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Effects of Prohexadione Calcium Applications on Growth and Yield Characteristics of Cucumber (*Cucumis sativus* L.)

(Kesan Penggunaan Kalsium Proheksadion kepada Ciri Pertumbuhan dan Hasil Timun (Cucumis sativus L.)

HAKAN BAŞAK*

ABSTRACT

This study was conducted to determine the effects of growth regulator prohexadione calcium (Pro-Ca) and inhibitor gibberellic acid on some parameters (yield, pigment levels of leaves, nutrient contents, proline, enzyme activities and fruit quality) when applied at different doses (0, 15, 30, 45, and 60 mg L⁻¹). Chemicals were applied after transplanting seedlings. A total of 12 plots including 3 plants for each dose were used in randomised plot experimental design. Although it has a positive effect on leaf proline content and enzyme activities, 45, and 60 mg L⁻¹ doses of Pro-Ca had negative effects on morphological parameters (excluding stem diameter), yield, leaf pigment (excluding chlorophyll a) and nutrient contents compared to control. High Pro-Ca doses generally affected the fruit quality properties positively while 15 mg L⁻¹ of Pro-Ca did not affect other parameters, except decreasing fruit yield. A 30 mg L⁻¹ of Pro-Ca dose on the examined parameters. The highest vegetative development, yield, nutrient uptake and proline content were obtained from the plants subjected to 30 mg L⁻¹ of Pro-Ca dose. Moreover, this dose increased fruit firmness, TSS, EC values and enzyme activities compared to control. Being an important quality criterion in cucumber storage after harvest, the weight loss rate 29.4% was lower in fruits obtained from the plant having 30 mg L⁻¹ of Pro-Ca dose compared to that of the control plants. Therefore, 30 mg L⁻¹ of Pro-Ca application, beside its positive effects on plant growth, yield and fruit quality, can be suggested for also increase shelf life of fruits in cucumber cultivation in greenhouse.

Keywords: Cucumber; fruit quality; growth; prohexadione calcium; yield

ABSTRAK

Penyelidikan ini dijalankan untuk mengkaji kesan penggunaan pengatur pertumbuhan kalsium proheksadion (Pro-Ca) dan perencat asid giberelik pada beberapa parameter (hasil, tahap pigmen daun-daun, kandungan nutrien, prolina, aktiviti enzim dan kualiti buah) apabila diaplikasikan pada dos yang berbeza $(0, 15, 30, 45 \text{ dan } 60 \text{ mg } L^{-1})$. Bahan kimia diaplikasikan selepas pindah tanam anak-anak benih. Sejumlah 12 plot tanaman termasuk 3 tumbuhan untuk setiap dos telah digunakan dalam reka bentuk uji kaji plot rawak. Walaupun terdapat kesan positif pada kandungan prolina daun dan aktiviti enzim, dos Pro-Ca 45 dan 60 mg L⁻¹ menunjukkan kesan negatif pada parameter morfologi (tidak termasuk diameter batang), hasil, pigmen daun (tidak termasuk klorofil a) dan kandungan nutrien berbanding dengan parameter terkawal. Dos Pro-Ca yang tinggi secara umumnya memberi kesan yang positif kepada kualiti buah manakala Pro-Ca dengan 15 mg L^{-1} tidak memberi kesan kepada parameter yang lain, kecuali penurunan hasil buah. Dos Pro-Ca 30 mg L⁻¹ menunjukkan dos yang paling positif pada parameter yang dikaji. Pembangunan vegetatif, hasil, pengambilan nutrien dan kandungan prolina yang tertinggi telah diperoleh daripada tumbuhan yang dikenakan dos Pro- Ca 30 mg L¹. Malah, dos tersebut meningkatkan kepejalan buah, TSS, nilai EC dan aktiviti enzim jika dibandingkan dengan parameter terkawal. Penyimpanan timun selepas penuaian merupakan kriteria yang amat penting, kadar penurunan berat sebanyak 29.4% adalah lebih rendah pada buah yang diperoleh daripada tumbuhan yang menerima dos Pro-Ca $30 \text{ mg } L^{-1}$ jika dibandingkan dengan tumbuhan yang terkawal. Oleh itu, aplikasi Pro-Ca dengan dos $30 \text{ mg } L^{-1}$, selain kesan positif terhadap pertumbuhan tumbuhan, hasil dan kualiti buah, ia juga menunjukkan peningkatan hayat simpan buah buahan dalam penuaian timun dalam rumah hijau.

Kata kunci: Hasil; kalsium proheksadion; kualiti buah; pertumbuhan; timun

INTRODUCTION

The growth and development in plants are known to be regulated by hormones. Gibberellins (GAs) increase internode length by affecting both cell division and cell length (de Saint Germain et al. 2013). In order to control the vegetative growth in seedlings and their generative development, plant growth retardants (PGRs) such as daminozide, chlormequat chloride, uniconazole, ethephon, trinexapac-ethyl, paclobutrazole and prohexadionecalcium (Pro-Ca) are used (Demir & Başak 2008; Ugur & Kavak 2007). Since the cucumber fruits of parthenocarpic cultivars in greenhouse are formed in the leaf axillaries in internodes located on the stem, the short lengths between internodes provide more fruit per unit stem length. Therefore, cucumber plants with short lengths between the internodes are more yield than those with longer internodes.

Pro-Ca is a plant growth regulator that inhibits gibberellin biosynthesis, reducing vegetative growth and internode length (Kim et al. 2007). Pro-Ca is a new generation growth retardant, which inhibits dioxygenase enzymes activity (Dragisic-Maksimovic et al. 2017). Besides controlling growth in plants, Pro-Ca is a plant bio-regulators and plant protectant that improves yield and fruit quality. It was reported that Pro-Ca increased the yield and quality of Chinese cabbage grown in cold and humid conditions (Kang et al. 2010), decreased the frost damage in tomato fruits (Aghdam 2013). It also reduced the oxidative stress by increasing the synthesis of the flavonoids that reduce the production of reactive oxygen species (ROS) (Ghiasi & Razavi 2013) and increased fruit weight, yield and leaf total soluble solids content in raspberry (Poledica et al. 2012).

Fard et al. (2015) observed that Pro-Ca treatment preserved the relative water content (RWC) and membrane stability in grass plants under drought stress. Bekheta et al. (2009) determined that proline, photosynthetic pigments, total carbohydrates and total soluble sugars were increased by Pro-Ca treatment in faba bean seedlings grown under salt stress, helping tolerance to stress conditions. It was reported that Pro-Ca preserved membrane integrity by reducing the accumulation of electrolyte leakage and malondialdehyde in tomato fruits, enhancing proline content and phenylalanine ammonia-lyase (PAL) enzyme activity and alleviating damage to cold after harvest (Aghdam 2013). With contributing to strengthening the natural defense mechanism by interfering with flavonoid metabolism in plants, Pro-Ca increases the level of luteoforol, a molecule with phytoalexin activity that increases resistance to pathogens (Spinelli et al. 2005).

Pro-Ca has a significant advantage compared to other PGRs due to its low toxicological effect and short half life in plants and soil (Bizjak et al. 2012). It was determined in different plant species that the application of lower doses of Pro-Ca in post-planting period had more positive effects on plant development, yield, and fruit quality compared to its higher doses (Altuntaş 2016; Kim et al. 2019).

Although many studies have been conducted to investigate the effectiveness of Pro-Ca application on cucumber seedlings in controlling growth and seedling quality, its effects on plant growth, yield and fruit quality after planting seedlings has not been sufficiently studied. . It was assumed that Pro-Ca, when applied to cucumber plants at lower doses after transplanting, may have positive effects on yield and fruit quality while it may reduce the lengths between the internodes in these plants. Thus, by using Pro-Ca, it was aimed to increase the yield and product quality to be obtained from the unit area. It is also expected that the positive effect of Pro-Ca on fruit quality can increase shelf life of cucumber fruits in storage and transport after harvest. Therefore, the effects of Pro-Ca applications at different doses to cucumber plants grown in cocopeat substrate with soilless agriculture technique on plant growth, yield, physiological properties and fruit quality were investigated in the current study.

MATERIALS AND METHODS

EXPERIMENTAL CONDITIONS AND PRO-CA TREATMENTS

This study was conducted in Soilless Agriculture Research Unit of Horticulture Department of Faculty of Agriculture of Kırşehir Ahi Evran University. Cevher F₁ cucumber cultivar seedlings (obtained from Yüksel Seeds A.Ş, Antalya, Turkey) were used as plant material. Cucumber seedlings were planted in cocopeat slabs $(100 \times 20 \times 16 \text{ cm})$ placed on 4 m long gutters on 27 April 2018. Each slab contained 3 plants. Pro-Ca (10% prohexadione-calcium, Velonta WG, BASF, Germany) was sprayed onto plant leaves at 0, 15, 30, 45, and 60 mg L⁻¹ doses twice - on the 10th day (on 7 May 2018); and on the 20th day after planting seedling (17 May 2018) (Table 1). Each dose was one treatment. In the current study, Pro-Ca application was performed twice with an interval of 10 days based on the supplier's suggestion that 'Velonta' should be applied twice by the producers to better control shoot development and to increase fruit quality (BASF 2019).

For each treatment dose, 12 plots including 3 plants were used in randomised plot experimental design. The

first and last plants of each gutter row were accepted as edge effects and discarded from the measurements and analyzes. The plants were irrigated with the pH and EC controlled automation system by changing their nutrient supply according to their requirements during development periods with some modifications in Hoagland nutrient solution (Table 2) (Gül et al. 2000). The range of plant nutrients was applied according to the growth of the plant.

Procedure	Date
Planting seedlings	27.04.2018
The first Pro-Ca application	07.05.2018
The second application	17.05.2018
The time (day) after Pro-Ca applications	
On 25th day, plant height and measurements of lengths between internodes	11.06.2018
On 50th day, plant height and measurements of lengths between internodes	06.07.2018
On 75th day, plant height and measurements of lengths between internodes	31.07.2018
On 100th day, completion of the experimental procedure of the study	25.08.2018

TABLE 1. The chronological procedure of the current study

TABLE 2. Chemical contents of nutrient solution (mg L⁻¹)

Elements	Ν	Р	K	Ca	Mg	Fe	Mn	Zn	В	Cu	Мо	EC (mS cm ⁻¹)
Concentration	184-259	29-46	228-350	152-190	36-53	2-3	0.75-1.5	0.5-1.0	0.4	0.1-0.2	0.05	1.8-2.4

GROWTH AND YIELD

At the end of the trial, 100 days after Pro-Ca applications (25 August 2018), plant height from soil level to shoot tip (cm), length between internodes of the third node (cm), stem diameter at 5 cm above ground level (mm), plant fresh and dry weights (g) were determined. The plant height and the length between internodes were measured for 15 plants selected from each Pro-Ca application group

on the 25th, 50th and 75th days (Table 1). All harvests made during the trial were weighed and recorded separately for each plant as yield per plant (g plant⁻¹).

POMOLOGICAL MEASUREMENT AND ANALYSIS

To determine the flesh firmness, the fruit peel was removed from three different regions and the measurement was made in Newton (N) unit using a penetrometer (PCE- FM200, Force Gauge) with a 7.9 mm tip. To determine total soluble solids (TSS), the juice of each cucumber was extracted and filtered on the filter paper, and the TSS was read as percent (%) using digital refractometer (Hanna HI 96801). The pH and EC (dS m⁻¹) values of fruit juices were determined by using table type pH meter (HI5221-02 Hanna) and EC meter (Mettler Toledo MC-126), respectively. To determine their fresh weight, 20 fresh fruits were selected randomly for each application group at harvest. Then, the weight difference (weight loss) between fresh and stored fruit samples in the incubator at 20 °C for 120 h was determined using the following formula:

Weight loss (%) = the fruit weight (g) of fruit after incubating at 20 °C for $120 \text{ h} \times 100 \text{ / fresh fruit weight (g)}$.

LEAF PIGMENT ANALYSIS

The leaf pigment contents were determined according to the method of Arnon (1949). Fresh leaf tissues (200 mg) were homogenized in acetone (80%). Homogenates were centrifuged and the absorbances of supernatants were determined at 645, 652, 663, and 470 nm. The amounts of pigments were calculated according to the formula of Lichtenthaler and Wellburn (1983).

MINERAL ANALYSIS

Nitrogen content was determined by Kjeldahl method (Bremner 1965). Phosphorus (P) was quantified by vanadomolybdophosphoric acid colorimetric method. The K, Ca, Mg, Fe, Zn, Mn and Cu contents were determined using an AAS (Atomic Absorption Spectrophotometer) (Kacar & Inal 2008).

ANTIOXIDATIVE ENZYMES AND PROLINE ANALYSIS

Superoxide dismutase (SOD) activity was assayed spectrophotometrically as the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm according to Beauchamp and Fridovich (1971). Peroxidase (POD) activity was assayed by measuring the rate change in absorbance at 470 nm using the method of Omran (1980). Catalase (CAT) enzyme activity was determined by measuring the initial rate of H_2O_2 disappearance at 240 nm using the extinction coefficient (Aebi 1984). The proline content (μ g g⁻¹ FW) was determined according to Bates et al. (1973).

STATISTICAL ANALYSIS

The data were analysed by GLM procedure of SPSS (Windows Version SPSS, release 20.00). Means were compared with Duncan's Multiple Range test in the same software.

RESULTS AND DISCUSSION

GROWTH PARAMETERS

According to plant height measurements on 25th, 50th, 75th, and 100th days after initiation of the study, Pro-Ca applications affected plant height significantly (P<0.001) (Figure 1). The plants subjected to Pro-Ca doses 45 and 60 mg L⁻¹ were shorter than the control plants at all height measurement times (P=0.000). The plants with 15 mg L⁻¹ Pro-Ca were shorter than the control on the 25th and 50th days, but this difference disappeared on the 75th day and afterwards. The higher plant length was obtained in the plants having 30 mg L⁻¹ Pro-Ca and control on the 25th and 50th days after application. Only plants with 30 mg L⁻¹ Pro-Ca dose were higher on the 75th and 100th days compared to the plants with other doses.

The reduction effect of Pro-Ca at 45 and 60 mg L⁻¹ on the length between the internodes was higher than other doses, but this effect partially disappeared on 100^{th} day in 45 mg L⁻¹ Pro-Ca treatment (Figure 2). Plants with 15 mg L⁻¹ Pro-Ca and the control had similar length between thier internodes statistically at all measurement times. On the 25th day, the length between internodes in 30 mg L⁻¹ Pro-Ca plants was lower than the control. However, this difference was small on the 50th day and higher than the control plants on the 75th day.

The effect of Pro-Ca on stem diameter was dose dependent (Table 3). Along with the increase in Pro-Ca doses, an increase in the stem diameter was determined, and the highest stem diameter (11.01 mm) was obtained by 60 mg L⁻¹ Pro-Ca application. Fresh stem weight was decreased by Pro-Ca applications, but the plants with 30 mg L⁻¹ Pro-Ca had fresh stem weights similar to those of the control plants (P<0.05). Among all applications, the highest fresh stem weight was 945.7 g in plants subjected to 30 mg L⁻¹ Pro-Ca. Similarly, the highest dry stem weight was 102.5 g in plants subjected to 30 mg L⁻¹ Pro-Ca applied plants did not decrease compared to the control as such the fresh stem weight,

but on the contrary slight increase though not statistically significant. The effect of Pro-Ca, which is a gibberellic acid inhibitor, on plants has not always prevented growth and development. In some cases, the effect of Pro-Ca is growth promoting depending on plant species, growth stage, season, application dose and number.

Altıntaş (2011) reported that a low dose 50 mg L⁻¹ of Pro-Ca increased plant height, while higher doses (100, 200, and 300 mg L⁻¹) decreased plant height significantly. Altuntaş (2016) stated that the highest plant height and stem diameter were obtained in tomato plants subjected to 15 mg Pro-Ca against other application doses (0, 5, 30, and 45 mg L⁻¹). Kim et al. (2010) determined Pro-Ca doses (100, 200, and 400 mg L⁻¹) caused decreases in plant height and length between internodes in *Chrysanthemum morifolium* R. cv Monalisa White compared to control.

Because of effect on gibberellin inhibition, Pro-Ca decreased plant height and length between internodes of tomatoes (Altintaş 2011), onion (El-Ghorab et al. 2019), strawberries (Kim et al. 2019) and eggplant (Özbay & Ergun 2015) without decreasing the number of leaves. In the current study, the similar observations were obtained until on the 50th day in 30 mg L⁻¹ Pro-Ca dose and until the end of the trial in 45 and 60 mg L⁻¹ Pro-Ca doses. Altuntaş (2016) reported that Pro-Ca doses applied to tomato plants at different growthy stages increased plant fresh and dry

weights, and the highest values were obtained at the dose $30 \text{ mg } \text{L}^{-1}$ Pro-Ca as observed in this study.

Similar to the findings in this study, Akdemir (2018) study in lettuce seedlings showed that the higher doses (100 and 150 mg L^{-1}) of Pro-Ca increased stem diameter and decreased plant height and stem fresh weight, while the low dose (50 mg L^{-1}) Pro-Ca increased stem fresh weight. Kim et al. (2019) reported that 50 mg L^{-1} Pro-Ca application after planting increased fresh and dry weights of strawberry plant compared to control.

Although the effect of Pro-Ca on vegetative growth inhibiton depends on its application doses, when the application is continued for 3-4 weeks, the inhibition of endogenous GA biosynthesis may decrease, and consequently vegetative growth accelerates again. Compared to control, the suppressive effects of Pro-Ca on growth parameters was low at lower doses 15 and 30 mg L⁻¹, but was high at higher doses 45 and 60 mg L⁻¹. According to the present findings, it can be said that Pro-Ca blocks cell elongation, but not cell division. At 45 and 60 mg L⁻¹ doses, plant height and length between internodes decreased compared to control, though the stem diameter, fresh and dry stem weights increased. This may be due to Pro-Ca's decreasing effect on gibberellic acid level in apical meristems and its increasing effect on cytokine level.

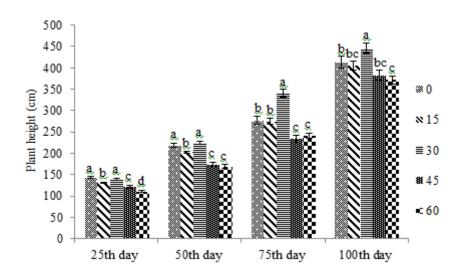


FIGURE 1. The effects of Pro-Ca (mg L⁻¹) treatments on plant height

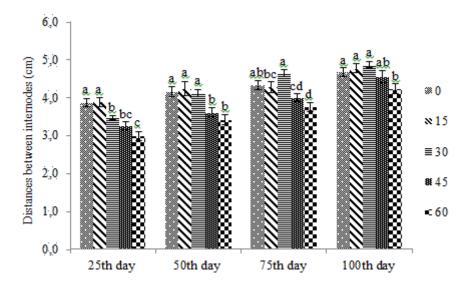


FIGURE 2. The effects of Pro-Ca (mg L⁻¹) treatments on the lengths between internodes

TABLE 3. The effects of Pro-0	Ca treatments on stem diamete	r, fresh and dry stem weights

	Pro-Ca treatments (mg L ⁻¹)								
	0	15	30	45	60	SEM	Р		
Stem diameter (mm)	9.57c*	9.71bc	10.16abc	10.56ab	11.01a	0.142	0.005		
Stem fresh weights (g)	892.5ab	826.8abc	945.7a	728.6c	757.9bc	23.10	0.012		
Stem dry weights (g)	84.19b	82.63b	102.47a	86.63b	88.59b	2.08	0.017		

*Means in each row with the different letters are significantly different (P < 0.05)

FRUIT QUALITY

Fruit firmness was increased by Pro-Ca application. Fruit firmness in the plants subjected to 30, 45, and 60 mg L⁻¹ Pro-Ca doses was significantly higher than those of the control and 15 mg L⁻¹ Pro-Ca applied plants (P<0.001) (Table 4). Pro-Ca doses increased significantly TSS, pH and EC values of cucumber fruit juice compared to those of the control (P<0.001). Among all Pro-Ca doses, the

highest TSS value (4.05%) was obtained in 45 mg L⁻¹ dose, the highest pH value (5.82) was in 60 mg L⁻¹ dose, and the highest EC value (4.50 dS m⁻¹) in 30 mg L⁻¹ Pro-Ca. Pro-Ca doses reduced the weight loss in cucumber fruits kept after harvest, except 15 mg L⁻¹ dose. Among all Pro-Ca doses, the lowest weight loss (20.1%) in cucumber fruits was obtained in 30 mg L⁻¹ Pro-Ca (Table 4). The observed weight losses in cucumber fruits were 29.4, 6, and 11.7% lower in the 30, 45, and 60 mg L⁻¹ Pro-Ca doses than that of the control, respectively. Similar to the findings in this study, Altuntas (2016) reported that Pro-Ca increased tomato fruit firnmess by obtaining the highest value at 30 mg L⁻¹ dose. Kang et al. (2010) reported that the increase of Pro-Ca doses increased the quality of Chinese cabbage by increasing total soluble sugar content and leaf hardness. Cucumber fruits have high Ca and K contents, increasing cell wall thickness and water holding capacity, extending the shelf life of fruits after harvest (Kaptan & Aydın 2007). In the current findings, the increase of Ca content in plants by application of 30, 45, and 60 mg L⁻¹ Pro-Ca doses increased significantly fruit firmness compared to the control. Moreover, these dose positive effects on cell wall thickness and resistance to stress reduced postharvest weight losses. Altuntas (2016) reported that Pro-Ca increased TSS contents of tomato fruits without statistical significance, and numerically higher values were obtained by 15 and 45 mg L⁻¹ doses. The increase in the total sugar and organic acid increases the taste and aroma in fruits. Pro-Ca decreases the amylase enzyme activity in plants and increases the total soluble sugar content of both fruit, and thus improves fruit quality (Rademacher et al. 2004).

The pH of fruit juice is an effective factor on taste of product. In vegetables, the pH is generally higher than 4.5. At low pH level, sourness increases in product. The products with high sugar content have low pH (Brown 2007). In the present findings, the increased pH, TSS and EC values by Pro-Ca applications contributed to taste and aroma, and consequently increased fruit quality.

FRUIT YIELD

The effect of Pro-Ca on yield was found to be variable. The highest yield per plant (9182.1 g) was obtained with 30 mg L⁻¹ of Pro-Ca. Yields of plants in other Pro-Ca doses were lower than the control (7976.9 g) (P<0.001) (Figure 3). It was reported that Pro-Ca increased productivity by affecting basic physiological events such as photosynthesis in different plant species besides growth inhibition. Consistent with the present findings, Özbay and Ergun (2015) reported that higher Pro-Ca doses (100 and 150 mg L⁻¹) decreased yield in eggplant, but no yield loss was seen with 50 mg L⁻¹ dose. The study of tomato showed that increase in Pro-Ca application dose and its application number significantly reduced total yield and early yield (Altıntaş 2011).

Asin et al. (2007) reported that Pro-Ca application suppresses growth with no negative effect on yield and flowering time. However, its positive effects on yield and fruit quality on apple and pear (Rademacher et al. 2004), tomato strawberry (Poledica et al. 2012) and onion (El-Ghorab et al. 2019) were reported. Kang et al. (2010) reported that Pro-Ca, when applied at different times and doses after planting, significantly increased yield by promoting growth parameters (head yield, leaf count, and head density) in Chinese cabbage.

Pro-Ca delays aging by reducing ethylene production by blocking 1-aminocyclopropane-1-carboxylate-oxidase (ACC-oxidase), an important enzyme in ethylene biosynthesis in plants. In the current study, when the general appearance of the plants were examined, it was observed that the plants treated with 30 mg L⁻¹ Pro-Ca showed less signs of aging compared to the other doses.

Pro-Ca as a gibberellic acid inhibitor may therefore have increased the yield at a dose of 30 mg L^{-1} . With the removal of the suppressive effect of 30 mg L^{-1} , Pro-Ca on plant growth in the later stages increased nutrient intake, leaf pigmentation, proline content, enzyme activity, and photosynthesis ratio, triggering an increase in yield in tested plants.

LEAF PIGMENTATION

Pro-Ca affected leaf pigmentation in cucumber plants significantly (P<0.001). Chlorophyll a level was significantly higher in leaves of plants subjected to 30, 45 and 60 mg L⁻¹ doses of Pro-Ca compared to those of the control (P<0.001) (Table 5). Chlorophyll b contents in the 15 mg L⁻¹ and 30 mg L⁻¹ Pro-Ca applied plants were similar to those of the control, while these contents were lower in the leaves of 45 and 60 mg L⁻¹ Pro-Ca applied plants (P<0.001). The highest total chlorophyll content was obtained in 30 mg L⁻¹ Pro-Ca dose with 5.312 mg g⁻¹. The total chlorophyll contents were lower in the leaves of plants subjected to 45 and 60 mg L⁻¹ Pro-Ca doses compared to that of the control. Similarly, the highest carotenoid content (1.234 mg g⁻¹) was detected in plants treated with 30 mg L⁻¹ Pro-Ca dose, but was decreased by 45 and 60 mg L^{-1} doses.

Kang et al. (2010) demonstrated significant decreases in leaf length and width with the effect of Pro-Ca applications but a significant increase in leaf chlorophyll content. The effect of Pro-Ca on leaf pigment level was dose dependent. In the current study, increases in chlorophyll a/b ratio were obtained by the increasing Pro-Ca doses. The chlorophyll a/b values were 1.98, 2.05, 2.18, 3.68, and 3.43 in control, 15, 30, 45, and 60 mg L⁻¹ Pro-Ca doses, respectively. The increase in chlorophyll a/b ratio is considered as a sign that the plants are resistant to

stress conditions or away from stress (Chettri et al. 1998). The higher chlorophyll a/b values obtained with higher Pro-Ca doses may suggest that Pro-Ca helps the resistance of plants to stress conditions.

Carotenoids act as antioxidants by protecting plant cells from the damage of superoxide and singlet oxygen released under stress conditions. In addition, carotenoids inhibit chlorophyll destruction by neutralizing free oxygen radicals (Edge & Truscott 2018). In the present study, as in total chlorophyll contents, the caroteneoid contents were decreased at 45 and 60 mg L⁻¹ doses compared to that of the control.

Kim et al. (2010) reported that leaf chlorophyll content was increased by Pro-Ca as the total leaf area decreased. Similar finding was obtained in the present study that the highest amount of fruit yield with total chlorophyll and carotenoid contents per plant was obtained by 30 mg L⁻¹ Pro-Ca dose. In plants, leaf chlorophyll and carotenoid contents are directly affected by adequacy of the supply of nutrients. The present decreases in the chlorophyll and carotenoid contents in the leaves of plants subjected to 45 and 60 mg L⁻¹ Pro-Ca could be explained by the decreases in the concentrations of N, P and K, Mg and Fe. As well known, Mg is present in the structure of chlorophyll while Fe acts a role in its synthesis.

LEAF NUTRIENT CONTENTS

The effect of Pro-Ca on leaf nutrient contents was found to be significant (Table 6). The effects of Pro-Ca on concentrations of N, P, K, Mg, and Fe were similar. Higher concentrations of nutritional elements were observed in the leaves of plants subjected to 30 mg L-1 Pro-Ca, though there was no significant change in the leaves of plants subjected to 15 mg L⁻¹ Pro-Ca. Pro-Ca significantly reduced the mineral contents in cucumber leaves at 45 and 60 mg L⁻¹ doses, but it did not show effect at 15 mg L⁻¹ dose. However, 30 mg L⁻¹ dose had positive efficacy on intakes of these minerals. Ca content in leaves was increased significantly by Pro-Ca applications, and it was dose dependent, especially at 30, 45, and 60 mg L⁻¹ doses (P<0.000). The highest Zn content was found in plants applied with 30 mg L⁻¹ Pro-Ca dose, but no significant reduction was observed in plants applied with 45 and 60 mg L⁻¹ Pro-Ca dose. Mn content increased in leaves of plants applied with 30, 45, and 60 mg L⁻¹ Pro-Ca doses compared to the control, while the highest Mn content was obtained by 30 mg L-1 Pro-Ca dose. Cu content was

significantly decreased in 45 and 60 mg L^{-1} Pro-Ca doses (P<0.05), but not in the other doses.

Except for 15 mg L⁻¹ dose, Pro-Ca doses increased Ca content in leaves. Ca has an active role on cell division, and it is present in plant cell membrane as pectate and phospahate forms. It is also involved in the transport of carbohydrates and amino acids in plants (Freitas et al. 2012). Therefore, the increase in Ca content in plants with 30, 45, and 60 mg L⁻¹ Pro-Ca doses, most likely increased fruit firmness with plant growth and positively reduced post-harvest weight loss. It was reported that Pro-Ca application increased leaf nutrient contents, especially Ca, Fe and Mn (Altuntaş 2016). Similarly in the present study, Ca and Mn contents in the plants subjected to Pro-Ca doses were observed higher than the control.

El-Ghorab et al. (2019) reported that Pro-Ca application at 10, 20, and 30 mg L⁻¹ doses increased the N, P, K and S contents in onion, and this was more prominant for 20 and 30 mg L⁻¹ doses. In the current study, 30 mg L⁻¹ Pro-Ca dose had the highest increasing effect on all analysed macro and micro elements except for Cu. The high intakes of these elements, which are necessary for plant development, can be considered as one of the main reasons for positive contribution of 30 mg L⁻¹ Pro-Ca dose to the other examined parameters.

ENZYME ACTIVITIES AND PROLINE CONTENT

Pro-Ca increased SOD, CAT, and POD enzyme activities by stimulating the antioxidative defense system in cucumber plants (P<0.001) (Table 7). Except for 15 mg L⁻¹ dose, the increased Pro-Ca doses caused increases in these enzyme activities. While the highest SOD, CAT and POD activities were observed in plants applied with 60 mg L⁻¹ Pro-Ca dose, SOD, and POD activities were statistically similar in the plants treated with 45 and 60 mg L⁻¹ doses. Compared to the control, SOD activity in plants treated with 30 mg L⁻¹ Pro-Ca increased significantly, while the increase in CAT and POD activities was observed at a lower level. Except for 15 mg L⁻¹ dose, Pro-Ca application doses increased the content of proline, which is an important osmoprotectant, similar to the effects of Pro-Ca effects on enzyme activities (Table 7). On the other hand, unlike on enzyme activities, the highest proline content (195.1 µg per g fresh weight) was obtained by 30 mg L⁻¹ Pro-Ca. It was also observed that the highest dose of Pro-Ca decreased proline content, but it was still higher than that of the control.

Antioxidant enzymes, as an important ROS detoxification system in plant cells, protect plants against oxidative stress (Zhang et al. 2012). El-Ghorab et al. (2019) reported that Pro-Ca had positive effects on growth, development, yield, mineral content, and antioxidant activity in onion plant. Fard et al. (2015) reported that Pro-Ca increased SOD, ascorbate peroxidase (APX) and CAT activities, which are the antioxidant enzymes, in turf plants grown under both well irrigated and drought conditions.

The observed high enzyme activities might be resulted from the stress caused by the application of 45 and 60 mg L⁻¹ Pro-Ca doses since these high doses decreased leaf pigment level and nutrient contents. However, the positive effects of 30 mg L⁻¹ Pro-Ca dose on plant growth, fruit yield and quality showed that this dose increased enzyme activities without causing any kind of stress. Also, the increase in the enzyme activities with this dose was reflected in positive plant growth and yield.

Aghdam (2013) reported that Pro-Ca increased proline content of tomato leaves. Bekheta et al. (2009)

reported that Pro-Ca increased proline, photosynthetic pigments, total carbohydrates and total soluble sugars in faba bean seedlings grown under salt stress. Özbay and Süslüoğlu (2016) added Pro-Ca to the priming solution, and they observed an increase in germination performance of pepper seeds at low temperature by improving the proline, total carbohydrate and total soluble sugar content of the seeds. Borzouei et al. (2012) reported that the increase in SOD and proline levels increased resistance to stress conditions. Acting as an osmotic regulator between cytoplasm and vacuole, the proline contributes to the preservation of membrane integrity and protects plant tissues against stress conditions by detoxifying reactive oxygen species.

The increased leaf proline content with 30 mg L⁻¹ of Pro-Ca increased nutrient uptake in plants due to proline's osmoregulator role. The increased pigment level in plants that feed better increases photosynthesis, and consequently increases in photosynthesis that increases both vegetative and reproductive (fruit yield) developments.

	Pro-Ca treatments (mg L ⁻¹)								
	0	15	30	45	60	SEM	Р		
Fruit firmness (N)	46.24b*	48.92b	55.63a	57.70a	55.26a	1.019	0.000		
pH (%)	5.68c	5.79ab	5.76b	5.75b	5.82a	0.013	0.000		
TSS (%)	3.52b	3.92a	3.95a	4.05a	3.98a	0.048	0.000		
EC (dS m ⁻¹)	3.53c	3.98b	4.50a	4.28a	4.33a	0.087	0.000		
Weight loss (%)	28.45a	28.83a	20.10d	26.73b	25.11c	0.620	0.000		

TABLE 4. The effects of Pro-Ca treatments on pomological characteristics of fruits

*Means in each row with the different letters are significantly different (P<0.05)

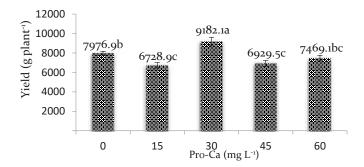


FIGURE 3. The effects of Pro-Ca treatments on yield (g plant⁻¹)

		Pro-C					
	0	15	30	45	60	SEM	Р
Chlorophyll <i>a</i>	2.750c*	2.765c	2.954a	2.862b	2.952a	0.022	0.000
Chlorophyll <i>b</i>	1.387a	1.349a	1.353a	0.777c	0.860b	0.062	0.000
Total chlorophyll	5.001b	4.971b	5.312a	3.808d	4.087c	0.137	0.000
Carotenoid	1.199a	1.202a	1.234a	1.046c	1.113b	0.017	0.000

TABLE 5. The effects of Pro-Ca treatments on leaf pigmentation (mg g^{-1} FW)

*Means in each row with the different letters are significantly different (P<0.05)

TABLE 6. The effects of Pro-Ca treatments on nutritional contents of cucumber leaves

Pro-Ca	N	Р	K	Са	Mg	Fe	Zn	Mn	Cu
(mg L ⁻¹) (ppm)	(%)	(%)	(%)	(%)	(%)	(mg kg ⁻¹)			
0	3.196b*	0.596b	4.185b	3.005b	0.435b	128.0b	46.42ab	37.20c	14.83a
15	2.943b	0.569b	4.015b	3.145b	0.413b	131.2b	42.79b	37.40c	13.76a
30	3.842a	0.683a	4.770a	4.437a	0.570a	150.4a	51.11a	46.39a	13.79a
45	2.250c	0.420c	3.330c	4.400a	0.278c	94.9c	45.50ab	42.89ab	10.38b
60	2.057c	0.473c	3.220c	4.460a	0.326c	107.7c	39.06b	40.88bc	10.25b
SEM	0.154	0.227	0.145	0.172	0.249	5.069	1.295	1.046	0.553
Р	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.008	0.004

*Means in each coloum with the different letters are significantly different (P<0.05)

TABLE 7. The effects of Pro-Ca treatments on enzyme activities and proline content

	_						
	0	15	30	45	60	SEM	Р
SOD (U.g ⁻¹ FW)	105.1c*	104.4c	127.4b	146.3a	149.3a	4.721	0.000
CAT (U.g ⁻¹ min ⁻¹)	21.84c	23.61c	27.71bc	32.13ab	34.89a	1.355	0.001
POD (U.g ⁻¹ min ⁻¹)	14.32b	14.50b	15.35ab	16.14a	16.24a	0.260	0.027
Proline (µg.g ⁻¹)	156.4c	146.4c	195.1a	189.1ab	175.6b	4.551	0.000

*Means in each row with the different letters are significantly different (P<0.05)

CONCLUSION

The present findings showed that Pro-Ca, which acts a gibberellic acid inhibitor not only shortens cell length, but also has positive effects on plant growth, yield and fruit quality at appropriate dosage and administration time. It was also showed that Pro-Ca application can increase the tolerance of biotic and abiotic stress conditions in plants by activating the enzymatic antioxidant defense system and increasing leaf proline content. To conclude, a 30 mg L⁻¹ of Pro-Ca application had positive effects on cucumber plant growth, yield and fruit quality. Therefore, this Pro-Ca dose can be used in greenhouse cucumber cultivation to reduce post-harvest weight loss and increase storage and transport time of cucumber fruits with short shelf life.

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