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Prohexadione-Ca Modifies Canopy, Antioxidant Levels and Enzymatic Activity on Jalapeño Pepper Grown in Greenhouse

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Authors' contributions

This work was carried out in collaboration between all authors. Author HR designed the study, wrote the protocol, and wrote the manuscript. Author LJR-P managed the experimental process, analyses of the study, performed the laboratory analysis and author JHR-A managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The use of growth retardants may be an alternative to improve yield and fruit quality of jalapeño pepper (*Capsicum annum* L.) growing in greenhouse; particularly when a high antioxidants fruit content is the main goal. Prohexadione calcium (P-Ca) is a bioregulator which reduces vegetative growth and increases antioxidants content in deciduous fruit species. The effect of P-Ca in jalapeño pepper is little known, therefore, the objective of this study was to evaluate the effect of P-Ca on the phenotype and fruit quality nutraceutical on jalapeño pepper hybrid Grande.
Study Design: A Completely Randomised Block Design was used, with four replicates per treatment. The results obtained were analyzed with the statistical program SAS (2009), for Windows version 9.1, and for the analysis of variance and comparison of means, the Tukey ($P \leq 0.05$) test was applied.
Place and Duration of Study: The experiment was established at the Universidad Autonoma

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Agraria Antonio Narro in Saltillo, Coahuila, Mexico, between April and July 2012.
Methodology: When seedlings had reached to 10 true leaves, a first foliar application and 15 days later, a second application of P-Ca at 0, 100, 200 and 300 mg L^{-1} were conducted.
Results: Application of Prohexadione-Ca reduced the final height and diameter of main stem; as well as yield. The P-Ca caused ripen fruits with significantly increase in the content of capsaicin, total carotenoids, vitamin C and catalase and peroxidase activity. Improvement in fruit quality compensates yield reduction since the benefit:cost (B:C) ratio in P-Ca fruits had a value of 0.46 as a result of a higher market price, whereas in control samples was 0.27.
Conclusion: Prohexadione-Ca has a potential to improve fruit quality in jalapeño pepper hybrid Grande growing under greenhouse conditions.

Keywords: Capsaicin; Capsicum annum L.; carotenoids; catalase; hormones; peroxidase.

1. INTRODUCTION

Jalapeño pepper is a vegetable of great consumption in Mexico and in many parts of the world used either as fresh or in cooking. The cultivation of this crop in Mexico makes a significative contribution to country´s economy since over 140,000 hectares are established yearly [1]. The medical recommendation of consumption of fruits and vegetables in the daily diet is multiplied exponentially now days. It is a subject of great importance within the quality of life and human health [1]. The modern horticultural science investigates physiological alternatives that would allow to improve the yield and fruit quality of cultivated crops, in particular through their content of minerals and antioxidants which contribution to the diet and health of man [2].

Capsaicin and vitamin C are natural fruit antioxidant substances which provide quality in the diet when consumed. In addition, they contribute as protectors for the consumer´s health [3]. In recent years it has been investigating in fruits and vegetables the presence of other compounds with high nutraceutical value related to carotenoids and enzymes such as catalase and peroxidase activity. These compounds as well as the capsaicin and vitamin C protect cells from free radicals [4].

Epidemiological studies suggest that diets rich in carotenoids are associated with the reduction in risk to develop certain types of cancer, heart disease and other diseases. It is well known that carotenoids can inhibit proliferation and cell transformation, as well as, the modulated expression of key genes in the prevention of certain types of cancer, and lymphomas [5]. Humans are unable to synthesize carotenoids, therefore, this depend from the diet they get with sufficient levels of these chemicals.

The bioregulators are clear alternatives used in the contemporary agriculture to modify temporarily the action of genes in plants in order to meet horticultural products demand by the market [6]. Most bioregulators used in horticulture now days are in armony with the environment. P-Ca (calcium 3-hydroxy-5-oxo-4-propionyl-cyclohex-3-enecarboxylate) is a growth retardant which has been classified as an environmentally friendly method and therefore it is a promising bioregulator for modern horticulture [7]. This compound has shown a substantial improvement in yield and fruit quality in several deciduous fruit species as well as in Mirador pepper [8]. The experience related to the impact of horticultural practices that alter the levels of antioxidants in plants is sparse. It has been reported that the increase in the levels of antioxidants in fruits and vegetables is a subject of great interest and represents an opportunity in the current strategy directed to improve the production systems in horticultural crops [9]. Therefore, the objective of this study was to evaluate the effect of prohexadione-calcium on plant phenotype and the content of total carotenoids, capsaicin, vitamin C, the activity of catalase and peroxidase in ripen fruits of jalapeño pepper hybrid Grande.

2. MATERIALS AND METHODS

2.1 Experimental Site and Design

The experiment was established at the Universidad Autonoma Agraria Antonio Narro in Saltillo, Coahuila, Mexico, in a greenhouse with a metallic overhead structure covered with white plastic (Caliber 720) on the roof and side-plates of polycarbonate. Seeds of jalapeño pepper Grande hybrid were used. The seeds were sown on April 15, 2012 in polystyrene trays with 200 cavities, using premier peat moss mix as a substrate. The transplanting was performed on

May 15, 2012 inside the greenhouse when plants reached 15 cm height into 20 liters plastic pots, using as a substrate, peat moss and perlite (50:50 v/v). The pots were distributed at a distance of 40 cm between plants and 80 cm between rows. The P-Ca treatments were: 0 (water-control), 100, 200 and 300 mg L^{-1}. When plants produced 10 true leaves, a first foliar spray of P-Ca was applied. A second application with same doses of P-Ca was conducted 15 days later. A Completely Randomised Blok Design was used to conduct the experiment. The phenotypic variables among treatments evaluated (10 replicates per treatment) were: Rate of growth in height and diameter of main stem, number of fruits and yield per plant. The content of capsaicin, total carotenoids, vitamin C; catalase and peroxidase activity in ripen fruits were evaluated using four replicates per treatment. The results obtained were analyzed with the statistical program SAS (2009) [10], for Windows version 9.1, and for the analysis of variance and comparison of means, the Tukey ($P \leq 0.05$) test was applied.

2.2 Horticultural Parameters

2.2.1 Rate of stem growth

Main stem growth was weekly measured between base and apical meristem from date of P-Ca first application until the end of the plant growing season using a tape scale 0 to 2 m.

2.2.2 Rate of growth of stalk diameter

This variable was weekly measured at the middle region of tissue using a vernier with a scale of 0 to 10 cm.

2.2.3 Number of fruits and yield

The number of fruits per plant was recorded at each of the seven harvest times and total yield was obtained using an Ohaus portable scale model SP602 with a capacity of 600 g.

2.3 Antioxidants

2.3.1 Capsaicin content

The content of capsaicin was determined using the method of spectrophotometry [11]. Ten ripen fruits were lyophilized and macerated in a mortar. One gram of sample was placed in an Erlenmeyer flask and 10 ml of absolute ethanol were added. The mixture was vortexed for 15 min, filtered with Whatman No. 1, paper and diluted to 25 ml with ethanol. The sample was transferred to a separation funnel and added 2.5 ml buffer solution at pH 2.8, 0.5 ml of ethanol, 20.5 ml of distilled water and 10 ml of adogen-toluene solution. The mixture was vortexed for 1 min. The absorbance corresponding to capsaicin in the organic phase was determinated in a spectrophotometer (Thermo Electron Corporation Biomate 5) at a wavelength of 286 nm. The readings were done in triplicate for each sample. In order to determine the content of capsaicin in the samples, a calibration curve was constructed using capsaicin standard (Sigma, Co) with a range of 0 - 0.40 mg ml^{-1}, previously dissolved in the referred solution.

2.3.2 Content of total carotenoids

Total carotenoids content was obtained by spectrophotometry [12], with some modifications. Ten grams of fresh ripen fruit were combined with 50 ml of acetone in an Erlenmeyer flask and kept for 24 h at 7°C. Sample was later grounded in a mortar, filtered in a textile mesh. Liquid phase was combined with 20 ml of petroleum ether and 100 ml of distilled water. The carotenoid containing layer was separated by adding 10 ml of NaOH (40%) and 20 ml sodium sulphate (10%). The mixture was filtered through a gauze containing anhydrous sodium sulfate. The filtered liquid phase containing the antioxidant was taken to a spectrophotometer (Thermo Electron Corporation Biomate 5) and absorbance was determined at 454 nm wavelength. The total carotenoids content was determined by the following formulae:

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

Where: % Abs = percent of absorbance, 3.857= standard factor, V = measured volume in probet, and W = sample weight in grams

2.3.3 Content of vitamin C

The content of vitamin C was determined following the methodology of titration [13]. Twenty grams of fresh fruit were grounded in a mortar, placed in a flask, 10 ml of hydrochloric acid (2 % v/v) and 40 ml of distilled water were added and mixture was later filtered through gauze. 10 ml of supernatant was titrated with 2.6 - modified dichloro phenol indophenol (1X10-3 N).

When solution reached a pink color, vitamin C content was determined using the following formula described by the authors of the referred method:

$$\text{Vitamin C}\left(\text{mg}\cdot 100\text{ g fw}\right)=\frac{\left(\text{ml used of 2,6 diclorophenolindophenol}\times 0.088\times \text{total volume}\times 100\right)}{\left(\text{aliquot volume}\times \text{weight of the sample}\right)}$$

2.4 Enzyme Activity

The enzyme activity was measured using 0.5 g of pericarp sample. Tissue was macerated in liquid nitrogen and soluble protein was extracted with HEPES-KOH 100 mM pH 7.5 in proportion 1:1 [14]. The soluble protein was separated by centrifugation at 11000 g for 10 min at 4°C. The supernatant fraction was used to quantify the protein content and enzyme activity in a spectrophotometer (Thermo Electron Corporation Genesys 10 uv). Catalase activity was quantified using the method modified of [15]. A reaction mixture containing potassium-sodium phosphate buffer pH 7 100 mM was combined with 20 µl of extract sample and H_2O_2 30 mM in to reach a total volume of 5 ml. The consumption of H_2O_2 was measured by their decrease to A_{240} ($P \leq$ 39.9 mM cm^{-1}) at intervals of 25 s during 3 min.

Peroxidase activity was determined using the modified technique of substrate oxidation [16]. The reaction mixture included 50 mM sodium phosphate buffer pH 7.0, 3.33 mM guaiacol (substrate), 5 mM H_2O_2 and 20 µl of the sample in a final volume of 5 ml; the reaction was carried out at 26-28°C. The oxidation of the substrate was determined by the increase in absorption at A_{470}.

3. RESULTS AND DISCUSSION

3.1 Growth Rate

The prohexadione calcium significantly reduced (*P≤0.05*) the rate of main stem growth (Fig. 1) and diameter (Fig. 2). This pattern was observed from the second week in any treatment with P-Ca until the end of the vegetative cycle. The effect observed with P-Ca on the reduction in the height of the plants is consistent with effects reported by [7] and [17]. This reduction seems to be directly related to the inhibition of the biosynthesis of gibberellins A_1, A_4 and A_7 by P-Ca at the apex [6]. It has been reported that the synthesis of these biologically active gibberellins is inhibited within a few hours after the P-Ca is taken by the shoot apex; whereas the stem growth reduction appears few days later [18].

3.2 Number of Fruits, Yield and Economic Impact

The number of fruits and yield per plant were lower *P≤0.05*) in plants treated with any P-Ca dosage with respect to the control plants (Table 1). This behavior was previously observed in grape [19]. This effect by P-Ca could be attributed to the fact that the growth retardant inhibits the synthesis of gibberellins at the apex and therefore less stem tissue growth takes place resulting in a drastic reduction in vegetative growth during the life cycle of the plant (Figs. 1 and 2), causing a decrease in length of shoots and therefore less surface tissue for the formation of flower buds and there after fruits [7]. Table 2 shows that although the yield cost per kilogram of fruits is higher in P-Ca samples, this adverse condition is compensated by the market price which doubles the one in control as a result of a higher antioxidant content in P-Ca fruits (Figs. 3 and 5). This difference is reflected in the benefit: cost ratio with a value of 0.46 in P-Ca fruits against 0.27 in control fruits. The reduction in yield caused by P-Ca is also linked to increments in fruit size and color intensity as it has been reported in Mirador pepper and tomato [7]. Improvement in fruit quality could compensate yield reduction since P-Ca fruits reached higher market price. This statement is supported by the fact that now days the consuming demand on vegetable commodities with a higher antioxidant content has been substantially increased even at a higher market price [2].

3.3 Antioxidants

3.3.1 Capsaicin

The fruits of plants treated with any concentration of P-Ca showed a higher content (*P≤0.05*) of capsaicin (Fig. 3). The treatment with P-Ca at 300 mg L^{-1} produced the highest level of this antioxidant. An increase in the levels of capsaicin in fruits of chile Mirador treated with P-ca at 175 mg L^{-1} has also been reported [7]. Although the mechanism of action of the retardant in this process has not been completely established, it has been speculated that the P-Ca exerts its effect by modifying the enzyme level in the flavonoid biosynthetic pathway generating modified flavonoids linked to the antioxidant activity in young tissue [6]. Considering this experience with the results of the present work, it is likely that the increase in capsaicin caused by treatments with P-Ca might be have taken place through a similar path of action.

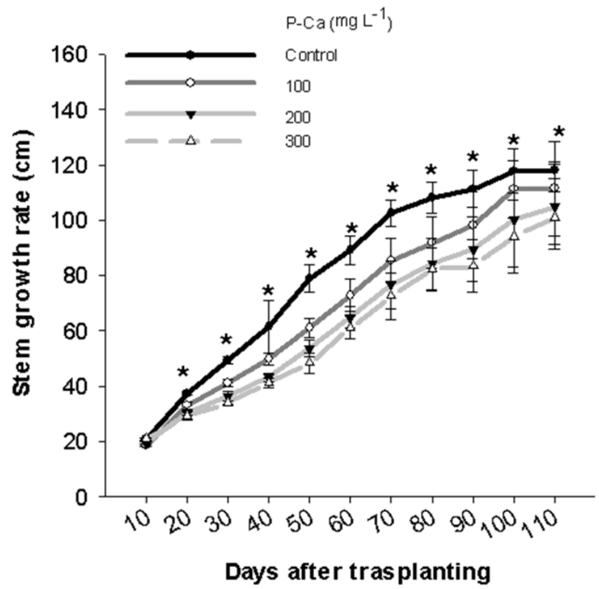

Fig. 1. Main stem growth rate of jalapeño pepper hybrid Grande after being treated with P-Ca. Each point represents the mean of ten replicates±standard error.
** indicates statistically significant difference (Tukey, P≤0.05).*

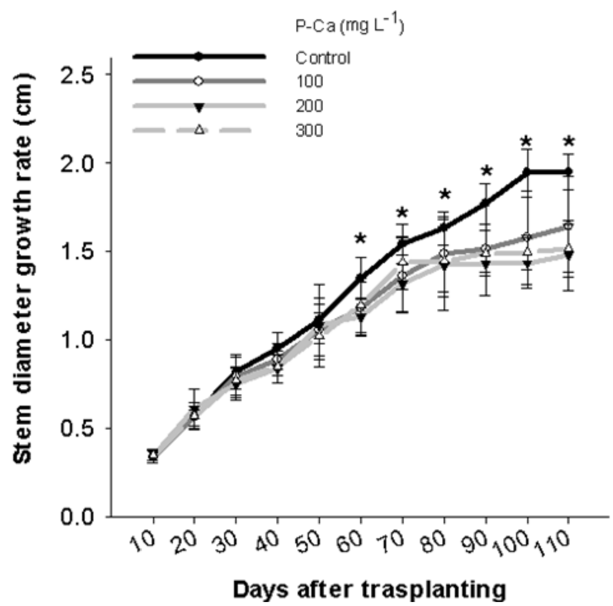

Fig. 2. Growth rate of stem diameter in jalapeño plants hybrid Grande after being treated with P-Ca. Each point represents the mean of ten replicates±standard error.
** indicates statistically significant difference (Tukey, P≤0.05)*

Table 1. Effect of P-Ca on number of fruits and yield in jalapeño pepper

	P-Ca mg L^{-1}				
Factor	**Control**	**100**	**200**	**300**	**V.C. %**
Number of fruits/Plant	66.6 a^Z	48.4 b	42.4 b	47.9 b	20.2*
Yield per plant (g)	1530.28 a	1019.95 b	836.41 b	860.98 b	17.1*

*g= grams; *: significanse at $P\leq0.05$; V.C.: variation coefficient; z In columns values with different letter are statistically different as Tukey at $P\leq0.05$*

Table 2. Economic impact for jalapeño pepper fruits treated with P-Ca

Concept	Control	$P\text{-}Ca^Z$ (30% antioxidant above control)
Grower price*/kg. $ USD	0.60	0.92
Cost/kg. $ USD	0.47	0.63
Benefit:Cost Ratio	0.27	0.46

**SAGARPA, México; ZMean: P-Ca Treatments*

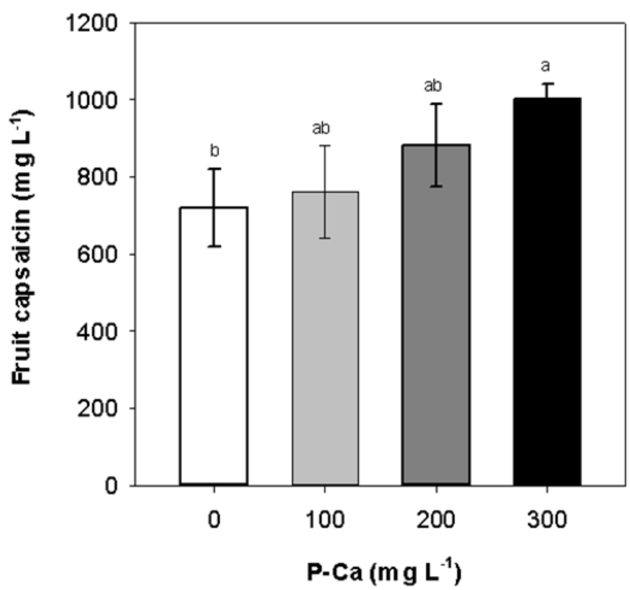

Fig. 3. Influence of prohexadione-ca on content of capsaicin in fruits of jalapeño pepper hybrid Grande. Each bar represents the average of 4 replications±standard error.
Bars with the same letter are equal (Tukey, $P\leq0.05$)

3.3.2 Total carotenoids

The P-Ca at any dosage caused a significant increase *$P\leq0.05$*) in the content of total carotenoids (Fig. 4). The treatment with P-Ca at 200 mg L^{-1} showed the highest concentration of those antioxidants. These results are consistent with those found in *Vicia faba* [20]. Carotenoids are a major source of provitamin A, which is related to the strengthening of the immune system in the human body [21]. Therefore, on the basis of this research, it is possible to suggest that P-Ca offers a promising alternative to improve jalapeño pepper fruit quality in terms of a higher content of total carotenoids.

3.3.3 Vitamin C

The Fig. 5 shows the content of vitamin C in fruits of jalapeño pepper Grande hybrid. It was observed that P-Ca applied at a concentrations of 200 and 300 mg $liter^{-1}$ significantly increased

($P \leq 0.05$) the content of that antioxidant. This effect is supported with the results in chile Mirador and tomato using P-Ca in the range of this study [22]. Vitamin C is an antioxidant which plays an important role in detoxification of activated oxygen and reacts directly with reactive oxygen molecules [13]. This compound contributes to a good health in humans and also strengthens the system of protection against diseases such as cancer, diabetes and blood pressure [1]. Therefore, increases in vitamin C by P-Ca in fruits of vegetable crops are considered an important alternative in modern technology [2].

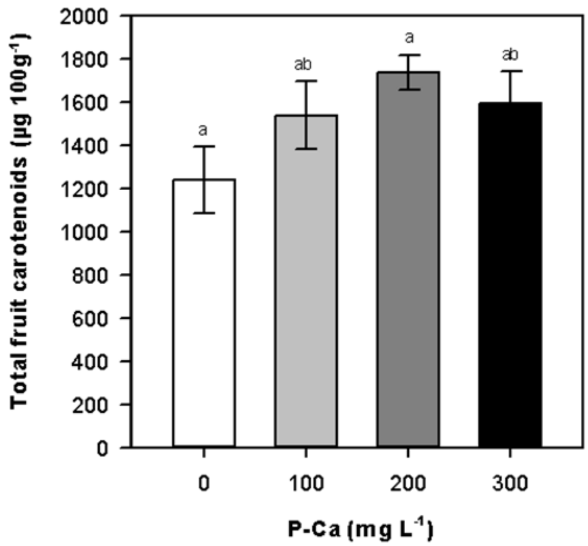

Fig. 4. Influence of prohexadione-ca in content of carotenoids in fruits of jalapeño pepper hybrid Grande. Each bar represents the average of 4 replicates±standard error

Bars with the same letter are equal (Tukey P≤0.05)

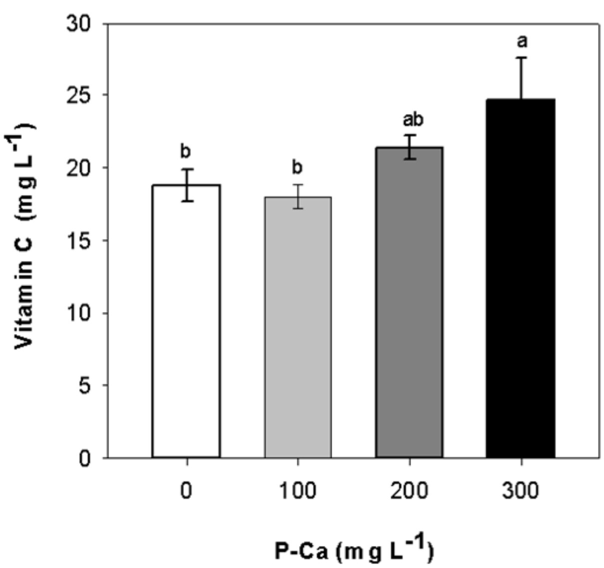

Fig. 5. Influence of prohexadione-ca in vitamin C content in fruits of jalapeño pepper hybrid Grande. Each bar represents the average of 4 replications±standard error

Bars with the same letter are equal (Tukey, P≤0.05)

3.4 Enzyme Activity

The activity of the enzyme catalase was significantly increased (*$P\leq0.05$*) in the fruits of plants treated with P-Ca at 200 and 300 mg L^{-1} (Fig. 6). The highest concentration of the growth retardant doubled the enzyme activity with respect to control samples. This result is similar to the one reported in peanut [23]. Catalase is an enzyme related to the cellular control on the levels of active oxygen species by catalyzing the dismutation of H_2O_2 into H_2O and O_2 [24]. An increase in catalase activity was observed during the ripening process of tomato fruits [25]. It is possible that P-Ca prolongs catalase synthesis as seen in apple [26].

Peroxidase activity increased (*P* =.05) in treatments with P-Ca at 200 and 300 mg L^{-1} (Fig. 7). This effect was also reported in onion and garlic [27]. The higher peroxidase activity observed in P-Ca treated plants could be related to an increase in the concentration of antioxidants shown in Figs. 3 and 4; as it has been discussed in ripening fruits of several deciduous fruit crops treated with P-Ca [28]. It would be of interest for future studies to search on the P-Ca involvement in the metabolic antioxidants pathway in Jalapeño pepper [29].

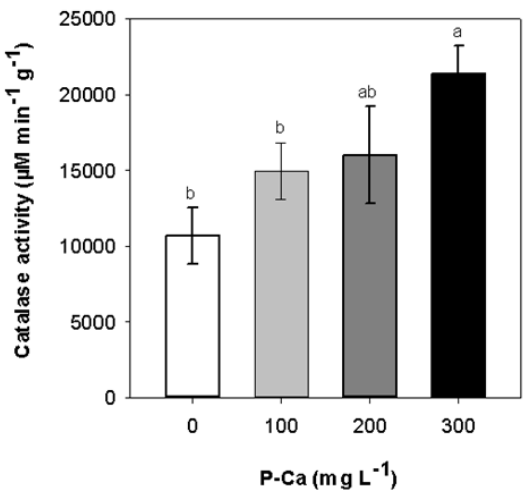

Fig. 6. Influence of prohexadione-Ca on catalase activity in fruits of jalapeño pepper hybrid Grande. Each bar represents the average of 4 replications±standard error.
Bars with the same letter are equal (Tukey, $P\leq0.05$)

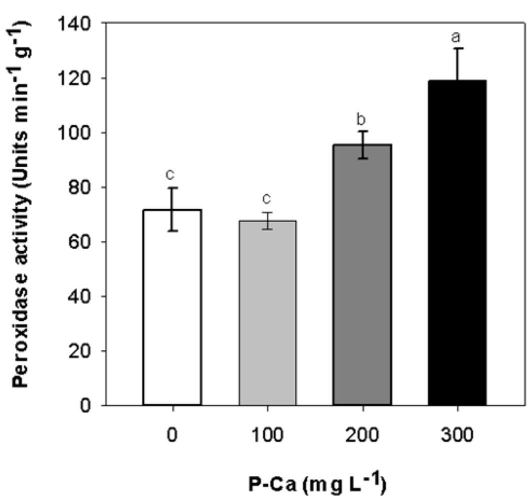

Fig. 7. Influence of prohexadione-ca on peroxidase activity in fruits of jalapeno pepper hybrid Grande. Each bar represents the average of four replications±standard error
Bars with the same letter are equal (Tukey, $P\leq0.05$)

4. CONCLUSIONS

Prohexadione - calcium reduces height and diameter of main stem, and yield; it increases the content of capsaicin, total carotenoids, vitamin C and enzyme activity in ripen fruits on Jalapeño pepper hybrid Grande.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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