). After organic solvent extraction and purification, the content of total carotenoids in the tissue sample was determined in the spectrophotometer at 454 nm and quantity of them established using the following formula:

$$\mu\text{g carotenoids / 100 g fruit} = \%\text{ Abs} \times 3.857 \times V \times 100 / W$$

Where: % Abs = percent of absorbance, V = measured volume in probet, and P = sample weight in grams.

Experimental design and statistical analysis: A randomized factorial design with seven replicates plants per treatment was used. The results were analyzed with the PROC ANOVA (SAS 9.1, SAS Inst., Cary, NC). Significance was calculated using the Tukey´s method.

Results and discussion

Plant growth, gibberellins and yield: The application of P-Ca at all of the concentrations evaluated, resulted in a significant reduction (P=0.05) of stem growth on most measuring dates (Fig. 1). At the end of the growing season, plant height in P-Ca treated plants at 100 and 175 mg L^{-1} was similar to control; whereas those with the higher P-Ca concentration remained with lower growth. Fig. 2 shows that stem diameter increased between 65 and 100 days after transplanting in most P-Ca treatments. This increment was significantly noticeable (P=0.05) at the end of the stem growth in P-Ca sprayed plants with 175 and 250 mg L^{-1}. The treatment with prohexadione-Ca at 175 mg L^{-1}caused changes in the endogenous gibberellins status at the apex (Table 1). P-Ca samples showed GA_9, GA_{20} and GA_{51}; whilst in control tissue GA_1, GA_4 and GA_7 were detected. The reduction in plant height and increment in stem diameter provoked by P-Ca (Figs. 1, 2) has also been observed in Mirador pepper (Ramirez *et al*., 2010) and apple trees (Costa *et al*., 2004). Prohexadione-Ca has been proven to be a strong retardant. This effect is explained in terms of its action as an inhibitor of the synthesis of gibberellins biologically active (Karhu and Hytönen, 2006; Rademacher, 2000). In this study, it was found that P-Ca inhibited at the apex the synthesis of gibberellins A_1, A_4 and A_7 (Table 1), which are

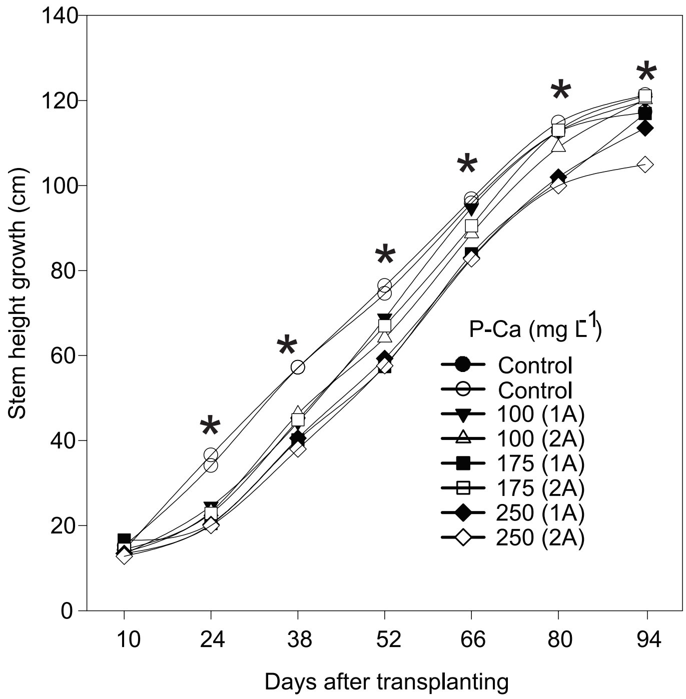

Fig. 1. Effect of prohexadione-Ca doses (mg L^{-1}) on stem height growth in habanero pepper (*C. chinense* Jacq.) cv. 'Jaguar**'**. Each point represents the mean of seven replicates. *Values statistically different at the Tukey´s P=0.05 level. A= Number of applications with P-Ca.

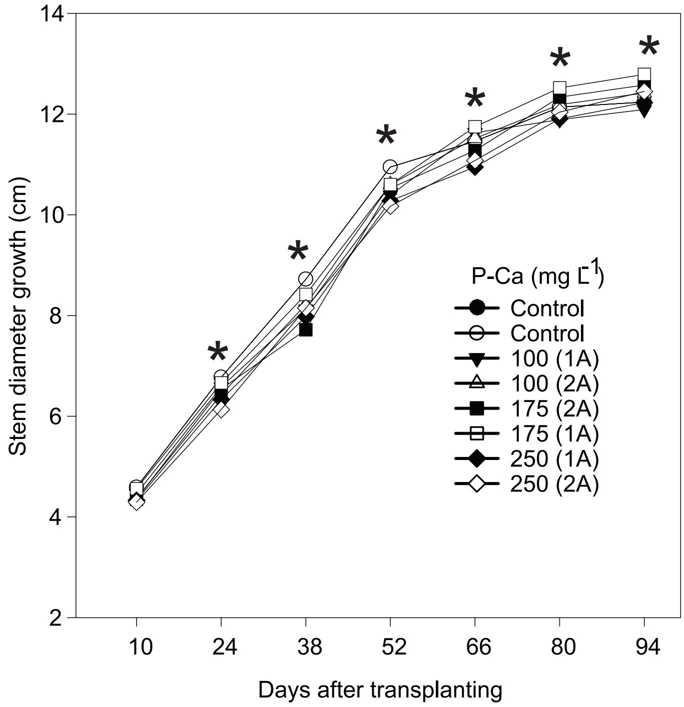

Fig. 2. Effect of prohexadione-Ca doses (mg L^{-1}) on stem diameter growth in habanero pepper (*C. chinense* Jacq.) cv. 'Jaguar**'**. Each point represents the mean of seven replicates.* Values statistically different at the Tukey´s P=0.05 level. A= Number of applications with P-Ca.

required for shoot growth (Ramirez *et al*., 2003). This metabolic gibberellin inhibition remains for a few days and its synthesis is restored soon after (Rademacher, 2004); the behavior which may explain the recovery in stem growth of those P-Ca treatments seen at the end of the growing season (Fig. 1). The increment in stem diameter seen in P-Ca treated plants (Fig. 2) has been explained as a result of an increase in cell division followed by an increase of assimilate flux moving into that growing tissue (Ramirez *et al*.,

Table 1. Gibberellins in stem apices of habanero pepper (*C. chinense* Jacq.) cv. 'Jaguar' four days after being sprayed with P-Ca at 175 mg L^{-1}

Gibberellins	KRI[a]	Principal ions and % relative intensity of base peak
	Control	
GA_1	2651	[506(M^+,100), 448(14), 377(15), 375(18)]
GA_4	2488	[418(M^+,21), 403(2), 400(12), 386(25), 284(100)]
GA_7	2416	[416(M^+,10), 193(12), 179(5), 155(13)]
	Prohexadione-Ca 175 mg L^{-1}	
GA_9	2295	[330(M^+,5), 217(37), 183(19), 159(45)]
GA_{20}	2468	[418(M^+,100), 403(17), 387(6), 375(82), 359(19)]
GA_{51}	2507	[418(M^+,4), 403(3), 386(15),371(3), 358(1)]

[a]kovats retention index. M^+ = Molecular ion.

2003). The effect of prohexadione-Ca on fruits and yield per plant is shown in Table 2. The most remarkable effect on increase in yield per plant (P=0.05) was observed when the growth retardant was applied once at 175 mg L^{-1}, where the fruit production per plant was 17% above control plants. Similar behavior was observed in the same treatment with respect to number of fruits per plant. The fact that P-Ca at 175 mg L^{-1} resulted in higher number of fruits and yield per plant, support the thesis that this given P-Ca concentration could be the optimum for habanero pepper plant under greenhouse conditions and also demonstrated for apple (Guak *et al.*, 2004) and berries (Schildberger *et al.*, 2011; Poledica *et al.*, 2012). Other P-Ca concentrations used in this study resulted in low or too high doses for this habanero pepper commodity. The increment in fruit number and yield seen in P-Ca treated plants may reflect that the reduction in vegetative growth (Fig. 1) resulted in an increase in flower bud induction by the presence of more cytokinins in the meristematic tissue (Ramirez *et al.*, 2010). This physiological condition also may promote more carbohydrates moving into developing fruitlets with a strong vascular connecting tissues which would avoid fruit drop (Costa *et al.*, 2004; Jordan *et al.,* 2001; Sridhar *et al.,* 2009).

Capsaicin and total carotenoids: The content of capsaicin increased significantly (P=0.05) in fruits collected from plants treated with any doses of prohexadione-Ca (Fig. 3). This effect was consistently higher when any concentration of P-Ca was sprayed only once. The highest increase in capsaicin occurred with P-Ca at 250 mg L^{-1}; in which the amount of the antioxidant

Table 2. Effect of prohexadione-Ca on fruit number and yield in habanero pepper (*C. chinense* Jacq.) cv. 'Jaguar'

Prohexadione-Ca (mg L^{-1})	Yield per plant (g)		Fruits per plant[y]	
	Applications			
	1	2	1	2
Control	764.13b[z]	760.03a	138.3b	143.75a
100	774.82b	698.88a	139.5b	175.95a
175	892.57a	541.01b	176.65a	109.85b
250	714.67b	401.54c	99.55b	84.4b
VC	15.99	19.37	31.88	34.9

VC: Variation coefficient. [z] Within columns values with same letter are not statistically different at the 0.05% probability level using the Tukey´s test. [y]Mean of seven plants.

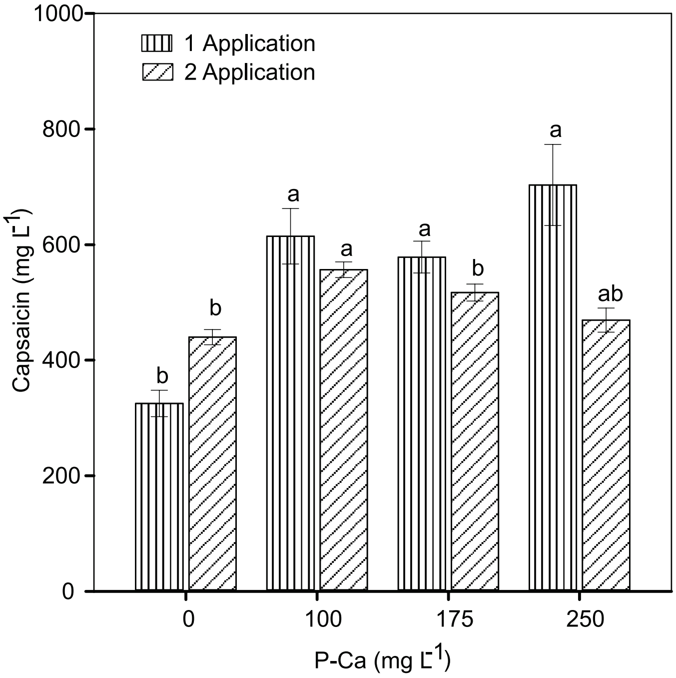

Fig. 3. Effect of prohexadione-Ca doses (mg L^{-1}) on capsaicin content in fruits of habanero pepper (*C. chinense* Jacq.) cv. 'Jaguar'. Each column represents the mean of three replicates ± standard error. Values with the same letter are statistically similar according to Tukey test at P=0.05.

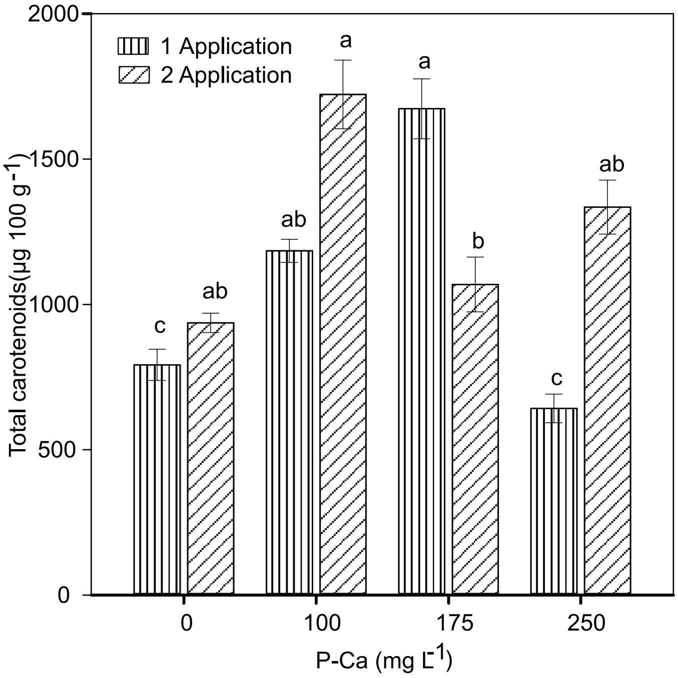

Fig. 4. Effect of prohexadione-Ca doses (mg L^{-1}) on total carotenoids content in fruits of habanero pepper (*C. chinense* Jacq.) cv. 'Jaguar'. Each column represents the mean of three replicates ± standard error. Values with the same letter are statistically similar according to Tukey test at P=0.05.

was double when compared with control samples. Information on the effects of P-Ca on capsaicin in habanero pepper is scarce. It has been proposed that the increment in fruit antioxidants such as capsaicin after the application of P-Ca may be due to the ability of this growth retardant to inhibit the production of cellular free radicals (Díaz *et al.,* 2004; Vázquez-Flota *et al.,* 2007) which are normally produced during fruit ripening. This metabolic process requires the action of enzymes such as catalase and peroxidase as it has been demonstrated in apple (Rademacher, 2000). The content of total carotenoids in fruits also showed a significant increase when the P-Ca was applied only once at any dose and with 100 mg L^{-1} sprayed twice (Fig. 4). These values represent a two fold increase when compared with control fruits. This effect has also been observed in beans (Bekheta *et al.,* 2009) and oranges (Graham and Smit, 2010). The promotion of carotenoids in the fruit by P-Ca could also be mediated through the hypothesis previously suggested for capsaicin. The increment in total carotenoids and capsaicin in fruits from P-Ca treated plants is an interesting contribution as an alternative to healthy food source since in recent years antioxidants consumption have been related to a high food quality (Da Silva *et al.,* 2014; Mc Cormick, 2012), as well as for cancer, diabetes and heart diseases protection (Howard *et al.,* 2000; Pramanik and Srivastava, 2013; Shaik *et al.,* 2013).

In conclusion, increased total carotenoids and capsaicin in fruits from P-Ca treated plants is an interesting contribution leading to high quality food production. More research is required to elucidate the mechanism of action by which higher carotenoid synthesis takes place as influenced by the growth retardant.

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