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Responses of the Photosynthetic Apparatus to Plant Growth Regulators in two Sunflower Cultivars (*Helianthus annuus* L.)

By

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Summary

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The aim of the work was to examine the effect of growth regulators GA_3, GA_4 (gibberellic acid) and Prohexadione-Calcium on growth, net photosynthetic rate (P_N), and chlorophyll fluorescence of *Helianthus annuus* (L.) plants. The experiment was also conducted to investigate the combined effects of exogenous hormones on photosystem II (PSII) activity, irradiance response curves for electron transport rate (ETR), non-photochemical quenching (q_N), photochemical quenching (q_P) and real photochemical efficiency of PSII (Φ_{PSII}) that were recorded under different photosynthetic active radiation. Exposure of sunflower plants to excess growth regulators (200 μM) in hydroponical culture led to inhibition of growth and decreased maximum quantum yield of primary photochemistry (Fv/Fm) under Prohexadione-Ca while GA_3 and GA_4 induced stem elongation and enhanced photosynthesis. In addition Prohexadione-Ca treated plants exhibited decreased photosynthetic pigments and photosynthetic rates, whereas application of hormones (GA_3 and GA_4) led to substantial preservation of chlorophylls and increases in chl a+b, net photosynthetic assimilation rate and stomatal conductance. Finally, plant biomass was reduced with Prohexadione-Ca. The alteration in chlorophyll a fluorescence characteristics ob-

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served under Prohexadione-Ca suggested a weakening of the photochemical processes near the PSII reaction centre.

Zusammenfassung

Giannakoula A. F. & Ilias I. F. 2011. Responses of the photosynthetic apparatus to plant growth regulators in two sunflower cultivars (*Helianthus annuus* L.). [Wachstumsregulatoren beeinflussen den Photosyntheseapparat in zwei Sonneblumen-Kultursorten (*Helianthus annuus* L.)]. – Phyton (Horn, Austria) 51 (2): 245–259.

In der vorliegenden Arbeit wurde der Effekt der Wachstumsregulatoren GA_3, GA_4 (Gibberellinsäure) und Prohexadione-Calcium (Hemmer der Gibberellinsäuresynthese) auf das Wachstum, die Nettophotosyntheserate (P_N) und die Chlorophyllfluoreszenz von *Helianthus annuus* (L.) untersucht. Die Experimente wurden auch durchgeführt, um zu erkennen, ob es Effekte durch exogene Hormone auf die Aktivität des Photosystems II (PSII), auf die Elektronentransportrate (ETR), auf das nicht-photochemische (q_N) und photochemische Quenchen (q_P) und die tatsächliche photochemische Effizienz des PSII – bei unterschiedlich photosynthetisch aktiven Strahlungsbedingen gemessen – gibt. Eine Behandlung der Sonnenblumen mit zusätzlichen Wachstumsregulatoren (200 μM) in der Hydrokultur führte unter dem Einfluss von Prohexadione-Ca zu Wachstumshemmung und einer geringeren maximalen Effizienz der primären Photosynthese (Fv/Fm), während GA_3 und GA_4 längere Sprosse und eine erhöhte Photosyntheserate bewirkten. Ferner zeigten Prohexadione-Ca behandelte Pflanzen eine Abnahme der Photosynthesepigmente und der Photosyntheseraten, während GA_3 und GA_4 die Chlorophylle schützen und Chl a+b zunahm, ebenso war die P_N gesteigert und die stomatäre Leitfähigkeit erhöht. Durch den Einfluss von Prohexadione-Ca war die Biomasse geringer. Die Veränderung der Charakteristika der Chlorophyll a-Fluoreszenz unter Prohexadione-Ca Einfluss lassen die Vermutung zu, dass es in der Nähe des PSII Reaktionszentrums zu einer negativen Beeinflussung der photochemischen Prozesse kommt.

Abbreviations: Chl chlorophyll; chl fluorescence-chlorophyll fluorescence; GA_3, GA_3-gibberellic acid; Prohex-Ca-Prohexadione-Calcium; PSII-photosystem II; RCs – reaction centres; PPFD – photon flux density; PAR-photosynthetically active radiation; PSI-photosystem I; ROS-reactive oxygen species; PGRs-plant growth regulators.

Introduction

Sunflower is one of the most ancient crops naturalized by man. It is planted throughout the world for its seeds which are used for human consumption and as a source of oil. In spite of its importance for human, lately its plant biomass is used as a source of bioenergy. So, it is of great importance to obtain the maximum biomass production of the crop. Regarding plant growth regulators, it has been observed that they affect growth process. They bring about many morphological and physiological changes in plants (Davies 1995), which may cause change in biomass production. Various plant regulators such as gibberellins affect plant growth and de-

velopment processes (OUZOUNIDOU & al. 2008). Plant hormones modify crop canopy and play an important role in controlling crop growth (RIES & HOUTZ 1983, CHEEMA & al. 1987, KHAN & al. 2000) through increase in the redistribution of photosynthesis. Gibberellins (GAs) are a family of plant hormones that mediate many responses in plants, from seed germination to defoliation (HISAMATSU & al. 2000, ILIAS & al. 2007). On the other hand Prohex-Ca blocks GA biosynthesis between kaurene and kaurenoid acid.

Prohexadione-Ca [CAS name: cyclohexenecarboxylic acid. 3.5-dioxo-4-(1-oxopropyl)-ion (1-) calcium, calcium salt] is a new plant growth retardant which inhibits the biosynthesis of gibberellin resulting in reduced internode length and vegetative growth.

Foliar absorption is the only significant means of plant uptake. Uptake is generally complete within eight hours following application. Results indicate that acropetal movement within the plant is important while basipetal movement is limited. Applications of Prohex-Ca at rates of 63 mg l^{-1} to 500 mg l^{-1} on vigorous trees have provided excellent control of vegetative growth and maintain plant size and form at desirable level (LATIMER 1991). Prohex-Ca effectively reduces the level of gibberellin in the plant for three to four weeks following application (BROWN & al. 1997). Prohex-Ca does not persist in the plant or affect vegetative growth the following season. However, where the proper balance between vegetative growth and crop is achieved, one application may provide season-long control of vegetative growth. Due to its short-term effect and lack of persistence.

Carotenoids and chlorophyll absorb radiant energy, which is used for photosynthesis and a part of this energy is emitted as chlorophyll fluorescence (PAPAGEORGIOU 1975). In many situations there is an inverse relationship between the photosynthetic activity and the in vivo fluorescence of the chl (KRAUSS & WEISS 1991, PEREIRA & al. 2000). Fluorescence increases under various conditions and there are changes in the characteristics related to fluorescence, such as initial fluorescence (Fo), maximum fluorescence (Fm), variable fluorescence (Fv) and the ratio between them (LICHTENTHALER & RINDERLE 1988, BINDER & FIELDER 1996). The yield of chl fluorescence emission from photosynthesis organisms is determined by two distinct processes, photochemical (q_P) and non photochemical quenching (q_N) (GENTY & al. 1989, HAVAUX & al. 1991). However, little is known about the effects of plant growth regulators on the sunflower photosynthetic apparatus. The effects of plant growth regulators and their relationship with photosynthesis and biomass production have been studied earlier but it isn't very clear how it affects plants. Therefore, the present investigation on sunflower (*Helianthus annuus* L.) aims to understand the effect of growth regulators treatment to leaf dry mass and biomass production. A change in P_N, ETR, q_p, q_N, following the loss of photosynthetic pigments

and growth regulators treatment and its relationship with photosynthetic function have also been investigated. The chl fluorescence parameters which indicate whether the PSII of photosynthesis operates normally (HAVAUX & LANNOYE 1985, LARSSON & al. 1998) have not been studied adequately under application (200 mg l^{-1}) of GA_3 and Prohex-Ca.

Material and Methods

Plant Cultivation and Growing Conditions

Helianthus annuus L. cv. 'Aida' and 'Zebilon' were used in all experiments. Seeds were sterilized with 4% NaCl for 10 minutes, washed with distilled water and then germinated in darkness at 25° C in a germination chamber. The seeds were randomly placed in petri dishes on filter paper moistened with de-ionized water for three days (until they have root length about 1.5 cm). The seedlings were transplanted and further cultivated for about 21 days until they had four mature leaves (in 30 polyethylene pots for each treatment and three plants in each pot). Plants were irrigated with a modified Hoagland's nutrient solution containing [μM]: KCl: 50, H_3BO_3: 25, $MnSO_4XH_2O$: 2, FeEDTA: 20, $CuSO_4x5H_2O$: 0.5, $ZnSO_4x7H_2O$: 2, $(NH_4)_2MO_7O_{24}x4H_2O$: 0.5.Nitrogen, phosphorus, potassium, calcium, magnesium and sulphur were supplied from KNO_3, Ca $(NO_3)_2{\cdot}4H_2O$, $NH_4H_2PO_4$ and $MgSO_4x7H_2O$ at concentrations [mM]: N:16, P:0.2, K:0.6, Ca:0.4, Mg;0.1, S:0.1. The nutrient solution (pH 5.5–6.0) was renewed every two days. GA_3, GA_4 and Prohex-Ca (BAS 125 10W, BASF Corp., Research Trianle Park. N,C) were dissolved in 1 mM ethanol (95% ethanol), respectively and diluted with distilled water to a final stock concentration of 300 μM/L. Two concentrations of GA_3, GA_4 (100 μM and 200 μM each) and Prohex-Ca (100, 200 mg l^{-1}) were used respectively. Control plants were treated with water. The seedlings were grown in a cultivation chamber under controlled environmental conditions with relative humidity $65 \pm 2/75 \pm 2$% day/night, temperature $22 \pm 1/20 \pm 1$°C (day/night) and 16/8 h photoperiod. During the entire period plants obtained light from 4 white fluorescents tubes (4x18W, Osram, Germany). The young plants exposed to photon flux density (PPFD) of 350 $\mu mol.m^{-2}{\cdot}s^{-1}$. A set of 30 plants in each plot was foliar sprayed (main axis) with a low pressure hand-wand sprayer to run off, two times at 2-weeks intervals with each of the above solutions. Control plants (thirty plants in each plot) were treated with water and surfactant (*Syngenta*, Ontario, Canada). PGRs concentrations and spraying time have been selected after preliminary experiments.

At the end of the treatment exposure, the plants were harvested and root and shoot elongation was determined and expressed as percentage. Following the 21-day period of GA_3, GA_4 and Prohex-Ca treatment, the plants were washed thoroughly with distilled water and then oven-dried (24h, 80°C).

Biomass

Following the 21-day period of GA_4, GA_3 and Prohex-Ca treatment, the plants were removed from the water, causing minimum damage to the roots, washed thoroughly with distilled water, blotted dry and fresh weight determination. After, the oven-dried samples in an electric oven (for 24 h, 80 °C) and dry weight determination (oven-dried for 24h, 80 °C).

Chlorophyll Fluorescence

At the termination of the experiment, the youngest and fully expanded leaf blade of 5 plants from each treatment was used for determination of various parameters related to PSII activity. In vivo PSII-Chl fluorescence was measured by a modulated (1.6 kHz) and low-intensity beam from light-emitting diodes (excitation wavelength 655 nm. detection above 700 nm) using a portable pulse-amplitude-modulated fluorometer (*PAM-2000; Walz*, Effeltrich, Germany), as described by SCHREIBER & al. 1986. The minimum fluorescence yield (F_0) of the plants adapted to darkness was determined under weak red modulated radiation. The middle part of the front was held in the leaf clip of the fluorometer at a standard distance from the optic fibre probe and a weak 5-s far-red (735 nm) pulse was sent to fully oxidize the electron transport chain. The maximum fluorescence yield (F_m) of the dark-adapted plants was reached by exposing PSII to a saturating pulse (0.8 s) of 'white light'. The difference between F_m and F_0 gave variable fluorescence (F_v). The maximum quantum 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. Ratio between the parameters F_v and F_0 (F_v/F_0) were also calculated.

After these dark measurements the plants were exposed to increasing actinic irradiances (66, 96, 136, 226, 336, 536, 811, 1211, 1911 and 3111 μmol m^{-2} s^{-1}). 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. The value of Φ_{PS2} which is the average photochemical efficiency of PSII units in the light (including closed and open ones) was determined by the equation: $\Phi_{PSII} = \Delta F/F_m' = (F_m'-F_t)/F_m'$ (GENTY & al. 1989) with measurements under actinic irradiation of the steady state fluorescence yield (F_t) and of the maximum fluorescence yield (F_m') obtained using a 0.8s saturating pulse. Additionally ETR and thus the overall photosynthetic capacity in vivo (GENTY & al. 1989) was calculated by the equation: $ETR = \Phi_{PSII} \times PAR \times 0.5 \times 0.84$, where PAR was the absorbed photosynthetic flux density (μmol m^{-2} s^{-1}) measured using an integrating sphere and 0.5 is a constant that accounts for partitioning of energy between PSII and PSI (MAXWELL & JOHNSON 2000). Furthermore, q_N.which is related to energy dissipation by other means than photochemistry and fluorescence, was calculated according to the equation: $q_N = (F_m-F_m')/F_m'$, using the initial F_m measured after the long darkness period and using the F_m' measured after irradiation (BILGER & al. 1995). Another widely used fluorescence parameter measuring photochemistry is q_P which was calculated as: $q_P = (F_m'-F_t')/(F_m'-F_0')$ (MAXWELL & JOHNSON 2000).

Chlorophyll Content

Chl of the same leaves, which were used for the measurements of various fluorescence parameters, was extracted with ethanol (96 %) after incubation in a water bath (78 °C). Measurements for chl concentration were made on the outermost and occasionally the second outermost leaves that curled to form the head. Chlorophyll a

and b were estimated spectrophotometrically (Perkin Elmer lambda Bio 20 UV-vis spectrophotometer). Chl amount was calculated according to WINTERMANS & MOTS 1965 and expressed on a dry mass (DM) basis.

Photosynthesis Measurements

At the termination of the experiment, the photosynthetic rate (Pn) (_mol CO_2 m^{-2} s^{-1}), stomatal conductance (mmolm^{-2} s^{-1}) and intercellular CO_2 concentration (Ci) of all plants were measured using the using an infrared gas analyzer (IRGA, Li-COR, 6200Lincoln, USA) portable measuring device. An old leaf, located between the middle and the base of the scion's shoots of each plant, was used for the above measurements (MATRAKA & al. 2010). Measurements were performed in the morning (9.30–10.30 a.m.) at steady light intensity (> 900 _molm^{-2} s^{-1}), while leaf temperature varied between 24 and 25 °C. Photosynthetic water use efficiency (WUE) was calculated using the data on photosynthetic rate and stomatal conductance (DAS & al. 1999). Photosynthetically active radiation (PAR) and light interception from top to the leaf that was used for photosynthesis measurement was recorded by photometer (LI 189, Lincoln, NE). PAR at full sunlight during sampling was 1100 mmol m^{-2} s^{-1}.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using the SPSS 11.0.1 for Windows statistical package (*SPSS*, Chicago, USA). For comparison of the means, the Duncan's multiple range tests ($p \leq 0.05$) were employed.

Results

Growth

The observations recorded on root length, root fresh and dry mass, shoot and leaves length, shoot and leaf fresh, dry mass and plant biomass were found to decrease with Prohex-Ca treatment (Table 1,2). The extent of reduction in the root was not significant at 100 mg l^{-1} of Prohex-Ca whereas upper area (shoot+leaves) reduced more at 200 mg l^{-1} of Prohex-Ca. Application of GA_3 and GA_4 were found most effective. During 10 days of growth maximum growth and also fresh and dry weight were found in GA_3 and GA_4 treatments.

Chlorophyll Concentration

Chl a+b concentration was negatively affected by high concentrations of Prohex-Ca (200 mg l^{-1}) whereas GA_3 and GA_4 (200 mM each) applications increase chl a+b concentration. From the other hand low concentrations of GA_3, GA_4 and Prohex-Ca (100 mg l^{-1}) didn't significantly affect total chlorophyll concentration (Table 6).

In Vivo Fluorescence Measurements

The efficiency of photochemistry Fv/Fm was declined especially in the application of 200 mg l^{-1} of Prohex-Ca showing alterations of PSII RCs

Table 1. Effects of GA_3, GA_4 (100, 200 mM each) and Prohexadione-Ca (100, 200 mgl^{-1}) on root and shoot length of sunflower plants. For each treatment, the values of each parameter marked with the same letter do not differ from each other for $p \leq 0.05$ (Duncan's multiple range test). Each number is the mean of 5 plants.

Treatments	Root length (cm)	Root length 21th day (cm)	Shoot length (cm)	Shoot length 21th day (cm)
Control	4.95a	10.2a	5.21a	17.1b
	(100%)	(100%)	(100%)	(100%)
100 GA3	4.7a	8.8a	5.15	17.0b
	(94.9%)	(91.3%)	(98.8%)	(99.8%)
200 GA_3	5.15a	13.2a	5.14a	18.4b
	(104.7)	(120.8%)	(98.6%)	(107.6%)
100 GA_4	4.65a	10.0	5.05	17.6b
	(93.9%)	(98.0%)	(96.9%)	(102.9%)
200 GA_4	5.0a	14.2a	5.10a	19.3a
	(101.0%)	(139.2%)	(97.8%)	(112.8%)
100 Prohex-Ca	4.72a	9.3a	5.18a	15.4b
	(95.3%)	(92.5%)	(99.4%)	(90.0%)
200 Prohex-Ca	3.8b	7.3b	4.1b	13.5c
	(87.5%)	(78.8%)	(86.6%)	(78.9%)

Table 2. Effects of GA_3, GA_4 (100, 200 mM each) and Prohexadione-Ca (100, 200 mgl^{-1}) on shoot fresh and dry weight of sunflower plants. The values of each parameter marked with the same letter do not differ from each other for $p \leq 0.05$ (Duncan's multiple range test). Each number is the mean of 5 plants.

Treatments	Shoot fresh weight (mg)	Shoot dry weight (mg)	Root fresh weight (mg)	Root dry weight (mg)
Control	212a	530.09a	212a	307b
	(100%)	(100%)	(100%)	(100%)
100 GA_3	202a	520.90a	210a	334b
	(95.28%)	(94.5%)	(99.01%)	(108.1%)
200 GA_3	198a	560a	174c	375a
	(93.38%)	(105.0%)	(82,07%)	(122.3%)
100 GA_4	203a	565a	185c	360a
	(95.7%)	(106.6%)	(87.2%)	(117.2%)
200 GA_4	215a	627a	195c	399a
	(101.1%)	(118.3%)	(91.9%)	(129.93%)
100 Prohex-Ca	199a	454.4ab	191a	186c
	(93.8%)	(85.6%)	(90.01%)	(60.5%)
200 Prohex-Ca	194b	433.2b	179b	162c
	(91.5%)	(81.6%)	(84.1%)	(52.7%)

Table 6. Net photosynthetic assimilation rate (P_N) (μmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (gS) (mmol $m^{-2}s^{-1}$) and chlorophyll concentration (chl a+b) (μg cm^{-2}), as affected by GA_3, GA_4, (100, 200 mM each) and Prohexadione-Ca concentrations (100, 200 mg l^{-1}) in the nutrient solution. The values of each parameter marked with the same letter (s) do not differ from each other for $p \leq 0.05$ (Duncan's multiple range test). Each number is the mean of 5 plants

Treatment	P_N	g_S	Chl (a+b)
Control	5.7b	50.7b	10.44b
100 GA_3	7.8a	70a	11.46b
200 GA_3	8.3a	85a	12.53a
100 GA_4	7.1a	80a	11.3b
200 GA_4	8.9a	92a	13.1a
100 Prohex-Ca	4.3c	38c	9.64b
200 Prohex-Ca	3.5c	30c	8.94c

and an inhibition of enzymatic process in the Calvin cycle (Table 3). Also slight but not significant changes in the maximum quantum yield of primary phytochemistry (Fv/Fm) was observed on exposure to GA_3 and GA_4. In addition, the significant increase of Tm/Area in the concentration of 200 mg l^{-1} Prohex-Ca corresponded to disturbances (or damage) to the photosynthetic apparatus. The values of ETR and Φ_{PSII} were considerably higher in the GA_3, GA_4 treated plants than in control ones at 226 PAR [μmol m^{-2} s^{-1}]. Additionally the analytical fluorescence data presented in show that up to 226 [μmol m^{-2} s^{-1}] PAR, q_N was significantly higher under GA_3 and GA_4 compared to the other treatments.

Table 3. Chlorophyll fluorescent parameters as affected by GA_3, GA_4 (100, 200 mM each) and Prohexadione-Ca concentrations [100, 200 mM] in the nutrient solution at 226 PAR [μmol m^{-2} s^{-1}] where the sunflower plants were exposed. The values of each parameter marked with the same letter do not differ from each other for $p \leq 0.05$ (Duncan's multiple range test). Each number is the mean of 5 plants

Treatment	Fv/Fm	Fv/Fo	Tm/Area
Control	0.795 ± 0.03a	3.886 ± 0.09c	0.19 ± 0.01b
100 GA_3	0.801 ± 0.02a	4.015 ± 0.08b	0.24 ± 0.03b
200 GA_3	0.829 ± 0.03a	4.840 ± 0.06a	0.29 ± 0.01b
100 GA_4	0.799 ± 0.01a	3.993 ± 0.09b	0.19 ± 0.03b
200 GA_4	0.845 ± 0.03a	5.474 ± 0.08a	0.17 ± 0.02b
100 Prohex-Ca	0.775 ± 0.06a	3.456 ± 0.06c	0.32 ± 0.03b
200 Prohex-Ca	0.754 ± 0.02a	3.030 ± 0.04c	0.65 ± 0.03a

Net Photosynthetic Rate (PN)

Photosynthesis expressed as net photosynthetic assimilation rate and stomatal conductance in sunflower plants where 50% lower in treated

leaves with Prohex-Ca (200 mg l^{-1}) compared to control plants. However, 10% increase in net photosynthetic rate was observed in the treated leaves with GA_3 (100 mM) (Table 6). Moreover, GA_4 gave higher values for P_N, in comparison to GA_3. On the other hand stomatal conductance (gS) increased 30.4% in the treated leaves with GA_4 (100 mM) while plants treated with GA_3 (100 mM) gave lower values 21.8% in comparison to GA_4, respectively.

Discussion

The application of Prohex-Ca to sunflower plants resulted in characteristic alterations of their growth parameters. Treatment with Prohex-Ca growth retardant induced reduction of shoot length, leading to shorter plants (Table 1). Sunflower plants treated with GA_4 (200 mM) were up to 12% higher than the controls. There was no significant difference between GA_3 and GA_4 treatments (100 mM). Prohex-Ca (mg l^{-1}) affected plant height even more strikingly. This decrease in plant height was dose dependent; plants were 10% shorter in the 100 mg l^{-1} treatment and 23% shorter in the 200 mg l^{-1} treatment (Table 1). Similar reductions of vegetative growth due to growth retardant use have been observed in fruit trees (Rademacher & al. 2004), grain crops (Lee & al. 1998, Kofidis & al. 2008) and ornamentals (Pinto & al. 2005). Application of GA_3 improved growth compared to the control. 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. These findings are comparable to ours concerning melon fruits responses to various growth regulators (Ouzounidou & al. 2008) and to those of Nakayama & al. 1992 who found a reduction on rice height under Prohex-Ca application with a concomitant reduction of endogenous 'gibberellins' concentration. Mata & al. 2006 have also reported an inhibition of shoot elongation in apple trees after Prohex-Ca application. Prohex-Ca has a potential for effective control of vegetative growth in several plant species however, timing seems to be very important (Ilias & Rajapakse 2005).

Plant growth regulators treatments induced changes in photosynthetic efficiency of leaves. In other words, the decreased Φ_{PSII} of GA_3, GA_4 plants under low irradiances ($\leq I_{226}$) was due to the fact that high percentage of excess photon energy of PSII was dissipated as heat (q_N); under such irradiances there were no significant differences between the three treatments concerning the values of q_P (Table 4, 5). The increased losses of excitation energy of PSII in the form of heat are considered to be a photoprotection mechanism of plant cells.

Table 4. Electron transport rate (ETR), Φ_{PSII}. photochemical quenching (q_P) and non photochemical quenching (q_N) values as affected by GA_3, GA_4 (200 mM each) and Prohexadione-Ca concentrations (200 μM) in the nutrient solution at 226 PAR [μmol m^{-2} s^{-1}] where the sunflower plants were exposed. The values of each parameter marked with the same letter do not differ from each other for $p \leq 0.05$ (Duncan's multiple range test). Each number is the mean of 5 plants

Treatment	ETR	Φ_{PSII}	q_P	q_N
Control	23.4a	0.708a	0.916a	0.118a
200 GA_3	23.9a	0.724a	0.942a	0.125a
200 GA_4	24.4a	0.738a	0.965a	0.040a
200 Prohex-Ca	23.3a	0.706a	0.920a	0.124a

Table 5. Electron transport rate (ETR), Photochemical effiviency (Φ_{PSII}), photochemical quenching (q_P) and non photochemical quenching (q_N) values as affected GA_3, GA_4, (200 mM each) and Prohexadione-Ca concentrations (200 μM) in the nutrient solution at 811 PAR [μmol m^{-2} s^{-1}] where the sunflower plants were exposed. The values of each parameter marked with the same letter do not differ from each other for $p \leq 0.05$ (Duncan's multiple range test). Each number is the mean of 5 plants

Treatment	ETR	Φ_{PSII}	q_P	q_N
Control	75.0b	0.664b	0.901a	0.256a
200 GA_3	77.8b	0.689b	0.918a	0.204a
200 GA_4	79.5b	0.704b	0.947a	0.237a
200 Prohex-Ca	76.2b	0.675b	0.909a	0.152b

Also, the decreased ETR and Φ_{PSII} at GA_3, GA_4 plants were rather related to the negative effects of low availability on the reaction of water photolysis (Marschner 1995, Fageria & al. 1997, González & al. 2007) and on the functionality of the thylakoid-bound electron transport chain from PSII to PSI. Prohex-Ca treatments decrease P_N to a greater extent especially in high concentration (200 mg l^{-1}) suggesting that leaves treated with GA_3 or GA_4 are more photosynthetically active compared to Prohex-Ca treatments. Moreover stomatal conductance was to a similar degree depressed resulting in decrease in P_N. Application of exogenous GA_3 or GA_4 resulted in a substantial stability of LHC of PSII RCs in sunflower plants. Applications of GA_3 or GA_4 mainly increased P_N, Fv/Fm that was improved under high concentration of hormone exposure (200 mM). In another study, cut stock flowers grown on medium with higher concentrations of GA_3 or GA_4 had also better photochemical efficiency of PSII (Ferrante & al. 2009). Thus the tested regulators (GA_3 and GA_4) might trigger some protective mechanisms on photosynthetic apparatus resulting to the stability of PSII (Rademacher 2000, Ouzounidou & Ilias 2005) and also resulting in higher P_N in these treatments.

According to JORDI & al. 1995 GA_3 has been reported to delay the loss of chl. Our data are different, showing a sharp decrease of chl concentration. Even though chl 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 growth retardant application. The efficiency of photochemistry (F_v/F_m) declined showing alterations of PSII reaction centres and an inhibition of enzymatic process in the Calvin cycle of sunflower plants subjected by Prohex-Ca and also implying that the maximum quantum yield of PSII photochemistry was significantly influenced as the concentration of growth retardants increased, Fv/Fm values decreased. In another study, cucumber plants grown on medium with higher concentrations of growth regulators had also lower photochemical efficiency of PSII (BURZA & al. 1994). These large decreases in Fv/Fm in the growth retardant-treated coriander plants were accompanied by corresponding increases in Fv/Fo, indicating possible structural damage to the thylakoid membranes of the chloroplasts (PEREIRA & al. 2000). 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 (ILIAS & al. 2007). These organelles are very sensitive to early stages of deterioration in plant tissue (SOLOMOS 1983, DALLING & NETTLETON 1986). In our experiment, CO_2 production pattern of leaves was negatively related with leaves chl fluorescence (F_v/F_o), measured at the same time. Fluorescence changes in response to CO_2 have been found in broccoli (DEELL & TOIVONEN 2000). The decrease in net photosynthesis rate (P_N) of sunflower and the decrease in chl a+b content correlate well with the chl fluorescence under Prohex-Ca application, representing the beginning of senescence. Decreased photosynthetic activity and growth of tissues are followed by reduction of plant productivity.

The values of P_N declined in high concentrations of Prohex-Ca while stomatal conductance increased significantly. This indicates that stomata are not responsible for photosynthesis decline. Also P_N/E ratio (data not shown) declined significantly following the stress, indicating that the plants were not able to maintain a good efficiency in the use of water.

The improper function under Prohex-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 electrons and photon energy (CAKMAK & ROMHELD 1997), resulting thus 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 Prohex-Ca concentrations.

In general, the decrease of Φ_{PSII} and increase of q_N is associated with the xanthophyll pigment cycle that provides photoprotection of photosystem by the dissipation of excess absorbed photon energy (DEMMIG-ADAMS & ADAMS 1992). Furthermore the fluorescence parameters (F_v/F_m, F_v/F_o) measured in dark-adapted *Helianthus annuus* plants were not affected significantly by the low Prohex-Ca concentration (100 mg l^{-1}) in the nutrient solution. YOO & al. 2003 also reported that in many studies a decrease in the Φ_{PSII} has been observed but with no changes in F_v/F_m. The PAR at which the highest ETR was recorded or in other words the point of photon energy saturation beyond which any further increase of electromagnetic irradiation results in reduced ETR was significantly affected by Prohex-Ca treatment. Also the values of ETR, Φ_{PSII} q_N and q_P did not differ significantly between the treatments control and Prohex-Ca although leaf chl contents were considerably lower under Prohex-Ca than control plants.

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

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