

COMPARATIVE STUDY ON THE EFFECTS OF VARIOUS PLANT GROWTH REGULATORS ON GROWTH, QUALITY AND PHYSIOLOGY OF *CAPSICUM ANNUUM* L.

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Abstract

Pre- and post-harvest physiology and quality responses of green pepper (*Capsicum annuum* L. cv 'Standar p.13/0211003-01-Agris') on exogenous Gibberellic acid-GA₃ (100 μM), Prohexadione-Calcium (100 mg l⁻¹), Cycocel (100 mg l⁻¹) and Ethephon (100 mg l⁻¹) applied as foliar sprays, were investigated. Among PGRs, GA₃ @ 100μM was effective in promoting flowering and better for vegetative characteristics. Chlorophyll a+b concentration of leaves was markedly inhibited by GA₃, Prohexadione-Calcium, Cycocel and Ethephon application while, the maximum quantum yield of primary photochemistry (F_v/F_m) and the ratio F_v/F_o was slightly increased under GA₃. The above indices were significantly depressed under Cycocel, Ethephon and Prohexadione-Calcium. CO₂ production pattern was negatively related with chlorophyll fluorescence (F_v/F_o) yield and ascorbic acid content. The Brix content and the "maturity index" were depressed after Prohexadione-Calcium, Cycocel and Ethephon application. In general, the best green pepper appearance was found under control and GA₃ whereas, pre- and post-harvest physiology and quality characteristics dropped sharply and green peppers were not marketable under the three growth retardants.

Introduction

In recent years, interest and demand for peppers has increased dramatically worldwide and peppers have achieved major economic significance in the global market. They are considered to be good sources of various beneficial phytochemicals such as carotenoids, flavonoids and ascorbic acid. *Capsicum annuum* (Bell pepper) is among the most vegetable crops cultivated and produced under irrigated agriculture. It has great significance in many countries such as China, Mexico, Turkey, India, Hungary and Greece. The enormous genetic diversity available for pepper breeding offers a potential for the development of new varieties and hybrids. Sweet peppers in Greece are produced for both the fresh (green peppers) and processing (red peppers) markets. A good yield should be 800-1000 bu/A (Delfine *et al.*, 2000; Niklis *et al.*, 2002).

The manipulation of growth and increasing productivity and quality of vegetables is the basis for most plant-related research. As a result many compounds are used to accelerate flowering and fruiting in young plants. The most widely available plant growth regulator is GA₃ or gibberellic acid, which induces stem and internode elongation, seed germination, enzyme production during germination and fruit setting and growth (Davies, 1995). PGRs are also used to control vegetative growth (Latimer, 1991; Ouzounidou *et al.*, 2008). The most commonly used PGRs are those, which inhibit GA₃ biosynthesis. Prohexadione-Calcium (Prohexadione-Ca) blocks GA biosynthesis between kaurene and kaurenoic acid, whereas Ethephon is one of the growth retardants, which do not inhibit GA biosynthesis. It's mode of action acts *via* liberation of ethylene, which is absorbed by

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the plant and interferes in the growth process (Leclerc *et al.*, 2006). It is reported that Ethephon decreases photosynthesis by increasing ethylene levels (Davies, 1995). Plants treated with Cycocel, a compound that blocks GA biosynthesis, have shortened internodes and enhanced photosynthesis (Davies, 1995). Plant growth retardants generally have the greatest effects on expanding or elongating cells, where inhibition of GA synthesis rapidly causes reduction in stem elongation and leaf expansion (Tanimoto, 1987; Leclerc *et al.*, 2006). Flowering has been hastened or delayed by PGRs depending on species (Latimer, 1991).

Measurements of chlorophyll fluorescence are linearly correlated with the functionality of PSII, where F_o and F_m are the chlorophyll fluorescence yields corresponding to open and closed PSII reaction centres, respectively (Ilias *et al.*, 2007). Fluorescence method can be used in order to determine changes concerning different growth stages of vegetables (Ouzounidou *et al.*, 2008). In parallel, early changes of maturity of various tissue characteristics in vegetables have been identified, including respiration, vitamin C and chlorophyll content (Solomos, 1983). Changes in respiration rate and vitamin C are considered sensitive indicators of changes in tissue condition after harvest (Perrin & Gaye, 1986).

The varying responses of pepper to plant growth regulators have been reported by Chattopadhyay & Sen, (1974), Minraj & Shanmugavelu, (1987), Balraj *et al.*, (2002), Joshi *et al.*, (1999) and Chaudhary *et al.*, (2006). However, information regarding the effectiveness of PGRs on growth and other physiological parameters of commercial pepper cultivars is very little. The current research attempted to enhance our knowledge on growth, quality and yield response of green pepper plants and fruits to selected plant growth regulators; while, emphasis has been placed on the impact of the above substances on the physiology of pepper photosynthesis.

Materials and Methods

Plant material and cultivation: Green pepper (*Capsicum annuum* L., cv 'Standar p.13/0211003-01-Agris') seeds were germinated on a greenhouse mist bench (20s of mist every 30 minute) set at $22 \pm 2^\circ\text{C}$. Three weeks after complete germination uniform seedlings were transplanted individually into 300 ml plastic pots containing a commercial potting mix (Pot ground Klasmann-Dielmann GmbH, Art-Nr.4390, Germany). Two weeks later, plants were transplanted individually and randomly inside greenhouse, in 15 experimental plots. Experiment was established on a sandy loam soil whose physicochemical characteristics were silt 18%, clay 5.6%, sand 70.4%, organic matter 0.88%, CaCO_3 0.9%, E.C. $1.5 \mu\text{S cm}^{-1}$, and pH (1:2 H_2O) 7.4. Plants were acclimatized for one week in the greenhouse before being subjected to the treatments.

Plants were watered as required and fertilized at each irrigation with 300 ml of 60mg N- 26.2mg P- 49.8mg K water- soluble fertilizer (20-20-20 F-TOP Ledra Ltd, Thessaloniki) during the experiment. Plants were maintained in greenhouse under natural sunlight while average day and night temperatures were $30 \pm 2^\circ\text{C}$ and $28 \pm 2^\circ\text{C}$, respectively. Relative humidity was 70-80% while photosynthetically active radiation was $500\text{-}700 \mu\text{mol m}^{-2}\text{s}^{-1}$.

Applications of treatments: GA_3 at $100 \mu\text{M}$, Cycocel, Ethephon and Prohexadione-Ca (BAS 125 10W, BASF Corp., Research Triangle Park, N.C) at 100 mg l^{-1} respectively

were evaluated. Each solution contained 0.1% Agral 90 as a surfactant (*Syngenta*, Ontario, Canada). A set of 18 green pepper plants in each plot was sprayed to run off two times at 2-week intervals with each of the above solutions. First application of the above solutions was made 3 weeks after germination (plants had nine to ten leaves). Control plants were treated with water and surfactant. PGR concentrations and spraying time have been selected after preliminary experiments.

***In vivo* chlorophyll fluorescence measurements:** Fast chlorophyll fluorescence was measured on the upper surface of the latest fully expanded leaf and used for primary photochemistry detection. The chlorophyll fluorescence induction curve was monitored by a Plant Efficiency Analyzer (PEA, Hansatech Ltd King's Lynn, Norfolk, England) with 600 Wm^{-2} of red (630) light intensity (excitation intensity), and were left for 30 min., to dark adaptation, at room temperature. Different values were selected in order to determine any structural and functional changes of the photosynthetic apparatus as a result of the different growth regulator applications. The initial fluorescence intensity (F_o) when all reactions centres (RCs) are open, the maximal fluorescence intensity when all reactions are close (F_m), the variable fluorescence (F_v) and the time to reach the maximal fluorescence intensity (t_{\max}), were calculated. The indicators were measured at room temperature on intact leaves of five replicate plants from the five treatments (Ouzounidou & Ilias, 2005). Ratios F_v/F_m and F_v/F_o , which provide an estimation of the maximal photochemical efficiency of photosystem II and the apparent quantum yield of the photosynthesis rate (Ouzounidou *et al.*, 2006) were used, to evaluate alterations under our experimental conditions.

Leaf chlorophyll content: Chlorophyll a+b was extracted in 100% acetone. Absorbance was measured at 663 and 645 nm using an LKB Ultraspec II spectrophotometer (Ouzounidou *et al.*, 1997).

Respiration rate measurements: A sample of 5 fruits was used to measure respiration. Gas exchange measurements (CO_2) were made on individual fruits in glass jars. These jars were placed at 20°C . One millilitre of the gas sample was removed using a special syringe, after the jars had been closed for 1h, and injected into a gas-chromatograph Perkin-Elmer 8700 with a TC detector. All results are expressed as $\text{mgCO}_2 \text{ kg}^{-1}\text{h}^{-1}$.

Quality assessment: The ascorbic acid content of pepper was estimated by macerating the fruit sample mechanically with a stabilising agent (5% metaphosphoric acid) and titrating the filtered extract with 2,6 dichlorophenolindophenol. Acidity was measured by titration potentiometrically with alkali 0.1 NaOH to an end-point of 8.2. The acidity was calculated as anhydrous citric acid, which is the predominant acid. An automatic digital refractometer of the firm Index Instruments UK, type GPR 12-70, was used to determine the Soluble Solids of the fruit samples. Results were expressed as Brix at 20°C . Glucose, fructose and saccharose were determined with an HP 1100 Series High Performance Liquid Chromatograph (refractive index detector (RID) using a reverse phase column 250x4mm (Lichrosphere NH_2) bonded to microparticulate silica of 5 μm diameter maintained at 37°C . Injection of 20 mm^3 of sample solution into a mobile solvent of $\text{H}_2\text{O}/\text{AcCN}$ (25:75; v/v) with a flow rate of 1.1 $\text{cm}^3 \text{ min}^{-1}$ gave the optimum result (Ouzounidou *et al.*, 2008).

Experimental design and data analysis: Fifteen experimental plots (three replications for each treatment) were set up randomly inside the greenhouse using a randomized complete block design. Eighteen single plants were used in each treatment combination/replication. Each plot contained three rows with six plants per row spaced 50 cm apart within each row. Distance between rows was 60 cm while distance between plots was 100 cm. Days to anthesis (from germination), plant height, first internode's length, length of the first leaf, number of fruits per plant and fruit length were recorded. Leaf chlorophyll content, leaf chlorophyll fluorescence, respiration rate and some quality characteristics of the fruits were also measured at the same time. Data were subjected to analysis of variance (ANOVA) and the treatment means were compared using the LSD test at $p=0.05$, spss procedure.

Results

A significant acceleration by 8.3% and a significant retardation by 42% of the control in the time of flowering of green pepper plants under GA₃ and Ethephon application was recorded (Table 1). There was no significant delay under Prohexadione-Ca and Cycocel. With respect to the effect of PGRs, GA₃ exhibited better results to other growth regulators concerning plant height and number of fruits per plant. GA₃ promoted the elongation of the first internode by 30% of the control, but a significant inhibition under Cycocel and Ethephon by 33 and 42% respectively, was observed (Table 1). First leaf length was depressed markedly under Prohexadione-Ca, Cycocel and Ethephon supply. Chlorophyll a+b concentration of green pepper leaves was markedly inhibited by 34, 53, 43 and 50% under GA₃, Prohexadione-Ca, Cycocel and Ethephon application respectively. Chlorophyll a was more affected than Chl b (Fig. 1). On the contrary, the maximum quantum yield of primary photochemistry (F_v/F_m) and the ratio F_v/F_o was slightly increased under GA₃. The two chlorophyll fluorescence indices were significantly depressed under Cycocel, Ethephon and Prohexadione-Ca by 34, 57 and 67% of the control, respectively (Fig. 1).

Fluctuations of carbohydrates content of pepper fruits are given in Table 2; saccharose content doubled in response to the application of GA₃ and glucose was inhibited by 25% as a result of Ethephon application (Table 2). The Titratable Acidity, expressed as g citric acid kg⁻¹ fruit fresh weight, was significantly reduced (by 25%, $p<0.05$) by Ethephon exposure, moreover, total soluble solids (TSS) were markedly inhibited (by 25%, $p<0.05$) as a result of Prohexadione-Ca, Cycocel and Ethephon application (Table 2). In addition, the content of ascorbic acid increased by 13% of the control ($p<0.05$) under GA₃ but decreased sharply under the growth retardants (by 57% of the control under Ethephon supply, Table 2). A similar pattern was followed by the "maturity index" reflecting the ratio of TSS to the Acidity, revealing the highest drop (by 40%, $p<0.05$) under Ethephon application (Fig. 2). Under GA₃ treatment green pepper presented the lowest respiration rate, showing a significant decrease by 29% of the control. On the contrary, CO₂ production increased significantly by 11, 24 and 37% of the control under Prohexadione-Ca, Cycocel and Ethephon, respectively (Fig. 2).

Table 1. Days of flowering, plant height, first internode length, first leaf length, number of fruits per plant, and fruit length of green pepper plants as affected by the exogenous application of GA₃ (100µM), Prohexadione-Ca (100mg l⁻¹), Cycocel (100mg l⁻¹), and Ethephon (100mg l⁻¹).

Treatments	Days of flowering	Plant height (cm)	First internode length (cm)	First leaf length (cm)	Number of fruits/plant	Fruit length (cm)
Control	85	70.2 ± 3.1	12.0 ± 1.3	12.0 ± 0.9	110	6.3 ± 0.5
GA ₃	78*	81.3 ± 2.6*	15.5 ± 1.8*	12.5 ± 1.1 n.s.	124*	6.7 ± 0.1 n.s.
Prohexadione-Ca	88 n.s.	69.2 ± 3.1 n.s.	7.5 ± 1.0*	6.5 ± 0.9*	106 n.s.	6.1 ± 0.3 n.s.
Cycocel	87 n.s.	63.7 ± 2.9*	8.0 ± 0.9*	7.5 ± 1.0*	87*	5.7 ± 0.2*
Ethephon	121*	61.1 ± 2.8*	7.0 ± 0.8*	4.5 ± 0.4*	84*	5.5 ± 0.4*

Values are means ± standard error, n=54 *differences from control significant at p<0.05, n.s.: no significant difference.

Table 2. Sugar content (% DW), Titratable acidity (g citric acid kg⁻¹ DW), Soluble solids (Brix, % DW) and Ascorbic acid (g kg⁻¹ DW) of green pepper fruits as affected by the exogenous application of GA₃ (100µM), Prohexadione-Ca (100mg l⁻¹), Cycocel (100mg l⁻¹), and Ethephon (100mg l⁻¹).

Treatments	Fructose	Glucose	Saccharose	Titratable acidity	Soluble solids	Ascorbic acid
Control	1.3 ± 0.08	1.6 ± 0.10	0.2 ± 0.08	12.8 ± 0.2	4.0 ± 0.2	0.7 ± 0.01
GA ₃	1.3 ± 0.05	1.8 ± 0.09	0.5* ± 0.03	12.7 ± 0.3	4.2 ± 0.3	0.8* ± 0.08
Prohexadione-Ca	1.2 ± 0.07	1.6 ± 0.04	0.2 ± 0.07	11.5 ± 0.08	3.0* ± 0.2	0.5* ± 0.02
Cycocel	1.2 ± 0.03	1.6 ± 0.05	0.2 ± 0.06	13.3 ± 0.07	3.0* ± 0.3	0.2* ± 0.01
Ethephon	1.2 ± 0.04	1.2* ± 0.02	0.2 ± 0.03	16.1* ± 0.06	3.1* ± 0.3	0.3* ± 0.02

Values are means ± standard error, n=3, *differences from control significant at p<0.05.

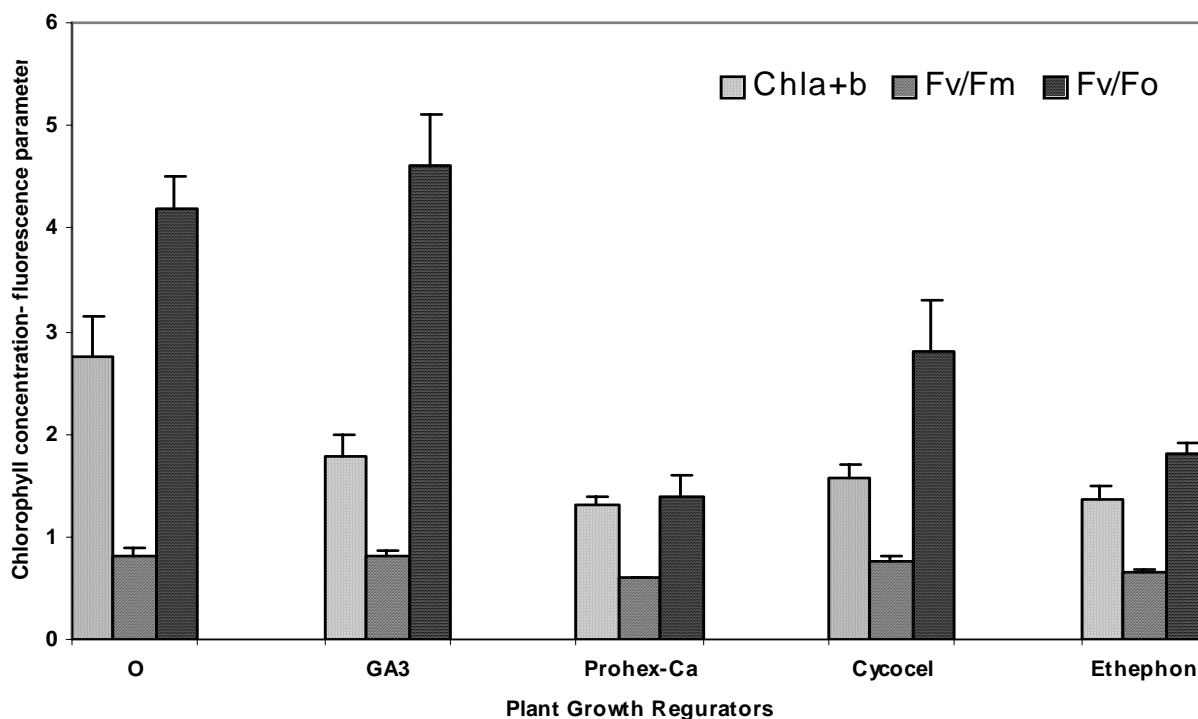


Fig. 1. Changes in chlorophyll a+b concentration (mg g^{-1} FW) and some chlorophyll fluorescence parameters ($n=5$,) of *Capsicum annuum* L. cv 'Standar p.13/0211003-01-Agris' fully expanded leaves, as a function of PGRs application. Vertical bars represent the standard error.

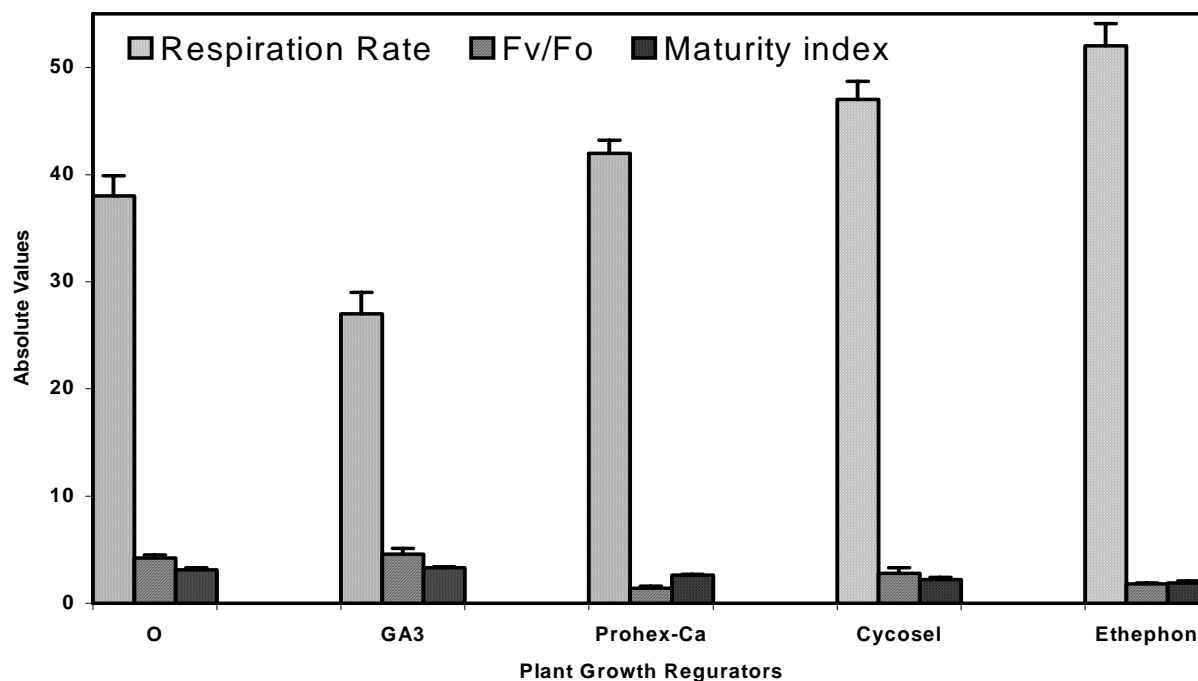


Fig. 2. CO_2 production ($\text{mgCO}_2 \text{ kg}^{-1}\text{h}^{-1}$) ($n=3$), F_v/F_o ($n=5$) and maturity index (Soluble Solids/Titratable Acidity) ($n=3$) of *Capsicum annuum* L. cv 'Standar p.13/0211003-01-Agris' as affected by the application of PGRs. Vertical bars represent the standard error.

Discussion

Improvement in pepper growth and yield under GA₃ application compared to the control was observed. This might be ascribed to more efficient utilization of food for reproductive growth (flowering and fruit set), higher photosynthetic efficiency and enhanced source to sink relationship of the plant, reduced respiration, enhanced translocation and accumulation of sugars and other metabolites. Inhibition of growth performance on exposure to the other PGRs occurred. Our findings are comparable to ours concerning the melon fruits responses to various growth regulators (Ouzounidou *et al.*, 2008) and to those of Nakayama *et al.*, (1992) who found a reduction on rice height under Prohexadione-Ca application with a concomitant reduction of endogenous gibberellin concentration. Mata *et al.*, (2006) have also reported, an inhibition of shoot elongation in apple trees after Prohexadione-Ca application. Prohexadione-Ca has a potential for effective control of vegetative growth in several plant species however, timing seems to be very important (Ilias & Rajapakse, 2005). It has been shown that multiple low-rate applications are more effective than a single, high-rate treatment (Brown *et al.*, 1997). In contrast, according to Hisamatsu *et al.*, (2000), PGRs which inhibit later stages of GA₃ biosynthesis, promoted stem elongation and flowering as well. Inhibition of both cell division and cell elongation has been found under the effect of Cycocel (Rajala & Peltonen-Sanio, 2001). Ethephon, an ethylene-releasing compound, can also be used to retard stem and leaf elongation, promote lateral branching, delay flowering date and diminish fruit size (Hayashi *et al.*, 2001; Ouzounidou *et al.*, 2008).

According to Jordi *et al.*, (1995) GA₃ has been reported to delay the loss of chlorophyll. Our data are different, showing a sharp decrease of chlorophyll concentration. Even though chlorophyll loss was a common feature, no visually apparent chlorosis or yellowing of the leaves, during PGRs application, was observed. In parallel, the chlorophyll fluorescence characteristics were negatively affected by the three growth retardants application. The efficiency of photochemistry (F_v/F_m) declined showing alterations of PSII reaction centers and an inhibition of enzymatic process in the Calvin cycle of pepper plants subjected by Prohexadione-Ca, Cycocel and Ethephon. The observed decline of variable fluorescence (F_v) represents a general decline in chloroplast function after exposure to PGRs. Measurements of respiration and F_v provide direct information on the functioning of mitochondria and chloroplasts, respectively (Ouzounidou *et al.*, 2006). These organelles are very sensitive to early stages of deterioration in plant tissue (Solomos, 1983; Dalling & Nettleton, 1986). In our experiment, CO₂ production pattern of fruits was negatively related with leaves chlorophyll fluorescence (F_v/F_o), measured at the same time. Fluorescence changes in response to CO₂ have been found in broccoli (Deell & Toivonen, 2000) and in maize (Ireland *et al.*, 1984). The increase in respiration rate of green pepper fruits and the decrease in “maturity index” content correlate well with the chlorophyll fluorescence under Prohexadione-Ca, Cycocel and Ethephon application, representing the reduction in freshness and the beginning of senescence. Decreased photosynthetic activity and /or growth of tissues are followed by reduction of plant productivity.

Besides elongation reduction, enhanced antioxidant activity following treatment with GA-synthesis inhibitors as well as a promotion of leaf pigments has been reported (Rademacher, 2000). Our findings are quite different, since under the application of the three growth retardants, a significant decrease in the ascorbic acid content of pepper was observed. Since horticultural maturity is defined as the development stage where

harvested fruit undergoes normal ripening and provides good eating quality, sugar content is likely to affect the eating quality of the fruit. The Brix content and the “maturity index” (TSS/Acidity) were depressed after Prohexadione-Ca, Cycocel and Ethephon application. In general, quality characteristics dropped sharply and green peppers were not marketable under the three growth retardants. However, it is important to note that peppers sprayed with GA₃ had higher concentrations of soluble solids and ascorbic acid. The augmentation of ascorbic acid content might be due to either increased ascorbic acid biosynthesis or to protection of synthesized ascorbic acid from oxidation through ascorbic acid oxidase. Ascorbic acid content of the chilli fruit was significantly increased with the application of GA₃ over other growth regulators (Chaudhary *et al.*, 2006). Gonzalez-Rossia *et al.*, (2007) also found an increased in quality characteristics of GA₃-treated peaches and nectarines; while changes on apple quality, like sugars, soluble solids and titratable acidity under Prohexadione–Ca were recorded by Mata *et al.*, (2006). Malic acid content gradually decreased and soluble solids content slightly increased during maturation under Cycocel, Ethephon and Prohexadione-Ca application in apples (Awad & Jager, 2002).

Overall, it is obvious that the application of GA₃, Prohexadione-Ca, Cycocel and Ethephon to green pepper plants leads to different alterations in developmental, pre- and post-harvest physiological and metabolic pathways. Based on plant and fruit performance it can be suggested that spraying with gibberellic acid, two times at two weeks intervals and three weeks after seed germination, maximizes the yield and achieves acceptable quality of *Capsicum*.

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