Chlorophyll Fluorescence and Photosystem II Activity of Tomato Leaves as Affected by Irradiance and Prohexadione-Calcium

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Abstract: The objective of this research was to investigate the effects of irradiances and growth retardant Prohexadione-Calcium on the growth and physiological parameters of tomatoes (*Lycopersicon esculentum* Mill, cvs 'Karla' and 'Hari Moran') grown under a glass greenhouse environment. Main stem length of tomatoes plants decreased in a quadratic pattern as the concentration of Prohexadione-Ca increased. High concentration ($300mgl^{-1}$) resulted in shorter plants than control plants. The application of $300mgl^{-1}$ of Prohexadione-Ca resulted in diminution of the leaf chlorophyll concentration in both cultivars. In addition high concentration significantly affected variable fluorescence Fv, maximum quantum yield of photosystem II (PSII) photochemistry (Fv/Fm), and the others chlorophyll fluorescence parameters (Fo, Fm, Fv/Fo). Since chlorophyll content and variable fluorescence decreased significantly but Fo did not change significantly the decrease of $t_{1/2}$ indicates negative changes on the acceptor side of PSII, which also may related to the diminution of the Calvin cycle. Furthermore, electron transport rate (ETR), real photochemical efficiency of PSII (Φ_{PSII}), photochemical quenching (q_P) and non-photochemical quenching (q_N), were recorded for cvs. 'Karla' and 'Hari Moran' under different irradiances (66, 96, 136, 226, 336, 536, 811, 1211, 1911 and 3111μmol m^{-2} s^{-1} (I_{66} to I_{3111}, respectively). Prohexadione-Ca in excessive concentration seems to disturb photosynthetic electron transport as well as real photochemical efficiency of PSII and photochemical quenching.

Key Words: *Lycopersicon*, Prohexadione-calcium, chlorophyll fluorescent, Growth inhibition growth retardant . photosystem2, photoinhibition

1. Introduction

Several plant growth retardants commonly used in greenhouse horticultural crops are reported to have many side effects. Most of the side effects are to control height of shoot growth without lowering plant productivity. Prohexadione- Calcium [CAS name: cyclohexenecarboxylic acid, 3,5-dioxo-4-(1-oxopropyl)-ion(1-) calcium, calcium salt] is a new plant growth retardant with low toxicity and limited persistence (Owens and Stover, 1999, Smitt et al., 2005), which inhibits the biosynthesis of gibberellin resulting in reduced internode's length and vegetative growth. It has been registered for growth control of rice (Evans et al., 1999), apples (Byers and Yoder, 1999; Medjdoub et al., 2004), pears (Costa et al., 2002) and recently on petunia and okra plants (Ilias & Rajapakse 2005, Ilias et al., 2007). Chlorophyll fluorescence measurement is rapid, extremely sensitive and non-destructive since it can performed on intact, attached leaves. The yield of chlorophyll fluorescence emission from photosynthesis organisms is determined by two distinct processes: photochemical (q_P) and non photochemical quenching (q_N) (Genty et al., 1989; Havaux et al., 1991). A relative new fluorescence method assessing the state of the photosynthetic apparatus of plants in the photon response curves where many physiological parameters associated with the function of PSII are recorded across different irradiances (White & Critchley, 1999). No information exists that demonstrates the effects of Prohexadione-Ca application on in vivo PSII activity parameters (in spite of their increased physiological significance) and also whether Prohexadione-Ca affects the irradiance in which the phenomenon of photoinhibition occurs. Instead, little information exists about the response of the photosynthetic apparatus to Prohexadione-Ca application (Ilias et al., 2007). The aim of the present experiment was to investigate the effects of Prohexadione-Ca on chlorophyll fluorescence and PSII activity of tomato plants under different irradiances.

2. Materials and methods

2.1. Plant material, culture conditions

The experiment was conducted from March 2006 to September 2006 in a greenhouse at the Technological Educational Institute of Thessaloniki in northern Greece. The site is located at 22°55′N, 40°38′E. The experiment was established on a sandy loam soil whose physico-chemical characteristics were silt 18%, clay 5.6%, sand 70.4%, organic matter 0.88%, $CaCO_3$ 0.9%, electrical conductivity 1.5 μS cm^{-1}, and pH (1:2 H_2O) 7.4. The region is characterised by continental climatic conditions. Seeds of tomato (*Lycopersicon esculentum* Mill) cvs. 'Karla' and 'Hari Moran' were sown in trays filled with a commercial germination mix and germinated on a greenhouse mist bench (20s of mist every 30 min) at temperatures of 22±2°C. At the three to four leaves' stage, uniform seedlings were transplanted individually and randomly inside greenhouse. Plants

were acclimated for two weeks in the greenhouse before treatments. Each plant was watered as required and fertilized weekly at each irrigation with 300 cm^3 of nutrient solution containing 60.0mg N, 26.2mg K, 49.8mg P (water-soluble fertilizer 20-20-20, *F-TOP Ledra*, Thessaloniki, Greece) during the experiment. Plants were maintained in the greenhouse under natural sunlight, relative humidity 70-80%, photosynthetically active radiation (PAR) was of 500-700μmol m^2 s^{-1} (measured by a *Li-6200* portable photosynthesis meter, *LiCor*, Lincoln, NE, USA), while average day and night temperatures were 32 ± 2 ^oC and 27 ± 2 ^oC, respectively.

2.2. Prohexadione-Calcium treatments

Prohexadione-Ca (BAS 125 10W, BASF Corp., Research Triangle Park, NC, USA) at 0, 100, 200, or 300 mgl^{-1} was evaluated. Each solution contained 0.1% Agral 90 as a surfactant (Syngenta, Montreal, Canada). A set of 6 plants from each plot was foliar sprayed with a low pressure hand-wand sprayer to run off two times at 10-day intervals with each of the above Prohexadione-Ca solutions. First application of Prohexadione-Ca were made 5 weeks after germination (plants had five to six leaves). Control plants (6 plants in each plot) were treated with water and surfactant.

2.3. Chlorophyll extraction

The chlorophyll of the same leaves was extracted with ethanol (96%) after incubation in a water bath (78°C). Total chlorophyll concentration was calculated from the equations given by Wintermans & Mots (1965).

2.4. Chlorophyll fluorescence parameters

An old leaf, located between the middle and the base of the scion's shoots of each plant, was used for the above measurements. In vivo PSII chlorophyll fluorescence was measured by a modulated (1.6kHz), low intensity beam from light emitting diodes (excitation wavelength 655nm, detection above 700nm) using a portable pulse-amplitude-modulated fluorometer (PAM-2000; Walz, Effeltrich, Germany as described by Schreiber et al. (1986). The minimum fluorescence yield (Fo) of the plants adapted to darkness was determined under weak red modulated radiation. The mid-part of the front was held in the leaf clip of the fluorometer at a standard distance from the optic fiber probe and a weak 5s far-red (735nm) pulse was sent to fully oxidize the electron transport chain. The maximum fluorescence yield (F_m) of the dark adapted plants was reached by exposed PSII to a saturating pulse (0.8s) of white light. The difference between F_m and F_0 gave the variable fluorescence (F_v). The maximum fluorescence yield of PSII photochemistry was calculated as the ratio of variable fluorescence to maximal fluorescence (F_v/F_m) and represents the efficiency of open PSII in the dark adapted state. The ratio between the parameters Fv and

Fo (Fv/Fo) was also calculated. After these dark measurements, the plants were exposed to increasing actinic irradiances (66, 96, 136, 226, 336, 536, 811, 1211, 1911 and 3111μmol m^{-2} s^{-1} marked as I with the respective index) At the end of each irradiation period that lasted 60s the operating PSII efficiency (Φ_{PSII}), the electron transport rate (ETR), q_P and q_N were determined according to Genty et al. (1989).

2.5. Gas-Exchange Measurements

Gas-exchange measurements were made on the third fully expanded leaf with a portable photosynthetic apparatus Li-6200 (Licor. Inc., Lincoln. USA) with IRGA. The carbon dioxide (CO_2) analyzer was calibrated with two standard CO_2/air mixtures. The lamina of the leaf was enclosed within a fun stirred ¼ L cuvette. The mean CO_2 concentration and leaf-to air vapor pressure deficit for all measurements were 350 μmol mol^{-1} and 20 mbar respectively. Measurements were made at 25°C under a photon flux density of about 600 μmol $m^{-2}s^{-1}$. A, E, Ci and Gw from gas-exchange measurements were calculated according to Von Caemmerer and Farquhar (1981).

2.6. Experimental Design, Data Collection, and Analysis

Twelve experimental plots (three replicates for each treatment) were set up randomly inside the greenhouse using a randomized complete block design. Each plot contains one row with six single plants. Distance between plots was 100cm while distance between plants within each row was 100cm. Main stem length (measured weekly from medium surface to apex), days to anthesis, total number of branches, stem and fruit diameter were recorded. Differences among treatment means were tested using Duncan's Multiple Range Test procedure at P =0.005.

3. Results and Discussion

In this study we identify the response of two tomato cultivars to Prohexadione-Ca, a growth regulator which applied as foliar spray. The application of Prohexadione-Ca reduces levels of GA_1 (highly active) and causes the accumulation of its precursor GA_{20} (inactive) (Evans et al. 1999). Shoot elongation was sensitive under excessive dosage of Prohexadione-Ca (Data not shown).

In general in our experiment, irrigation of the tomato (*Lycopersicon esculentum* Mill.) plants with 100-300 mg l^{-1} Prohexadione-Ca reduced leaf chlorophyll and the values of chlorophyll fluorescence parameters. These diminutions were greater and statistically significant only in the highest concentration (300mgl^{-1}). This could be explained by an inhibition of enzymatic processes in the Calvin cycle subjected by this substance.Furthermore, the diminution of the maximum quantum yield of PSII (F_v/F_m) means that the plants irrigated with excess of Prohexadione-Ca were under high concentrations where molecular O_2 operates as an alternative acceptor for non-utilized electrons and light energy (Larsson, et al., 1998; Cakmak .and Romheld, 1997; Papadakis et al., 2004), resulting thus in the generation of reactive oxygen species (Cakmak, 1994). The ability of reactive oxygen species to cause photooxidative damages in organic molecules could probably explain the structural damages in the chloroplasts, and the reductions of leaf chlorophyll.

The leaf concentrations of chlorophyll were affected by the application of Prohexadione-Ca

(Fig. 1, 2). Prohexadione-Ca resulted in a significant decline of the leaf concentration of the plants grown under the effect of various concentrations (100-300mgl^{-1}). However, significant decrease of leaf chlorophyll concentrations was found under 300 mgl^{-1} and not in lower concentration The ability of reactive oxygen species to cause photooxidative damages in organic molecules could probably explain the reductions of leaf chlorophyll. According to Pereira et al. (2000), one of the probable reasons for the reduction of photosynthesis is the structural damage of thylakoids, which affects the photosynthetic transport of electrons, as indicated by the reduction of the F_v/F_0 ratio, regardless if the decrease is due to an increase in *Fo* or a decrease in *Fv* (Havaux & Lannoye, 1985). The increase in *Fo* is characteristic of the destruction of PSII reaction centers (Pereira et al., 2000) Similarly, the index *Fm/Fo* showed parallel fluctuations reflecting disturbances in the structural integrity of the RCs of PSII under Prohexadione-Ca (Ouzounidou et al., 2003). Also the decline of Fv/Fm demonstrated that photochemistry of PSII, light-driven electron transport and enzymatic reactions requiring ATP and NADPH from chloroplasts were significantly affected by plant growth regulators. Prohexadione-Ca reduced the values of chlorophyll fluorescence parameters for both varieties. These diminutions were greater and statistically significant only in the highest concentration (300 mgl^{-1}). This could be explained by an inhibition of enzymatic processes in the Calvin cycle subjected by this substance. The irradiance at which the highest electron transport rate (ETR) was recorded or else the point of photon energy saturation beyond which any further increase of electromagnetic irradiation results in reduced ETR, was significantly affected by Prohexadione-Ca treatments. Prohexadione-Ca induced reductions in Photosynthesis (Table 1) can be partly associated with stomatal closure, though the reduced stomatal conductance did not reduce in the same extent intercellular CO_2concentration. However the reductions in PSII electron transport suggest an appreciable non-stomatal limitation to photosynthesis. Moreover Proxedione-Ca decreased photochemical quenching and increased the non photochemical dissipation of excitation energy.

The improper function under Proexadione-Ca treatment of the photosynthetic electron transport rate increases the probability of oxidative stress for leaf chloroplasts. Under such stress, molecular O_2 operates as an alternative acceptor for non-utilized electronsand photon energy (Cakmak and Romhed, 1997), resulting tus in the generation of reactive oxygen species (ROS)(Cakmak, 1994). The ability of ROS to cause photoinhibition damages to organic molecules could be probably explain the reductions of leaf Chl content specially under high Proexadione-Ca concentrations.

In conclusion, the combination of physiological and metabolic results presented in this work demonstrated that tomato plants are not tolerant to the effects of high concentrations of Prohexadione-Ca. Our data further reinforce the need for adequate amounts of plant regulators and multiple applications in order to succeed the optimum vegetative growth. However, the role of plant regulators is complicated biologically and biochemically and needs further research.

Acknowledgements

The authors would like to thank Ms Mastoraki Panagiota for her help during field measurements. The project is co-funded by the European Social Fund & National Resources- EPEAK II- ARCHIMIDIS.

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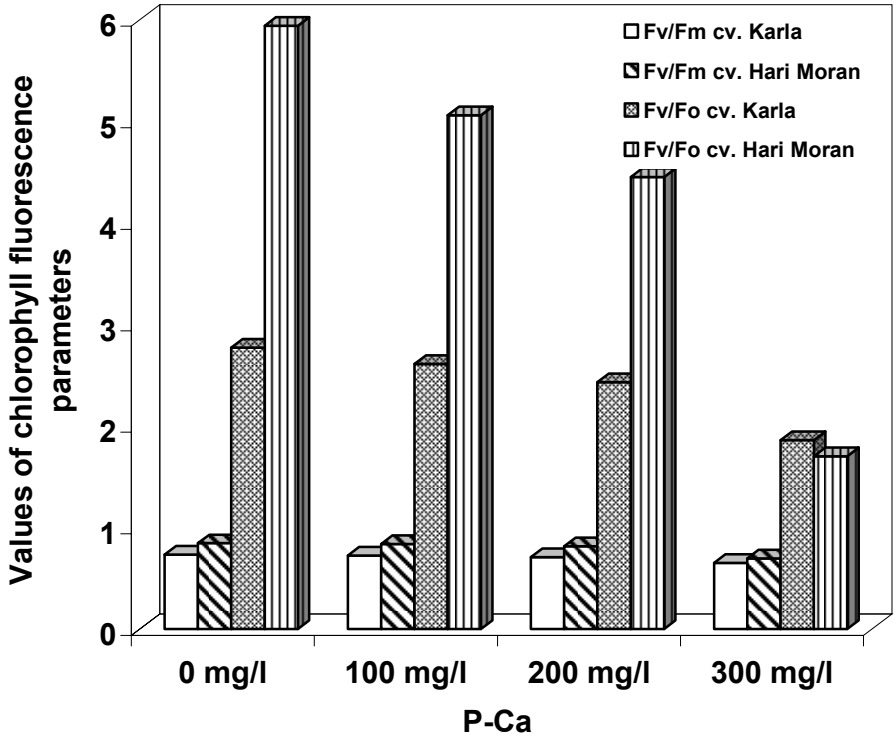
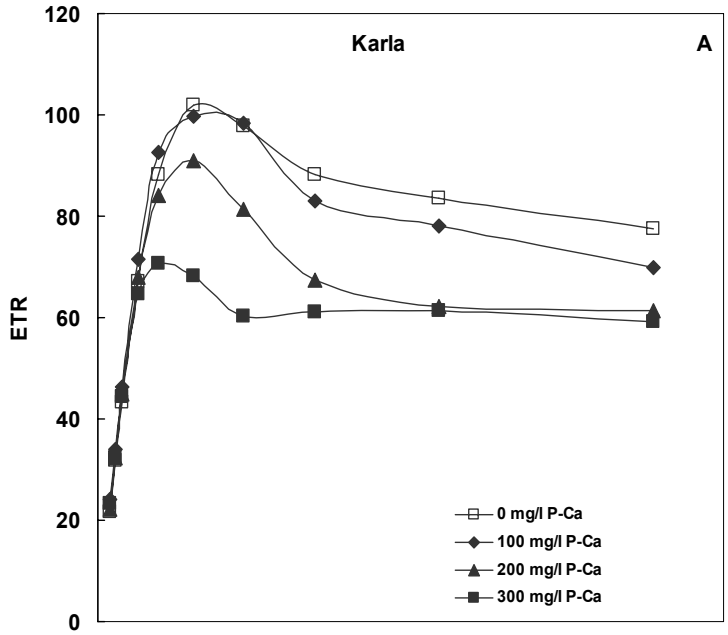
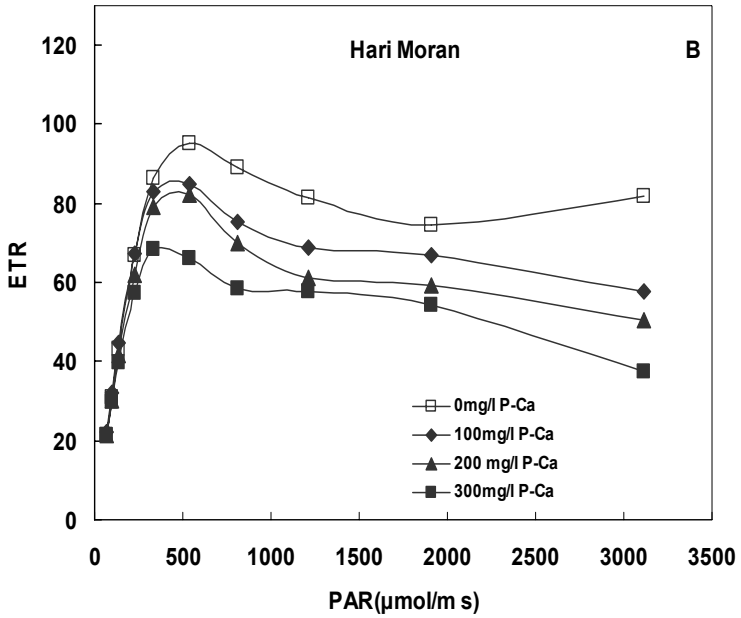


Figure 1. Effect of Prohexadione-Ca (P-Ca; 0 to 300 mgl^{-1}) on Fv/Fm and Fv/Fo ratio in two cultivars of tomato plants.

Figure 2. (A, B). Effect of Prohexadione-Calcium (P-Ca; 0 to 300 mgl^{-1}) on Electron Transport Rate (ETR) at different irradiances (PAR)(66, 96, 136, 226, 336, 536, 811, 1211, 1911 and 3111µmol/m^2 s), in two cultivars of tomato plants.

Table 1. Effects of Prohexadione-Ca (P-Ca; 0 to 300 mgl^{-1}) on gas exchange parameters in two cultivars of tomato plants. Each number is mean of four replications (n=4) In the column numbers flanked by the same letter are not statistically different for *P=0.05*





P-Ca (mg l^{-1})	Hari Moran			
	Gw	**E**	**Ci**	**Amax/E**
0 mg l^{-1}	260c	3.7b	280b	3.8b
100 mg l^{-1}	289b	3.9b	293a	2.1a
200 mg l^{-1}	240d	3.6b	279b	3.7b
300 mg l^{-1}	300a	4.1b	290a	2.2a
	Karla			
	Gw	**E**	**Ci**	**Amax/E**
0 mg l^{-1}	521a	6.8a	268a	3.2b
100 mg l^{-1}	477a	6.3a	264a	3.2b
200 mg l^{-1}	456a	6.1b	262a	3.3a
300 mg l^{-1}	343b	5.1c	270a	2.8c