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BRIEF COMMUNICATION

Effects of gibberellic acid and prohexadione-calcium on growth, chlorophyll fluorescence and quality of okra plant

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Abstract

The experiment was conducted to identify the response of three cultivars of okra [*Abelmoschus esculentus* (L.) Moench] to exogenous hormones [gibberellic acid-(GA_3) and prohexadione-Ca] applied as foliar spray. Stem and leaf dry masses and stem length were significantly enhanced by the application of exogenous GA_3, but prohexadione-Ca inhibited growth. Control and prohexadione-Ca treated okra plants took more time to bloom than did GA_3 treated plants. In the fruits of all the cultivars a decrease in fructose content was observed, while protein content remained almost unchanged after the application of the two growth regulators. The small changes in chlorophyll *a* fluorescence characteristics observed under prohexadione-Ca suggested a weakening of the photochemical processes near the photosystem 2 reaction centre. The lowering of ratio between maximum time to reach maximum fluorescence, F_m (T_{max}) and Area (sum of F_m - F_t for t = 0 to t = T_{max}) caused by GA_3 was probably due to the increase of Area rather than to changes in T_{max}.

Additional key words: *Abelmoschus esculentus*, fruit quality, photosynthetic quantum yield, photosystem 2, growth regulators.

Plant growth regulators (auxins, gibberellins, abscisic acid) are used in agricultural industry for stimulation and synchronization of flowering and fruit setting, promotion of rooting, reduction of vegetative growth, reduction of lodging of agronomic crops, or defoliation (Briant 1974). On the other hand, plant growth retardants, such as ancymidol, daminozide, paclobutrazol, chlormequat chloride, and uniconazole are used specifically to reduce vegetative growth and control plant size and shape (Latimer 1991).

Gibberellins (GAs) mediate many responses in plants from seed germination to senescence (Davies 1995). The most widely available compound is gibberellic acid (GA_3), which induces stem and internode elongation, seed germination, enzyme production during germination, and fruit setting and growth (Dijkstra and Kuiper 1989, Ross *et al.* 1990, Davies 1995). Prohexadione-Ca (Prohex-Ca) is a new plant growth retardant patented by *Kumiai Chemical Industry Co.* that was registered for growth control of rice (*Oryza sativa* L.) in Japan (Evans *et al.* 1999). Recently, it has been registered in the United States (under the trade name *Apogee*), as a replacement for daminozide, for use on apples (*Malus* × *domestica* Borkh.) because of its negligible toxicological effects on mammals and a half-life of few weeks in higher plants and less than a week in the soil. In addition, Prohex-Ca is a late stage GA biosynthetic inhibitor that blocks 3β-hydroxylation, but it also prevents catabolism of GA_1 to inactive GA_8 by blocking the 2β-hydroxylation (Brown *et al.* 1997). Only few reports are available on the use of Prohex-Ca on vegetable crops (Hisamatsu *et al.* 1998, 2000, Tatineni *et al.* 2000, Ilias and Rajapakse 2005) and they do not show a clear relationship between GAs and Prohex-Ca concentration and rates of photo-synthesis and growth. Application of GAs either enhanced photosynthesis and growth (Kwan 1996, Hayat *et al.* 2001, Yuan and Xu 2001), or stimulated growth but decreased the rate of photosynthesis (Dijkstra and Kuiper 1989). Inhibitors of GAs biosynthesis stimulated photo-synthesis and reduced growth (Thetford *et al.* 1995),

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Abbreviations: Area - summation of all values (F_m - F_t) for t = 0 to t = T_{max}; Chl - chlorophyll; GA_3 - gibberellic acid; Prohex-Ca - prohexadione-calcium; F_0 - minimal chlorophyll fluorescence when all dark-treated PS 2 reaction centres are open; F_m - maximal chlorophyll fluorescence in dark-acclimated leaves; F_v - variable fluorescence (F_m - F_0); F_v/F_m - maximal photochemical yield of PS 2 in dark-acclimated leaves; PS 2 - photosystem 2; RC - reaction centre; T_{max} = time to reach F_m.
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or caused reduction of both growth and photosynthetic rate (Bode and Wild 1984). Among the possible causes of the contradictory results are the different ways of measuring and expressing growth and photosynthesis and the different methods of application of GA_s and Prohex-Ca. With these considerations in mind, an attempt was made to evaluate the use of GA_3 and Prohex-Ca on okra plant. This annual vegetable edible crop is cultivated as a summer or a winter crop. The traditional uses of the plants, which are based on the primary products, *i.e.* the fruits and the green herb, are two-fold: medicinal and culinary. We have chosen three okra cultivars differing in growth. The specific objectives were to *1*) investigate the effects of GA_3 and Prohex-Ca on growth, physiology and fruit quality characteristics of three okra cvs., and *2*) examine the possibility of use of GA_3 and Prohex-Ca in order to improve okra productivity.

Experiments were conducted from April to August 2003 inside a greenhouse at the Technological Educational Institute of Thessaloniki in northern Greece. The site was located at 22°55´N, 40°38´E. Experiments were 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 okra [*Abelmoschus esculentus* (L.) Moench] cultivars Pileas, Psalidati, and Clemson Spineless were sown in trays filled with a commercial germination mix and germinated on a greenhouse mist bench (20 s of mist every 30 min) at a temperature of 22 ± 2 °C. At the three to four leaves' stage, uniform seedlings were transplanted individually and randomly inside greenhouse, in 27 experimental plots. Each of them contained one line of seven plants. The distance among plants was 40 cm while the distance between lines was 70 cm. Plants were acclimated for one week 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 60.0 mg N, 26.2 mg K, 49.8 mg P (water-soluble fertilizer 20-20-20, *F-TOP Ledra*, Thessaloniki, Greece) during the experiment. The photosynthetically active radiation (PAR) at plant height in the greenhouse was of 500 - 700 μmol m^{-2} s^{-1} (measured by a *Li-6200* portable photosynthesis meter, *LiCor*, Lincoln, NE, USA). Plants were maintained in the greenhouse under natural sunlight, average day/night temperatures were 35 ± 2 / 28± 2 °C. The experiments were terminated when all plants had fruits.

GA_3 was dissolved in 1 mM 95 % ethanol and diluted with distilled water to a final stock concentration of 100 μM and also Prohex-Ca (*BAS 125 10W, BASF Corp.*, Research Triangle Park, NC, USA) concentration was 100 μM. Each solution contained 0.1 % *Agral 90* as a surfactant (*Syngenta*, Ontario, Canada). First application of GA_3 and Prohex-Ca were made 3 weeks after germination (plants had 4 to 5 leaves). A set of 7 plants in each plot was foliar sprayed (main axis) to run off four times at 10-d intervals with each of the above solution. Control (C) plants (7 plants in each plot) were treated with water and surfactant.

Fast chlorophyll (Chl) fluorescence measurement was performed on pre-darkened leaves (30 min at room temperature) and excited with red (630 nm) radiation (600 W m^{-2} on the upper surface) using a Plant Efficiency Analyzer (PEA, *Hansatech*, King's Lynn, England). The following characteristics were calculated: F_0 = initial fluorescence when all reaction centres (RCs) are open, F_m = maximal measured fluorescence when all RCs are closed, F_v = variable fluorescence; $F_v = F_m - F_0$, T_{max} = time to reach the F_m, Area = summation of all values ($F_m - F_t$) for t = 0 to t = T_{max} (for detail see, Ouzounidou and Ilias 2005).

Main stem length (measured every 10-d from growing medium surface to apex), the number of days from sowing until the first flowering, fresh (FM) and dry masses (DM) of stems, leaves and seeds, were recorded for each plant. Determinations of water content, sugars, and proteins in mature okra fruits were carried out in three separate samples. Water content was determined using drying at 105 °C, while dry mass was determined after drying of stems and leaves at 90 °C for 2 d. Proteins were determined by the Kjeldahl method (Williams 1984). Sugar analysis was performed with a high performance liquid chromatograph (HPLC, *HP 1100*, Germany). Glucose, fructose, and saccharose were determined with a refractive index detector (RID) using a reverse phase column 250 × 4 mm (*Lichrosphere NH_2*) bonded to microparticulate silica of 5 μm diameter maintained at 37 °C. Injection of 20 mm^3 sample solution into a mobile solvent of H_2O/AcCN (25/75; v/v) with a flow rate of 1.1 cm^3 min^{-1} gave the optimum result (Manolopoulou and Papadopoulou 1998).

Application of GA_3 caused significant ($P < 0.05$) increase in DM of the stems by 10, 30, and 28 % of the control (C) in Pileas, Psalidati, and Clemson Spineless, respectively (Table 1). The leaf biomass also increased under GA_3 treatment; the highest one occurred in Psalidati (5.5 times more than C). GA_3 significantly increased stem length in Clemson Spineless by 22 % of C. Prohex-Ca induced significant depression of both leaf and stem DM of all cultivars (Table 1). The effect of Prohex-Ca on stem elongation was also inhibitory, showing the highest depression in Psalidati (Table 1). However, application of Prohex-Ca resulted in important increase of seed DM in Pileas and Psalidati. The time from germination to flowering was significantly shorter under GA_3 in Clemson Spineless (Table 1).

F_0 was significantly decreased under GA_3 treatment in Pileas and under Prohex-Ca in Clemson Spineless (Table 1). F_m in Pileas declined under both treatments, in Clemson Spineless increased under Prohex-Ca application. Significant decline in F_v/F_m was found only in Pileas under Prohex-Ca application. The ratio T_{max}/Area was significantly suppressed under GA_3 supply in Pileas and Psalidati (Table 1). The long-term exposure to plant regulators provoked alterations in the photo-chemical efficiency of PS 2 (F_v/F_0) that is more sensitive than the

Table 1. Effect of GA_3 (100 μM) and Prohex-Ca (100 μM) application on paramaters of growth, chlorophyll *a* fluorescence induction kinetics, and fruit quality of okra plants. Means of 21 plants (growth), five (fluorescence), three (fruit) and 100 seeds (seed DM) measurements. * - significant differences from control ($P \leq 0.05$).

Parameters	Pileas			Psalidati			Clemson Spineless		
	C	GA_3	Prohex-Ca	C	GA_3	Prohex-Ca	C	GA_3	Prohex-Ca
Seed DM [g]	9.9	9.5	11.7*	12.2	11.6	13.2*	12.2	11.0	11.7
Stem DM [g]	206.7	226.7*	66.2*	87.8	115.4*	61.8*	47.9	61.3*	35.9*
Leaf DM [g]	154.6	167.2*	61.8*	66.2	176.9*	56.4*	32.9	43.2*	18.5*
Stem length [cm]	147	156	122*	67	93*	51*	90	110*	91
Flowering time [d]	94	90	93	65	61	64	85	77*	85
F_0	0.708±0.09	0.690±0.08*	0.734±0.07	0.666±0.05	0.621±0.09	0.703±0.08	0.601±0.06	0.639±0.06	0.714±0.05*
F_m	3.754±0.09	3.500±0.08*	3.154±0.10*	3.467±0.07	3.433±0.08	3.600±0.08	3.223±0.05	3.226±0.08	3.500±0.10*
F_v/F_m	0.811	0.800	0.767*	0.807	0.818	0.804	0.813	0.804	0.782
F_v/F_0	4.3	4.0	3.3*	4.2	4.5	4.1	4.3	4.0	3.8*
T_{max}/Area	0.55	0.42*	0.56	0.60	0.40*	0.53	0.57	0.58	0.56
Area/F_v	19.6	23.2*	20.2	18.9	25.3*	20.1	19.1	21.0*	20.0
Water content [%]	81.1	82.9	83.2	87.5	86.0	85.3	87.1	83.3	87.9
Fructose [%(DM)]	0.30	0.22*	0.22*	0.21	0.24	0.18*	0.18	0.18	0.15*
Glucose [%(DM)]	0.28	0.27	0.24	0.23	0.20*	0.16*	0.21	0.23	0.18
Sucrose [%(DM)]	0.23	0.12*	0.24	0.11	0.13	0.18*	0.18	0.10*	0.16
Proteins [%(DM)]	0.64	0.53*	0.44*	0.35	0.41	0.41	0.31	0.43*	0.29

maximum quantum yield (Table 1). GA_3 treatment caused a severe increase on the ratio Area/F_v in the three cultivars, whereas Prohex-Ca treatment resulted in no significant increases (Table 1).

Water content of fruits was not changed under both the growth regulator treatments. A steady decrease in fructose content was observed after GA_3 and Prohex-Ca application (Table 1). Glucose content was reduced under both GA_3 and Prohex-Ca application only in Psalidati. In contrast, saccharose content declined under GA_3 treatment in Pileas and Clemson Spineless and significantly increased under Prohex-Ca treatment in Psalidati, while protein content declined in GA_3 and Prohex-Ca-treated Pileas, as well as in GA_3-treated Clemson Spineless (Table 1).

Our results are in agreement with those of Daykin *et al.* (1997) and Hisamatsu *et al. (*2000) showing that GA_3 stimulates both cell division and cell elongation. On the other hand, Prohex-Ca inhibits excessive vegetative growth, while it may cause a delay of senescence as a result of reduced ethylene production (Rademacher *et al.* 1992).

C and Prohex-Ca treated okra plants took more time to bloom than did GA_3 treated plants. Williams *et al.* (1999) demonstrated that Prohex-Ca was ineffective in promoting precocious flowering in *Eucalyptus*, in contrast, Hisamatsu *et al.* (1998) found that both GA_3 and Prohex-Ca significantly accelerated the flowering season in *Matthiola incana* plants.

According to Davies (1995) the mode of GA_3 action is the influence of hydrolytic enzymes of starch, fructans, and sucrose. Thus, we postulate that the reduced contents of sugars observed under GA_3 application could be the result of affection of sugar hydrolysis and metabolism by GA_3. The tendency to increase protein content under GA_3 application, may be a result of higher N assimilation, it is correlated well with the increased okra growth. In parallel, a clear tendency of reduction of the above parameters under Prohex-Ca application was observed.

Gibberellins strongly promote plant development and growth of stems, leaves, and roots (Tanimoto 1987, Nagel *et al.* 2001). However, one question still remains, if this increase in size is due to increased photosynthetic rates or is it due to more efficient utilization of photosynthetic products. The weak changes of Chl fluorescence characteristics caused by the two growth regulators show that the two substances probably act *via* specific physiological and metabolic pathways.

In conclusion, both GA_3 and Prohex-Ca lead to some alterations in the physiological and metabolic pathways that resulted in growth and quality changes of okra plants. Prohex-Ca was effective in reducing stem elongation of two okra cultivars while GA_3 promoted stem elongation.

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