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DIFFERENTIAL RESPONSES OF ONION AND GARLIC AGAINST PLANT GROWTH REGULATORS

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Abstract

The effects of Gibberellic acid-GA_3, Prohexadione-Calcium, and Ethephon pre-harvest application on yield, biomass production, photosynthetic function, lipid peroxidation and quality characteristics of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) plants were investigated. Shoot length and biomass of onion and garlic, expressed either in fresh or dry weight, increased significantly under GA_3, while a progressive decrease under Prohex-Ca and Ethephon occurred. Higher MDA (lipid peroxidation) values were recorded after Prohex-Ca and Ethephon supply on onion and garlic plants; it seems that GA_3 treatment prevents lipid peroxidation as measured with the help of the TBARS method. Plants treated with Prohex-Ca and Ethephon revealed higher peroxidase activity compared to control and GA_3 treated plants. Considering the results of MDA content and peroxidase activities it can be assumed that GA_3 treated plants are slightly protected from the natural course of oxidative stress, which occurs during ageing as observed for control samples. The fluctuations of chlorophyll fluorescence parameters represent a general decline in chloroplasts function after plant growth regulators exposure, whereas in combination to the suppressed chlorophyll content, structural malformations of photosystems may also occur. The production of ascorbic acid, glucose and fructose content seems to be enhanced under GA_3 in both species, while their values were depressed under Prohex-Ca and Ethephon. Overall, only GA_3 supply leads to a vigorous onion and garlic growth and yield.

Introduction

Onion, (*Allium cepa* L.) is a biennial of the *Alliacea* family. The edible portions of the bulb are the fleshy leaf bases and compact stem. Green and spring onions are eaten for their immature bulb and green foliage (Adamicki, 1998). Spring onion is a salad vegetable consumed worldwide and it is favorable because of its volatile flavors released during tissue disruption (Abbey & Joyce, 2004). Major onion producers are China, India, U.S., Turkey, Japan and Spain. Most studies on physiological stress effects on growth and quality of *Allium* spp., have been focused on bulb onions (Benkeblia & Shiomi, 2004). Studies on spring onions have received little attention.

Garlic (*Allium sativum* L.) is also a member of the family *Alliaceae* and is produced as an annual crop for seed, fresh market and proceed products (Cantwell, 2000). Because of its characteristic pungent flavour, garlic has been cultivated since antiquity as a vegetable and flavoring agent. Garlic also has many medicinal properties; it acts as an antimicrobial agent and improves blood sugar metabolism, it prevents atherosclerosis and coronary heart disease by reducing platelet aggregation, promoting fibrinolysis, and lowering blood triglyceride and cholesterol levels. *Allium* vegetables, particularly common onion and garlic, are extremely abundant in flavonoids, especially quercetin (Patil *et al*., 1995, Sellappan & Akoh, 2002).

Plant growth regulators (PGRs) have a particularly interesting role in modern agriculture (Ashraf *et al*., 2011). In Greece and others European countries the PGRs are commonly used on food crops (melon, pepper, celery etc) in order to improve and accelerate plant productivity. The knowledge of their metabolic and transport pathways will lead to new opportunities to manipulate regulator levels and thus regulate plant growth.

Gibberellins play a major role in diverse growth processes including seed development, organ elongation, senescence and control of flowering time (Ouzounidou *et al.,* 2008; Yamaguchi, 2008; Yu *et al.,* 2009). GAs are synthesized from geranylgeranyl diphosphate in a multi-enzyme pathway that is subject to complex regulation. Further, GA levels are influenced by other hormones such as ethylene (Santner *et al*., 2009). Prohexadione-Calcium also, inhibits late stages of GA biosynthesis e.g. hydroxylation of GA_{20} to GA_1 and reduces the vegetative growth of the plant (Brown *et al.,* 1997; Kim *et al.,* 2007). The gaseous hormone ethylene plays a key role in plant senescence and fruit ripening (Santner *et al.,* 2009). Ethephon, an ethylene-realising compound, can induce leaf senescence as well as generation of reactive oxygen species, which in turn leads to cell death (Chen *et al*., 2010).

The onset and progression of plant growth and senescence can be influenced by exogenous and endogenous factors affecting the photosynthetic efficiency, the enzyme activities and the nutritional value (Yu *et al*., 2010). GA_3 inhibits senescence mainly by a modulation of lipid peroxidation through maintaining high levels of such cellular scavengers as SOD and catalase (Dhindsa *et al*., 1982). It is reported that Ethephon decreases photosynthesis by increasing ethylene levels (Davies, 1995), while, other substances like Prohexadione Calcium and Cycocel block plant growth development by the inhibition of GAs biosynthesis (Ouzounidou *et a*l., 2010).

The objectives of this study were to study the effects of certain PGRs pre-harvest application on yield, biomass production, photosynthetic function, lipid peroxidation and quality characteristics of onion and garlic plants.

Materials and Methods

Local bulbs of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) were sown in a greenhouse experiments at the Technological Educational Institute of Thessaloniki (northern Greece) (22°55΄E, 40°38΄N) to determine the effect of pre-harvest application of GA_3, Prohexadione-Calcium and Ethephon on plant growth,

photosynthesis, biochemistry and quality. Bulbs were sown individually and randomly inside greenhouse, in experimental plots. Experiments were 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%, electrical conductivity 1.5μS cm^{-1}, and pH (1:2 H_2O) 7.4. The region is characterised by continental climatic conditions. 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 and 49.8 mg P or P_2O_5 (water-soluble fertilizer 20-20-20, *F-TOP Ledra*, Thessaloniki, Greece) during the experiments. 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 30 ± 2 / 26± 2 °C. Green onions and garlic plants were harvested after seven or eight weeks period in two stages with no bulbs and immature bulbs and green foliage for salad use.

In all experiments with onion and garlic, Ethephon at 100 mg l^{-1}, Prohexadione-Calcium (Prohex-Ca), (*BAS 125 10W, BASF Corp.*, Research Triangle Park, NC, USA) at 200 mg l^{-1} and GA_3 at 100 μM were evaluated. GA_3 was dissolved in 1 mM 95 % ethanol and diluted with distilled water to a final stock concentration of 100 μM). Each solution contained 0.1 % Agral 90 as a surfactant (Syngenta, Ontario, Canada). First application of Ethephon, GA_3 and Prohex-Ca were made 3 weeks after germination. A set of 30 plants in each plot was foliar sprayed (main axis) 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. PGR concentrations and spraying time have been selected after preliminary experiments.

The level of lipid peroxidation in plant tissue was measured as malondialdehyde (MDA) content determined by reaction with 2-thiobarbituric acid (TBA)-reactive substances according to Hodges *et al.,* (1999) (modified the first method by Heath & Packer, (1968). Tissue was homogenized in TBA solution comprised of 20.0% (w/v) trichloroacetic acid and 0.01% butylated hydroxytoluene, at 4°C Absorbances were read at 440, 532, and 600 nm. The concentration of MDA was calculated from the difference of the absorbance at 400, 532 and 600 nm using the extinction coefficient of 157 $mmol^{-1}cm^{-1}$ and expressed as nmol MDA g^{-1} fresh weight.

Fresh tissue was homogenized in an ice-cooled mortar with 2 ml Tris-HCl buffer (0.1M, pH 7.5) containing 1mM Phenyl methyl sulfonic fluoride (PMSF) and 5% (w/v) Polyvinyl-polypyrolidone (PVPP). The homogenate was centrifuged at 15.000 rpm for 15 min at 4°C. The supernatant was used for enzyme determination. Total peroxidase activity was determined spectrophotometrically by monitoring the formation of an indamine dye from 3-dimethylamino-benzoic acid (DMAB) and 3-methyl-2-benzothiazolinone hydrozone hydrochloride monohydrate (MBTH) at 590 nm in the presence of H_2O_2 according to the method of Ngo & Lenhoff, (1980).

In vivo PSII chlorophyll fluorescence was measured in a fully expanded leaf by a modulated (1.6 kHz), low intensity beam from light emitting diodes (excitation wavelength 655nm, detection above 700nm) using a portable pulse-amplitude-modulated fluorometer (PEA-Hansatech; Walz, Germany). The following fluorescence indices were calculated: the initial fluorescence intensity (Fo) when all reactions centers (RCs) are open, the maximal fluorescence intensity (Fm), when all reactions are close, the variable fluorescence (F_v), the ratios Fv/Fm and Fv/Fo (Ouzounidou *et al.,* 2006). Chlorophyll a+b was extracted in 100% acetone as described by Ouzounidou *et al.,* (1997).

Samples of 50 g of green onion and garlic were closed in small glass jars (0.5 L). Gas exchange measurements (CO_2) were made by gas-chromatographer Perkin-Elmer 8700 with a TC detector on individual plant in glass jars at 20°C. Respiration rate is expressed as $mgCO_2$ $kg^{-1}h^{-1}$ (Ouzounidou *et al.,* 2010).

Chemical analysis was realized during the two growth periods. The first, when plants had no bulbs and the second, when the plants had white immature bulbs. The ascorbic acid content of onion and garlic was estimated by macerating the plant sample mechanically with a stabilising agent (5% metaphosphoric acid) and titrating the filtered extract with 2,6 dichlorophenolindophenol. Glucose, fructose and sucrose 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 H_2O/AcCN (25:75; v/v) with a flow rate of 1.1cm^3 min^{-1} gave the optimum result (Ouzounidou *et al.,* 2008). The moisture content was determinated according to the AOAC method (Anon., 1990).

Fifteen experimental plots (three replications for each treatment) were set up randomly inside the greenhouse using a randomized complete block design for each onion and garlic plants. Thirty single plants were used in each treatment combination/ replication. Each plot contained three rows with 10 plants per row spaced 15 cm apart within each row. Distance between rows was 35 cm while distance between plots was 60 cm.

Main stem length (measured from growing medium surface to apex) and shoot fresh weight (stem and leaves) were recorded at the end of the experiments. Shoot dry weight were recorded 2-3 weeks after harvest. Broadleaf and grass weed species were removed by hand-weeding. Insect management and other cultural practices were carried out according to the recommended production practices. For the onion and garlic data (plant height, shoot fresh and dry weight and yield) were subjected to analysis of variance (ANOVA) and the treatments were compared using Fisher's Protected LSD test (p <0.05) and SPSS program.

Results and Discussion

Growth and chlorophyll concentration: During maturity, a passes through a series of changes in colour, texture and flavour indicating that compositional changes are taking place. The pre-harvest application of plant growth regulators, influenced in a different way the course of plant development as it was reported by Ouzounidou *et al.,* (2008; 2010) for other plant species.

GA_3 promoted the total stem elongation of onion and garlic by 35% and 25% of the control respectively ($p<0.05$, Table 1); whereas a significant inhibition of the shoot length with application of Prohex-Ca by 11% and 12% and Ethephon by 35% and 25% for onion and garlic respectively, was observed (Table 1). Shoot biomass of onion and garlic, expressed either in fresh or dry weight, increased significantly under GA_3, while a progressive decrease under Prohex-Ca and Ethephon occurred as compared to the control (Table 1, $p<0.05$). Generally, during the maturity, plant biomass increased considerably, however in our case, only GA_3 supply leads to a vigorous plant growth and yield. Our results are in agreement with those of Daykin *et al.*, (1997) and Hisamatsu *et al.,* (1998) showing that GA_3 stimulates both cell division and cell elongation.

Table 1. Changes of shoot elongation (n=15), fresh (n=15) and dry biomass (n=15) and chlorophyll concentration (n=3, mg g^{-1} F.W.) of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) plants after plant growth regulators application.

Treatments	Shoot length (cm)		Shoot fresh mass (g)		Shoot dry mass (g)		Chlorophyll (a+b)	
	Onion	Garlic	Onion	Garlic	Onion	Garlic	Onion	Garlic
Control	16.80[b]	21.80[b]	55.10[b]	14.55[b]	5.38[b]	2.89[b]	12.46[a]	10.44[a]
GA_3	22.70[a]	27.35[a]	62.60[a]	19.67[a]	6.87[a]	3.99[a]	12.53[a]	10.67[a]
Prohex-Ca	14.90[c]	19.08[c]	24.83[c]	11.31[c]	2.78[c]	2.01[c]	11.30[b]	9.96[a]
Ethephon	10.93[d]	16.40[d]	8.93[d]	10.60[d]	0.95[d]	1.40[d]	9.64[c]	8.97[b]

Means in the same column followed by different letters are significantly different ($p<0.05$)

Chlorophyll a+b concentration of onion and garlic, remained almost unchanged with GA_3, while it was negatively affected by 9% and 5% of the control after Prohex-Ca treatment and significantly by 23% and 14% under Ethephon pre-harvest application (Table 1). Chlorophyll a was more affected than chlorophyll b during PGRs exposure (data not shown). Our results agree with Chen *et al.,* (2010) and Ouzounidou *et al.,* (2008) findings in sweet potato and green pepper leaves and suggest that Ethephon, an ethylene-releasing compound, can induce leaf senescence and chloroplast breakdown. Growth regulators reduce chlorophyll content by inhibition of cytochrome P450-dependent hydroxylation reactions in chlorophyll biosynthesis (Davis *et al*., 1988). According to Arteca, (1996), Ouzounidou & Ilias, (2005) and Shah *et al.,* (2007) GA_3 induces enhancement of ultrastructural morphogenesis of plastids, which coupled with the retention of chlorophyll, delay plant senescence.

Biochemistry and respiration rate: One reason for growth retardation may be the oxidative damage indicated by an increase in the H_2O_2 content and lipid peroxidation degree. Furthermore, there is a negative correlation between shoot fresh weight and H_2O_2 content, fresh weight and the content of thiobarbituric acid-reacting substances (TBARS) (Stacy *et al.,* 1996). The malondialdehye (MDA) or thiobarbituric acid reactive substances (TBARS) assay has been used extensively since the 1950s to estimate peroxidation of lipids in membrane and biological systems (Sinnhuber *et al.,* 1958; Heath & Packer, 1968; Pelle *et al.,* 1990; Du & Bramlage, 1992; DeLong & Stefen, 1997).

In the present study, the control onion and garlic plants showed enhanced lipid oxidation, estimated as the MDA level, which was 3 and 2 times higher than that observed in GA_3 treated plants (Table 2). Higher MDA values were recorded after Prohex-Ca and Ethephon pre-harvest supply on onion plants by 20 to 38% of the control and by 27 and 4% on garlic plants, respectively, (Table 2). Concluding, it seems that GA_3 treatment prevents lipid peroxidation as measured with the help of the TBARS method.

Peroxidase activity for both plant species under GA_3 treatment, was similar to the control, but significantly higher (up to 2-times) for plants treated with Prohex-Ca and Ethephon (Table 2). Plants treated with Prohex-Ca and Ethephon revealed higher enzymatic activity compared to control and GA_3 treated plants. Growth regulators enhance activity of antioxidant enzymes such as ascorbate peroxidase, glutathione reductase, catalase and peroxidase, which protects plants from a variety of stresses (Dwyer *et al.,* 2001; Fletcher *et al.,* 2000). Considering the results of MDA content and peroxidase activities it can be assumed that GA_3 treated plants are slightly protected from the natural course of oxidative stress, which occurs during ageing as observed for control samples. Similar results were found by Yu *et al.,* (2009) in *Paris polyphylla* after GA_3 treatment; the lipid peroxidation and membrane deterioration is lower than in the control. As a result senescence was retarded.

Under GA_3 treatment garlic presented the lowest respiration rate, showing a significant decrease by 11% of the control; onion respiration rate remained almost unchanged. Significantly higher respiration rates were revealed by the plants under Prohex-Ca and Ethephon treatments by 17 to 58% of the control (Table 2). Changes in respiration rate and vitamin C are considered sensitive indicators of changes in tissue condition after harvest (Perrin & Gaye, 1986; Ouzounidou *et al.,* 2008). The increase in respiration rate of onion and garlic plants and the decrease in ascorbic acid content (Table 3) correlate well with the chlorophyll loss (Table 1) under Prohex-Ca, and Ethephon application, representing the reduction in freshness and the beginning of senescence.

Chlorophyll fluorescence: Fluctuations of chlorophyll fluorescence indices in onion and garlic leaves are given in Fig. 1. The pre-harvest PGRs application induced differential changes in minimum, variable and maximum fluorescence (Fig 1A). The maximum quantum yield of primary photochemistry (*Fv/Fm*) was enhanced by 8 and 4% of the control under GA_3 application in onion and garlic; whereas it was significantly decreased with application of Ethephon by 10% in both onion and garlic. Prohex-Ca displayed lower decrease by 5% and 9% of the control, in onion and garlic, respectively (Fig 1B). The *Fv/Fo* ratio revealed higher sensitivity, showing significant increase by 49 and 22% of the control in onion and garlic with GA_3 application and an inhibition by 20 to 35% of the control under Prohex-Ca and Ethephon application (Fig 1B). The observed decline of variable fluorescence (Fv) represents a general decline in chloroplast function after exposure to PGRs (Ouzounidou *et al.,* 2006). The slight decrease in F_v/F_m in both species, indicates that growth retardants diminished reoxidation of Q_A^- and started to inactivate the RC of PSII; while an inhibition of enzymatic process in the Calvin cycle of the plants subjected by Prohexadione-Ca and Ethephon may have occurred (Ouzounidou *et al.,* 2008). *Fv/Fm* was reduced less than *Fv/Fo* pointing to disturbances in the photosynthetic electron transport or damage to the thylakoid structure in the donor side of PS2 (Ouzounidou *et al.,* 1997; Drazkiewicz & Baszynski, 2008). It can also be suggested that treating plants with growth regulators contributes to inefficient energy metabolism in plants.

Table 2. Changes of lipid peroxidation MDA (n=3, nmol/g F.W.), peroxidase activity (n=3, units mg^{-1} protein) and respiration rate (n=3, $mgCO_2\ kg^{-1}h^{-1}$) of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) plants after plant growth regulators application.

Treatments	MDA		PA		Respiration rate	
	Onion	Garlic	Onion	Garlic	Onion	Garlic
Control	7.80 [c]	10.93 [b]	0.25 [c]	0.25 [c]	40[c]	36[d]
GA_3	2.46 [d]	5.14 [c]	0.27 [c]	0.25 [c]	38[c]	32[c]
Prohex-Ca	9.31 [b]	13.88 [a]	0.48 [b]	0.50 [b]	49[b]	42[b]
Ethephon	10.76 [a]	11.34 [b]	0.89 [a]	0.77 [a]	63[a]	56[a]

Means in the same column followed by different letters are significantly different (p<0.05)

Table 3. Plant growth regulators effects on moisture (% fresh mass), ascorbic acid (% F.M.), glucose, fructose and sucrose (% D.M.) content of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) measured on two growth stages.

Treatments	Moisture		Ascorbic acid		Glucose		Fructose		Sucrose		(F+G)/S	
	Onion	Garlic	Onion	Garlic	Onion	Garlic	Onion	Garlic	Onion	Garlic	Onion	Garlic
Control no bulbs	91.4a	86.6a	9.0ab	6.1c	3.0b	6.8a	3.0a	5.7a	1.4c	2.4b	4.29c	5.21ab
With bulbs	90.3a	86.9a	9.6a	7.0b	3.3b	7.2a	3.0b	5.6a	2.0b	2.8a	3.15d	4.57c
GA_3 no bulbs	90.2a	81.8a	10.2a	8.8a	3.3b	7.0a	3.0a	5.9a	2.0b	2.7a	3.50d	4.78c
With bulbs	87.4a	81.7a	9.3a	8.5a	3.8a	7.4a	3.6a	6.2a	2.3a	3.0a	3.22d	4.53c
Prohex-Ca no bulbs	91.7a	88.6a	8.4b	6.4c	3.b	5.2c	2.9a	4.9b	1.0d	1.9cb	5.90b	5.32a
With bulbs	88.7a	82.4a	7.4c	7.3b	2.7c	6.0b	2.9b	4.8b	1.5c	2.1b	3.73d	5.14b
Ethephonno bulbs	88.6a	86.6a	5.1e	5.2d	2.7c	4.3d	2.9a	4.8b	0.5e	1.6d	11.20a	5.69a
With bulbs	87.3a	84.1a	6.4d	4.5d	1.9d	6.1b	2.2c	5.0c	0.9d	1.9cb	4.56c	5.84a

Means (n=3) in the same column followed by different letters are significantly different (p<0.05)

Plant quality characteristics: The Table 3 sets out the changes in the quality characteristics after PGR pre-harvest application. Both species possessed moisture content much greater than 80% in the stage with no bulbs formation, which slightly diminished in the stage with immature bulbs. It is well known that moisture decreased during ripening due to the higher respiration rates (Vekiari *et al.,* 2004).

The content of ascorbic acid under GA_3 in garlic with no bulbs and with immature bulbs, increased by 44 and 21% of the control (p<0.05), respectively (Table 3), but decreased sharply under the Ethephon (by 25 and 36% of the control respectively, Table 3). A significant increase in ascorbic acid content in onion plants with no bulbs by 13% under GA_3 application was observed, while Prohex-Ca and Ethephon reduced the ascorbic acid content in a greater degree. Ascorbic acid content of the chilli and pepper fruits was significantly increased with the application of GA_3 over other growth regulators (Chaudhary *et al.,* 2006; Ouzounidou *et al.,* 2010). 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. Glucose and fructose content seems to be enhanced under GA_3 application, whereas their values were depressed under Prohex-Ca and Ethephon in both species (Table 3). Sucrose concentration was more sensitive to PGRs exposure since it displayed the highest changes. In garlic plants with no bulbs sucrose was increased by 13% of the control and was decreased by 33% on exposure to Ethephon. In onion plants treated with GA_3 a significant increase in sucrose content by 43% and a high drop by 64% of the control in Ethephon treated plants was measured (Table 3).

Furthermore, the glucose to fructose ratio (G/F) remained almost constant, however it tends to decrease under Ethephon treatment for both species. The reducing sugars to sucrose (G + F / S) ratio was slightly suppressed

by GA_3 supply in both onion and garlic plants (Table 3). That ratio sharply increased by 2.6 times and 45% of the control under Ethephon in onion plants with no bulbs and with immature bulbs and by 9 and 28% of the control in garlic plants, respectively (Table 3). Prohex-Ca application inhibited the ratio to a lesser extent than Ethephon. During ripening the sucrose content was increased, while the concentrations of glucose and fructose were decreased. Sucrose biosynthesis may be correlated by the two monosaccharides metabolism as well as by the breakdown of different carbohydrates. Alterations of sugars, soluble solid, titratable acidity and vitamin C have been reported by Awad & Jager, (2002), Mata *et al.,* (2006), Gonzalez-Rossia *et al.,* (2007) and Ouzounidou *et al.,* (2010) under GA_3, Cycocel, Ethephon and Prohex-Ca application. These changes can be attributed to alterations in the activity of different enzymes, related to the maturity, during PGR treatment.

Summing up, the higher yield and better quality of onion and garlic can be achieved by 100μM GA_3 pre-harvest application.

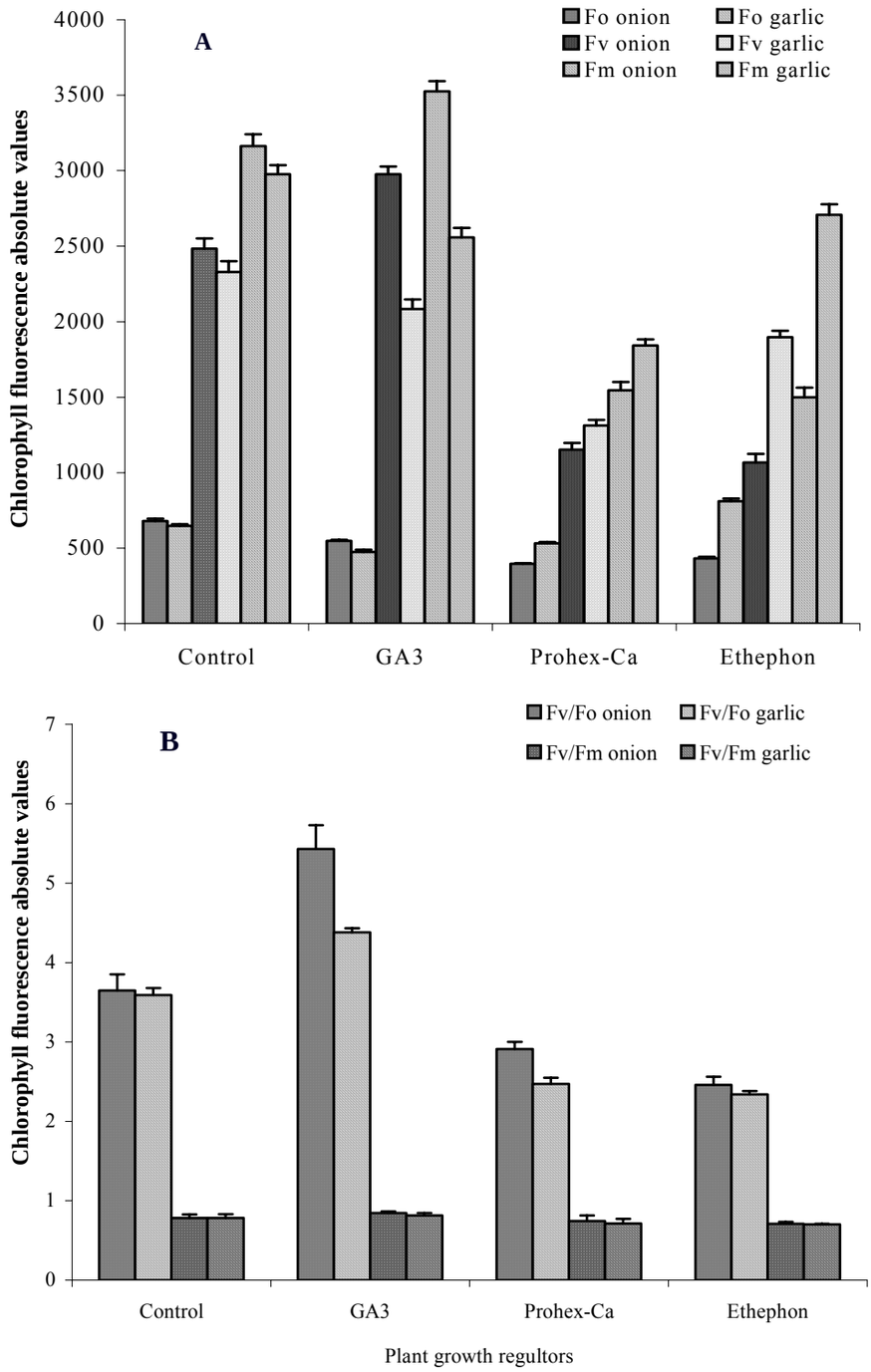

Fig. 1. A. Initial chlorophyll fluorescence (Fo), variable chlorophyll fluorescence (Fv) and maximum chlorophyll fluorescence (Fm) fluctuations and B. Fv/Fo and Fv/Fm ratio changes of onion and garlic leaves as a function of plant growth regulators pre-harvest application. Vertical bars represent the standard error, n=3.

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