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PLANT GROWTH REGULATORS TREATMENTS MODULATE GROWTH, PHYSIOLOGY AND QUALITY CHARACTERISTICS OF *CUCUMIS MELO* L. PLANTS

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Abstract

Our objective was to identify the effects of Gibberellic acid (GA_3), Prohexadione-Calcium (Prohex-Ca), Cycocel and Ethephon applied as foliar sprays, on pre- and post-harvest physiology and quality characteristics of melon (*Cucumis melo* L.). GA_3 promoted melon growth, while a significant inhibition with Cycocel and Ethephon was observed. The chlorophyll a+b concentration as well as the chlorophyll fluorescence characteristics were negatively affected by Prohex-Ca, Cycocel and Ethephon application. The maximum quantum yield of PSII (Fv/Fm) was declined showing an impairment of the primary photochemical efficiency of the photosynthetic apparatus during the time course of the experiment. The significant decrease of Fv/Fo is an indicator of structural damage, which occur in the thylakoids and affects the photosynthetic electron transport. With GA_3 application, fructose, glucose and soluble solids remained unchanged, whereas ascorbic acid content increased significantly. With the retardants a significant decrease in sugars, soluble solids and ascorbic acid content and an increase in respiration rate and in titratable acidity of fruits, was induced. Less soluble solids accumulation in melon from plants treated with growth retardants could be a consequence of delayed maturity, a fact that it can be proved by the lower maturity index.

Introduction

The manipulation of growth and increasing productivity of plants, is the basis for most plant-related research. Economic studies have demonstrated the marked advances of early fruit production. As a result many techniques are used to accelerate and increase food crops productivity. Gibberellins (GAs) are a family of plant hormones that mediate many responses in plants, from seed germination to senescence. The most widely available compound is GA_3 or gibberellic acid, which induces stem and internode elongation, seed germination, enzyme production during germination and fruit setting and growth (Davies, 1995). Plant growth regulators (PGRs) are also used in order to control vegetative growth (Latimer, 1991). The most commonly used PGRs are those which inhibit GA_3 biosynthesis. Cycocel is an onium compound, which blocks GA biosynthesis at the step between geranylgeranyl pyrophosphate and copalyl pyrophosphate. Plants treated with this compound, have shortened internodes and enhanced photosynthesis. Prohexadione-Calcium inhibits late stages of GA biosynthesis e.g., hydroxylation of GA_{20} to GA_1 (Brown *et al*., 1997), whereas Ethephon is one of the growth retardants which do not inhibit GA biosynthesis. Its mode of action is *via* liberation of ethylene, which is absorbed by the plant and interferes in the growth process. It is reported that Ethephon decreases photosynthesis by increasing ethylene levels (Davies, 1995). Species

vary in responsiveness to PGRs and optimum rates may vary with cultivar or growing condition (Latimer, 1991). 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). Flowering has been hastened or delayed by PGRs depending on species (Latimer, 1991). In Greece and others European countries the above growth regulators are commonly used on food crops (melon, pepper, celery etc) in order improve and accelerate plant productivity.

Rapid nondestructive methods are needed to assess changes in quality of vegetables (Ilias & Ouzounidou, 2007). Early changes 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). In parallel, a few reports have been published concerning the use of chlorophyll fluorescence measurements in vegetables. Toivonen (1992) demonstrated a strong association between chlorophyll fluorescence changes and declines in both respiration and vitamin C content in broccoli. The chlorophyll fluorescence method generally compares well with other quality assessment methods (Ouzounidou *et al*., 2006).

We selected several methods and characteristics in order to identify the effects of growth regulators (Gibberellic acid, Prohexadione-Calcium, Cycocel, Ethephon) on growth, pre- and post-harvest physiology and quality of *Cucumis melo* L., cv Galia. Galia melon cultivar, known for its excellent flavour, is widely cultivated in the Northern Greece and has an important market interest.

Materials and Methods

Plant material and cultivation: Melon (*Cucumis melo* L. c.v. Galia) seeds were germinated on a greenhouse mist bench (20s of mist every 30 min) set at 22 ± 2 ^{o}C. Three weeks after complete germination uniform seedlings were transplanted individually into 300ml 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 green house, 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 μS 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 60 mg N-26.2 mg P-49.8 mg K water- soluble fertilizer (20-20-20 F-TOP Ledra Ltd, Thessaloniki) during the experiments. Plants maintained in greenhouse under natural sunlight while average day and night temperatures were 30 ± 2^{o}C and 28 ± 2^{o}C, respectively. Relative humidity 50-95%; photosynthetically active radiation of day time maximum intensities at plant height in the greenhouse 500-700 μmol $m^{-2}s^{-1}$. The experiment was terminated when plants from each treatment had developed at least one fruit.

Applications of treatments: GA_3 at 100 μM, Cycocel, Ethephon and Prohex-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 (Syngent Ltd, UK). A set of 12 melon 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 6 weeks after sowing (plants had six to seven leaves). Control plants were treated with water and surfactant.

***In vivo* chlorophyll fluorescence measurements:** Fast chlorophyll fluorescence was measured on the upper surface of the third 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), after were left for 30 min., to dark adaptation, at room temperature. Measurements were made between 9:00 and 12:00 h at the harvest time. The following fluorescence indices were calculated: the initial fluorescence intensity (Fo) when all reactions centers (RCs) are open, the maximal fluorescence intensity when all reactions are close (Fm), 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). We used the ratios Fv/Fm and Fv/Fo, 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) to evaluate alterations under our experimental conditions.

Leaf chlorophyll content: Chlorophyll a+b was measured at the third fully expanded leaf at the harvest time and it 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 3 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^{o}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 Thermal Conductivity (TC) Detector. All results are expressed as mg $CO_2Kg^{-1}h^{-1}$

Chemical analysis: The ascorbic acid content of melon 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 malic 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^{o}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 of Lichrosphere NH_2 bonded to microparticulate silica of 5 μm diameter maintained at 37^{o}C. Injection of 20 μl of sample solution into a mobile solvent of H_2O/AcCN: 25:75 (v/v) with a flow rate of 1.1ml min^{-1} gave the optimum result) (Manolopoulou & Papadopoulou, 1998). All measurements of chemical data were performed at the harvest time.

Experimental design and data analysis: Fifteen experimental plots (three replications for each treatment) were set up randomly inside the greenhouse using a randomized block design. Twelve single plants were used in each treatment combination/replication. Each plot contained three rows with four plants per row spaced 50 cm apart within each row. Distance between rows was 90 cm while distance between plots was 100 cm. Days to anthesis (from sowing), stem length, first internode's length, length of the first leaf, at the harvest time, were recorded. At the same time, leaf chlorophyll content, chlorophyll fluorescence, fruit diameter and some quality characteristics, were measured. Analysis of regression and variance were performed on the data using Duncan's multirange test for means separation.

Results

The growth regulators tested didn't affect the date of flowering of melon plants (Table 1). GA_3 promoted the total stem length as well as the elongation of the first internode and leaf by 70, 17 and 11% of the control respectively; whereas a significant inhibition of the above lengths with application of Cycocel by 29, 22 and 18% and Ethephon by 18, 17 and 15% respectively, was observed (Fig. 1). Chlorophyll a+b concentration remained unaffected with GA_3, while it was inhibited markedly by 37, 30 and 30% with Prohex-Ca, Cycocel and Ethephon application (Table 2). In the same way, the maximum quantum yield of primary photochemistry (Fv/Fm) was significantly decreased with application of Cycocel, Ethephon and Prohex-Ca by 7, 10 and 30% of the control, respectively. The Fv/Fo ratio revealed more sensitivity, showing significant increase (24% of the control) with GA_3 application and an almost total inhibition by 69% of the control with Prohex-Ca (Table 2). In addition, the significant increase of Tm/Area (which was 100% of the control with Prohex-Ca) corresponded to disturbances in or damage to the photosynthetic apparatus (Table 2). Changes on fruit performance of treated plants were recorded (Table 1). GA_3 enhanced fruit diameter by 56% of the control, while with Cycocel application the highest diameter inhibition by 35% of the control was revealed. In addition, with the application of GA_3 fructose, glucose and the Soluble Solids of melon fruits remained unchanged, as compared to the control, while Titratable acidity decreased by 34% and ascorbic acid content increased significantly (Table 1, Fig. 2). On the contrary, with application of growth retardants, especially of Cycocel and Ethephon, a significant decrease in sugars, SS and ascorbic acid content and a tendency to increase the Titratable Acidity of fruits was induced (Table 1, Fig. 2). The so-called "maturity ratio" reflecting the ratio of SS to the Acidity, was significantly reduced with Cycocel and Ethephon by 60 and 18% of the control respectively, while with GA_3 was increased by 37%, showing a better quality of the fruits due to its application (Fig. 2). Changes in the respiration rate of melon fruit after PGR application are also detected and they are given in Table 3. With GA_3 application melon fruit presented the lowest respiration rate, showing a significant decrease by 37% of the control. CO_2 production increased significantly by 10, 32 and 58% of the control with Prohex-Ca, Cycocel and Ethephon, respectively (Table 3).

Table 1 Effect of GA_3 (100μM), Prohex-Ca (100mg l^{-1}), Cycocel (100mg l^{-1}) and Ethephon (100mg l^{-1}) application on time of anthesis of melon plants (n=36) grown in greenhouse conditions, diameter, sugars and titratable acidity of melon fruits (n=3).

Growth regulator	Flowering time (d)	Fruit diameter (cm)	Fructose (% FW)	Glucose (% FW)	Saccharose (% FW)	Titratable acidity (g $100g^{-1}$ FW)
Control	62a	9.5b	2.3a	2.4a	4.0a	0.91a
GA_3	60a	14.8a	2.5a	2.6a	3.1b	0.60c
Prohex-Ca	62a	8.1c	2.3a	2.4a	3.5ab	0.73b
Cycocel	62a	6.2d	1.6b	1.7b	0.8d	0.96a
Ethephon	66a	7.8c	1.6b	1.7b	2.4c	0.96a

Means followed by different letters in the same column for each treatment differ significantly ($p<0.05$).

Table 2. Changes of chlorophyll a+b concentration (mg g^{-1} FW) and some chlorophyll fluorescence parameters (n=5) of melon fully expanded leaves, as a function of PGRs application.

Growth regulator	Chla+b	Fv/Fm	Tm/Area	Fv/Fo
Control	3.04a	0.802a	0.43b	4.1b
GA_3	2.89a	0.840a	0.43b	5.1a
Prohex-Ca	1.90b	0.565c	0.87a	1.3d
Cycocel	2.10b	0.748b	0.70a	2.9c
Ethephon	2.08b	0.728b	0.80a	2.6c

Means followed by different letters in the same column for each treatment differ significantly ($p<0.05$).

Table 3 Effect of GA_3 (100μM), Prohex-Ca (100mg l^{-1}), Cycocel (100mg l^{-1}) and Ethephon (100mg l^{-1}) application on respiration rate of melon plants (n=3) expressed as mgCO_2 kg^{-1} h^{-1}.

Growth regulator	Respiration rate	% change of control
Control	19d	0
GA_3	12e	-37
Prohex-Ca	21c	+10
Cycocel	25b	+32
Ethephon	30a	+58

Means followed by different letters in the same column for each treatment differ significantly ($p<0.05$).

Discussion

Stem length, first internode and first leaf length of melon plants were significantly enhanced by the application of GA_3; on the contrary, inhibition of growth performance on exposure to the other PGRs was observed. It is known that GA_3 supply induces cell elongation and/or cell division, whereas, the GA_3 biosynthesis inhibitors, like Prohex-Ca and Cycocel, inhibits plant growth (Rademacher, 2000). Our data are in accordance with the findings of Nakayama *et al*., (1992) where a reduction on plant height with Prohex-Ca application in rice plants with a concomitant reduction of endogenous gibberellin concentration was found. However, Hisamatsu *et al.*, (2000) found that PGRs, which inhibit later stages of GA_3 biosynthesis, promoted stem elongation and flowering. Prohex-Ca has a potential for effective control of vegetative growth in several plant species (Brown *et al*., 1997). Inhibition of both cell division and cell elongation has been found with the application of Cycocel (Rajala & Peltonen-Sainio, 2001), resulting in a

production of shorter leaves and shoots in melon. In addition, Ethephon, an ethylene-releasing compound, can also be used to retard stem elongation, promote lateral branching, and manipulate flowering date. Since ethylene acts as an antigibberellin (Hayashi *et al.*, 2001), Ethephon can affect plant height after absorption. The observed inhibition of melon growth induced by Ethephon can be also attributed to the cessation or retardation of the mitotic processes in the meristems of the root and shoot (Burg, 1973).

In melon leaves, even though no chlorotic or yellowing phenomena were observed, a significant chlorophyll loss with Prohex-Ca, Cycocel and Ethephon was detected. Our results are in agreement with those of Jordi *et al.*, (1995). They reported that GA_3 delay the loss of chlorophyll, but it is unclear whether GA_3 acted indirectly on chlorophyll loss by affecting the endogenous cytokinin concentration in the leaves. In our study, not only the chlorophyll a+b concentration but also the chlorophyll fluorescence characteristics were negatively affected by Prohex-Ca, Cycocel and Ethephon application. The maximum quantum yield of PSII (Fv/Fm) was declined showing an impairment of the primary photochemical efficiency of the photosynthetic apparatus during the time course of the experiment. The reduced chlorophyll content is one of the reasons for the decreased photosynthetic efficiency (Ouzounidou *et al*., 2006). In addition, the significant decrease of Fv/Fo is an indicator of structural damage, which occur in the thylakoids and affects the photosynthetic transport of electrons. The increase in Fo observed with Prohex-Ca, Cycocel and Ethephon application is characteristic of the destruction of the PSII reaction centers of melon leaves (Ouzounidou *et al*., 1997). Chlorophyll fluorescence indices provide direct information on functionality and the effectiveness of photosynthesis (Lichtenthaler *et al.*, 2005), thus, we can postulate that an inhibition of enzymatic processes in the Calvin cycle of melon subjected by the above PGRs, was also occurred. More, changes in Fv reflect early stages of chloroplast deterioration, presumably associated with water loss, and not senescence, hence reflecting a decline in freshness. Measurements of respiration and Fv provide direct information on the functioning of the mitochondria and chloroplast, respectively (Solomos, 1983, Dalling & Nettleton, 1986). The increase in respiration rate of melon fruits and the decrease in ascorbic acid content correlate well with the chlorophyll loss with Prohex-Ca, Cycocel and Ethephon application, representing the reduction in freshness and the beginning of senescence.

Beside the length reduction, enhanced antioxidant activity following treatment with GA inhibition has been reported in several plants as well as a promotion of leaf pigments has been observed (Rademacher, 2000). Our findings are quite different, since with the application of the three growth retardants a significant decrease in the ascorbic acid content of melon was observed. In parallel, the SS and the "maturity ratio" were depressed as well. Based to our chemical data, a better quality status of the fruits with GA_3 application was detected. As melon is a fruit and humans eat melon for pleasure, the sweetness is a very important quality factor. Less soluble solids accumulation in melon from plants treated with growth retardants could be a consequence of delayed maturity, a fact that it can be proved by the lower maturity index. In general, quality characteristics dropped sharply and melons are not marketable with the three growth retardants. Gonzalez-Rossia *et al.*, (2007) found increased in quality characteristics of GA_3-treated peaches and nectarines. Some changes on apple quality, like sugars, soluble solids and titratable acidity under Prohexadione-Ca were recorded by Mata *et al.*, (2006). On the contrary, malic acid content gradually decreased and soluble solids content slightly increased during maturation with Cycocel, Ethephon and Prohex-Ca application in apples (Awad & Jager, 2002).

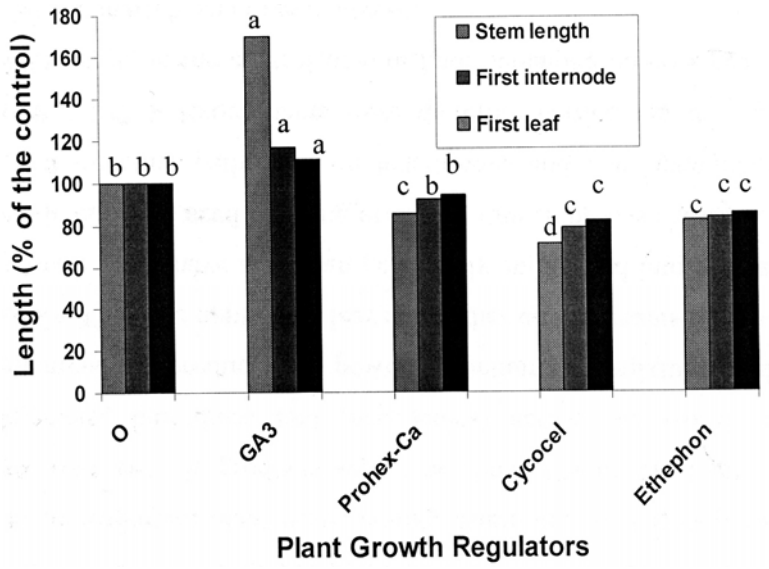

Fig. 1. Changes of stem length, first internode and first leaf elongation (n=36) of *Cucumis melo* L. cv Galia plant, expressed as a percentage of the control values, as a function of PGRs application. Means with different letter in the same parameter (stem, first internode and first leaf length) for each treatment differ significantly ($p<0.05$). Control lengths: stem, 128.8cm; first internode, 3.6 cm; first leaf, 9.0cm. (For details see Materials and Methods).

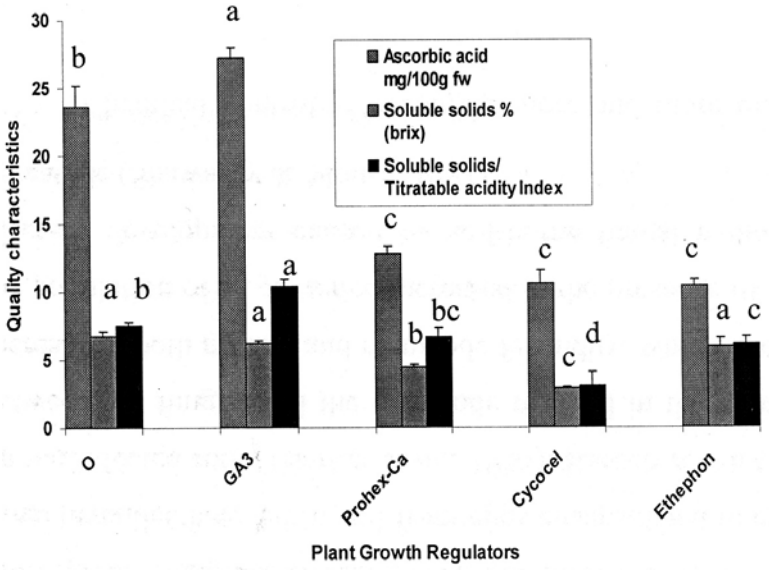

Fig. 2. Changes of some quality characteristics (n=3) of *Cucumis melo* L. cv Galia fruit, as a function of PGRs application. Means with different letter in the same parameter (ascorbic acid, soluble solids, soluble solids/titratable acidity) for each treatment differ significantly ($p<0.05$). (For details see Materials and Methods).

Based to our data, Prohex-Ca was found to be milder on growth, maturity and respiration ratio as compared to Cycocel and Ethephon. The relatively low efficacy of Prohex-Ca may be due to its rapid degradation compared to the others PGRs. Overall, it is obvious that the application of GA_3, Prohex-Ca, Cycocel and Ethephon to melon plants leads to different alterations in developmental, pre- and post-harvest physiological and metabolic pathways. Melon fruits seem to improve their quality and delay the senescence with pre-harvest GA_3 application.

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