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Ethylene synthesis and photosynthetic responses in bean and maize plants exposed to auxins[1]

Síntese de etileno e respostas fotossintéticas em plantas de feijão e milho expostos a auxinas

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Abstract - Auxins IAA (indole-3-acetic acid), 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) were applied on maize (*Zea mays*, monocot) and bean (*Phaseolus vulgaris*, dicot) plants, with the goal to understand the mechanisms that lead to different responses in relation to the metabolism of ethylene. Maize plants treated with auxins did not produce ethylene, whereas beans produced a lot of it after the treatment. After being sprayed with auxins, maize did not produced any 1-Aminocyclopropane-1-carboxylic acid (ACC), which was observed on beans. The maximum quantum yield of the photosystem II (F_v/F_m ratio) and the levels of photosynthetic pigments were not altered in maize plants treated with auxins. Bean plants showed significant decreases in both variables after the treatment with 2,4-D and 2,4,5-T, but not with IAA. The reduction of chlorophyll levels in bean plants treated with 2,4-D and 2,4,5-T was related to the observed chlorosis, since there was a more accentuated degradation of chlorophylls than carotenoids. Xantophylls also had a more accentuated degradation than alpha and beta carotene in bean plants treated with 2,4-D and 2,4,5-T. When aminoethoxyvinylglycine (AVG) and Co^{2+} were provided to bean plants together with auxins, there was no fall in the F_v/F_m ratio and in the pigment levels, except for the alpha carotene.
Keywords: herbicide; *Phaseolus vulgaris*; senescence; vegetal hormones; *Zea mays*

Resumo - As auxinas AIA (ácido indol-3-acético), 2,4-D (ácido 2,4-diclorofenoxiacético) e 2,4,5-T (ácido 2,4,5-triclorofenoxiacético) foram aplicadas em plantas de milho (*Zea mays*, monocotiledônea) e feijão (*Phaseolus vulgaris*, dicotiledônea), visando compreender os mecanismos que levam a respostas diferenciais das plantas de milho e feijão em relação ao metabolismo do etileno. Plantas de milho tratadas com as auxinas não produziram etileno, já o feijão produziu bastante após o tratamento. Após pulverização com as auxinas, o milho não exibiu produção do ácido 1-carboxílico-1-amino-ciclopropano (ACC), fato observado no feijoeiro O rendimento quântico máximo do fotossistema II (razão F_v/F_m) e os níveis dos pigmentos

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fotossintéticos não foram alterados em plantas de milho tratadas com as auxinas. As plantas de feijão mostraram quedas significativas em ambas variáveis após o tratamento com 2,4-D e 2,4,5-T, mas não com o AIA. A redução nos níveis das clorofilas em plantas de feijão tratadas com 2,4-D e 2,4,5-T relacionou-se com a clorose observada, uma vez que ocorreu uma degradação mais acentuada das clorofilas do que dos carotenoides. As xantofilas também tiveram uma degradação mais acentuada do que o alfa e beta caroteno em plantas de feijão tratadas com 2,4-D e 2,4,5-T. Quando aminoetoxivinilglicina (AVG) e Co^{2+} foram fornecidos às plantas de feijão, conjuntamente com as auxinas, não ocorreu queda na razão F_v/F_m e nem no nível dos pigmentos, com exceção do alfa caroteno.
Palavras-chaves: herbicida; *Phaseolus vulgaris*; senescência; hormônios vegetais; *Zea mays*

Introduction

The regulation of metabolism, growth and development, of the morphogenesis and the responses to biotic and abiotic stresses in plants is coordinated by signaling molecules, among which there are phytohormones. Auxins include an important class of phytohormones; the indole-3-acetic acid (IAA) is the natural auxin with the greatest abundance and physiologic importance. When applied at low concentrations, auxins promote cell growth by division and elongation; they are used in the cultivation of callus, cells and tissues, at levels between 10^{-6} to 10^{-8} M.

In addition to this, they are responsible for the coordination of a series of development processes in plants, such as the regulation of the apical dominance, the formation of adventitious roots and the vascular differentiation, among others (Taiz and Zeiger, 2010). Auxins are, moreover, space coordinators of the plant development. The differential distribution of this phytohormone in plant tissues controls a great variety of development processes, enabling the adaptation of plants to different environmental conditions (Vanneste and Friml, 2009).

At relatively high concentrations, auxins present herbicide effect on dicots. On the other hand, monocots present responses to treatments with auxins, due to mechanisms such as the limited distribution of the applied auxins, quick auxin inactivation and differentiated perception of auxins in relation to dicots (Kelley and Riechers, 2007). Such facts increase the importance of studying the effects of ethylene production in response to auxins in monocots, in order to compare them with the symptoms observed on dicots. Herbicide symptoms include leaf and stem epinasty, inhibition of root and shoot growth, decrease in the elongation of internodes, leaf area reduction and abscission. These symptoms are followed by accelerated leaf senescence, progressive chlorosis and by the destabilization of the membrane and vascular system integrity, leading to desiccation, necrosis and death of the plant (Grossmann 2010).

The most important chemical classes of the synthetic herbicide auxins include phenolcarboxylic acids (2,4-dichlorophenoxyacetic acid - 2,4-D), benzoic acid, pyridines and quinolines. When at high concentrations, these compounds promote the same kind of response in plants as IAA. Such responses are also similar to the ones observed in lines of *Arabidopsis thaliana* transgenic plants, super expressing the IAA (Mashiguchi et al. 2011). Natural auxins are subjected to a quicker inactivation, by degradation and conjugation, compared to synthetic herbicide auxins (Woodward and Bartel, 2005). These, in turn, present a more durable and intense effect on plants, due to high stability, lower enzymatic degradation and also to the fact that they are not subjected to many homeostatic and metabolic mechanisms that may affect the IAA (Grossmann 2010).

As demonstrated in a variety of dicotyledonous species and in auxin supeproducing transgenic plants (Argueso et al.

2007), auxins stimulate the biosynthesis of ethylene, so that their effects may be related to the morpho-physiologic effects of ethylene (Dayan et al. 2010). With the establishment of the Yang cycle, it was possible to determine that the key-enzyme of the ethylene biosynthesis is the synthase of the 1-Aminocyclopropane-1-carboxylic acid (ACS); the 1-Aminocyclopropane-1-carboxylic acid (ACC) is the immediate precursor of ethylene in its biosynthetic route. The expression and activity of the ACS enzyme are significantly increased in dicots treated with auxins at relatively high concentrations (Kraft et al. 2007; Guilfoyle 2007), which would explain the action of the herbicide auxins on sensitive plants (dicots). Isoforms of this enzyme are coded by the multigene family ACS, to which the genes of the initial responses to auxins belong. These isoforms are expressed in a differentiated way or, in addition, are regulated post-transcriptionally or post-translationally by the IAA and by herbicide auxins, a few minutes after their application (Stepanova et al. 2007; El-Sharkawy et al. 2008). Interactions between IAA and ethylene have been studied a lot, for example the regulation of the shoot and root growth depends on balances between these two hormones (Pierik et al. 2009).

In light of the aforementioned, this work has the goal to investigate the action mechanism of auxins at high concentrations, which lead to a differential response in monocots and dicots.

Material and Methods

Plant material and establishment of experimental conditions

Maize (*Zea mays*) variety UFV-M100 Nativo and bean (*Phaseolus vulgaris*) cultivar Ouro Vermelho plants were cultivated in greenhouses in Viçosa (30°45' S, 42°15' W), from July 2011 to January 2013. Plants were used in the experiments two weeks after germination, when they were submitted to the different treatments. Plastic 0.5 dm^3 planters were used, and also plastic trays and TROPSTRATO HT substrate (Vida Verde Indústria e Comércio de Insumos Orgânicos Ltda, Mogi Mirim - SP).

Maize and bean plants were treated with a natural (IAA) and two synthetic (2,4-D; 2,4,5-T) auxins at the concentration of 10^{-3} M, in a pH 7.0 solution, in order to follow the development of characteristic symptoms. Control sample plants were treated with deionized water. Hormonal solutions were prepared using Sigma Aldrich auxins with 99% purity.

Inhibition of ethylene biosynthesis

In order to investigate the action of the herbicide auxins with the biosynthesis of ethylene, bean and maize plants were treated with IAA, 2,4-D and 2,4,5-T and they were also treated with some biosynthesis inhibitors, such as AVG 10^{-3} M (inhibiting the ACS activity) and Co^{2+} 10^{-3} M, which is an inhibitor of the 1-Aminocyclopropane-1-carboxylic acid (ACO) oxidase activity. Both inhibitors were provided in a solution to the cut base of plant stems, via vacuum infiltration (- 0.07 MPa) for 3 minutes, with a 4 min interval without vacuum and, after that, another vacuum infiltration for 3 more minutes. Plants remained in the solutions for 2 hours, in order to guarantee the absorption of the compounds. After two hours, plants were transferred to plastic cups with tap water, where they received auxin spraying.

Determination of ethylene levels

The ethylene emanated by plants in response to the application of auxins was quantified, as a way to speculate about the association of ethylene with the other variables under study, such as pigment concentration and the F_v/F_m ratio.

After two hours from the auxin spraying, bean leaves and leaf sections from maize plants, of approximately 2 g of fresh mass (FM), were placed in 250 cm^3 Erlenmeyer flasks containing, at the bottom, two layers of Whatman n. 1 filter paper dampened with 4 cm^3 of deionized water. The internal atmosphere of the flasks was

homogenized, occasionally, with a long-needle syringe perforating the seal.

The ethylene emanated by plants was quantified according to the technique described by Silva et al. (2014). Samples of 1 cm^3 of the free atmosphere of the Erlenmeyer flasks were collected with the help of disposable plastic syringes with volume of 1 cm^3 (29 G ½'' needles). Samples were collected 2, 12, 24 and 48 h after placing the leaves in the Erlenmeyer flasks. It was then possible to obtain the total ethylene accumulated in each collection time and also the ethylene production rate, dividing the total accumulated ethylene by the time during which the leaves were kept in the Erlenmeyer flasks.

Subsequently, they were injected in a Hewlett-Packard 5890 series II gas chromatograph, equipped with a flame ionization detector and stainless steel column (1.0 m x 6.0 mm), wrapped with Porapak-N (80-100 mesh). Nitrogen was the carrier gas, in a 30 cm^3 min^{-1} flux. Hydrogen and air flows were kept at 30 and 320 cm^3 min^{-1}, respectively. Temperature of the column, injector and detector were kept at 60, 110 and 150°C, respectively. The quantification of ethylene was determined from the ethylene peak areas, calculated with the N2000 online Chromatostation software, associated to the chromatograph, by comparison with areas from the ethylene standard.

Quantification of ACC

In order to evaluate the ACS activity in response to auxins, the ACC determination was performed on bean and maize plants. ACC was quantified on plants according to the technique described by Gallardo et al. (1991), with some changes. Samples were collected 2, 12, 24 and 48 h after spraying with auxins. The extraction of ACC was made by grinding the leaf samples (approximately 2.0 g of FM) in a mortar with 10 cm^3 of ethanol (80%) and polyvinylpolypyrrolidone (PVPP 5 % m/v). The extract was centrifuged at 28000 *g* for 20 minutes, at 4°C and the supernatant was submitted to evaporation in a rotating evaporator, at 45°C until dryness. The residue was re-suspended in 4 cm^3 of distillated water (original extract).

Half of the original extract was used to quantify the free ACC, by chemical conversion to ethylene, following the technique described by Lizada and Yang (1979). A 0.5 cm^3 aliquot of the extract was taken in a test 6 cm^3 test tube with 0.1 cm^3 of mercury chloride ($HgCl_2$) (5 µmol), to which water was added, until a volume of 0.8 cm^3. The test tube was sealed with a latex sealer and kept in ice bank. After that, 0.2 cm^3 of a cooled mixture of NaOCl (5 %) and saturated NaOH (v/v 2:1) were injected in the sealed test tube, shaken in a vortex mixer for 5 seconds and incubated in ice bank for 2.5 minutes. After another tube shake for 5 seconds, a gas sample of 1.0 cm^3 of the tube atmosphere was removed for the quantification of ethylene, according to the aforementioned technique.

The other half of the original extract was used to determine the total ACC (free ACC + conjugated ACC). After the acid hydrolysis with HCl (2 N), at 100°C, for 3 hours, the extract was neutralized with NaOH (2 N) and evaporated after dryness and adding, subsequently, 2.0 cm^3 of water. The quantification of total ACC followed the same procedure as the quantification of free ACC. With the difference between the ACC contents before (free) and after the hydrolysis (total ACC), the conjugated ACC in the extract was determined.

Chloroplast pigments

In order to investigate the apparent accelerated senescence in bean plants treated with auxins, in addition to the pigment content, the maximum quantum yield of the photosystem II (F_v/F_m ratio) was followed, as a senescence diagnose of the leaves (Kusaba et al. 2007); these variables were related to the functions of chloroplasts.

Leaf samples of approximately 2 g of FM were collected three days after the spraying

with auxins and were stored in a freezer at - 80° C. For the analyses, 80 mg of the frozen material were macerated in 2 cm^3 of acetone 85%; then, the extract was centrifuged for 10 minutes at 15000 g at 4°C. The supernatant was collected and the volume was adjusted, with acetone 85%, to 3 cm^3. The determination of pigment contents in the samples and in the chlorophyll standards (a, b and total) and of carotenoids (violaxanthin, antheraxanthin, zeaxanthin, lutein, neoxanthin, α-carotene and β-carotene), was made in a high performance liquid chromatograph (HPLC, Hewlett-Packard version 1050, Germany), with a C18 reversed-phase column (Waters Spherisorb® 5µm ODS2 4.6x250 mm Analytical Column) and equipped with an ultraviolet and visible radiation detector, using a solvent system composed by acetonitrile : water: triethylamine (9:1: 0.01 and pure ethyl acetate.

The complete chromatographic run occurred in 27 min, at 440 nm, in a flow of 1.2 cm^3 min^{-1} and using 20 mm^3 of sample; the initial solvent system was composed by 75% of the group acetonitrile : water : triethylamine and 25% of ethyl acetate. For 10 minutes, a gradient took the solvent compound to 59% of acetonitrile : water : triethylamine and 41% of ethyl acetate. For the following 10 minutes, another gradient took the solvent compound to 0% of acetonitrile : water : triethylamine and 100% of ethyl acetate. This last condition lasted for 2 more minutes. Finally, for 5 minutes, there was the post-run, where the initial condition was re-established for a new run (Ramalho et al. 1997).

Standards of the photosynthetic pigments were used (DHI Laboratory Products, Horsholm, Denmark). Using standards, it was possible to obtain peaks (points in mvolt min^{-1}) calculated with the software N2000 online Chromatostation, associated to the chromatograph. In the end, the concentrations were calculated by comparing the obtained areas with the patterns; the results were presented in mg g^{-1} of FM.

Maximum quantum yield of the Photosystem II

The fluorescence of chlorophyll a was evaluated using a fluorometer with modulated pulse width (Mini-PAM, H. Walz, Effeltrich, Germany). After being adapted to the dark for 30 minutes, leaf tissues were initially exposed to a weak pulse of distant-red light (0.03 µmol m^{-2} s^{-1}), in order to determine the initial fluorescence (F_0). After that, a saturating light pulse, with an irradiance of 6000µmol (photons) m^{-2} s^{-1} and duration of 0.8 s, was applied to estimate the maximum emitted fluorescence (F_m). In addition, it was possible to estimate the maximum photochemical effectiveness of the PSII (F_v/F_m), starting from the simple ratio between the variable fluorescence (F_v), which is the subtraction of F_0 from F_m, and the (F_m) (Klughammer and Schreiber, 2008); this variable is provided by the fluorometer.

Experimental design

The experiments were designed in a completely randomized way, with five replications per treatment. In order to determine the ethylene levels, the sample unit was a closed 250 cm^3 Erlenmeyer flask, containing bean leaves (first leaf pair emitted before trefoils) or sections of maize leaves (bigger and fully developed leaves). In this experiment, maize and bean plants were treated with IAA, IAA + AVG + Co^2, 2,4-D, 2,4-D + AVG + Co^{2+}, 2,4,5-T, 2,4,5-T + AVG + Co^{2+} and the control sample plants were treated with water and water + AVG + Co^{2+}. The t test was used in this experiment to compare the effect of AVG + Co^{2+} in the production rate of ethylene for each analysis period after the treatment with auxins. Comparisons were made only between each auxin plus the treatment with AVG + Co^{2+}.

In the experiments of the chemical analyses (quantification of ACC and photosynthetic pigments), five samples of approximately 2.0 g of FM were analyzed. In the experiment where the production of free and total ACC was evaluated, the treatments were

IAA, 2,4-D and 2,4,5-T and the control sample plants were treated with water. Again, the t test was used in this experiment to compare the production rate of free and total ACC for each analysis period after the treatment with auxins. In this experiment of the photosynthetic pigments, the treatments were IAA, IAA + AVG + Co^2, 2,4-D, 2,4-D + AVG + Co^{2+}, 2,4,5-T, 2,4,5-T + AVG + Co^{2+} and the control sample plants were treated with water and water + AVG + Co^{2+}. The Scott-Knott test was used to group the averages in different groups, since they were compared all at once.

In the experiments to determine the fluorescence parameters of chlorophyll a, the sample unit was a section of a maize leaf and a bean leaf. The treatments were IAA, IAA + AVG + Co^2, 2,4-D, 2,4-D + AVG + Co^{2+}, 2,4,5-T, 2,4,5-T + AVG + Co^{2+} and the control sample plants were treated with water and water + AVG + Co^{2+}. Plants were evaluated for 3 days in a row after the respective treatments. The Tukey's test was used separately for each auxin. The effect of the auxin treatment on plants, of auxin plus AVG + Co^{2+}, control sample (water) and control sample plus AVG + Co^{2+} were compared. The evaluation was made 3 times, once a day for 3 days.

Differences between the averages were detected primarily performing the ANOVA, followed by the t, Tukey's and Scott-Knott tests, depending on the evaluation type, at 5% level of probability. The normality of values was analyzed using the Lilliefors test. All tests were performed by the SAEG program.

Results and Discussion

Ethylene emanation in treated plants

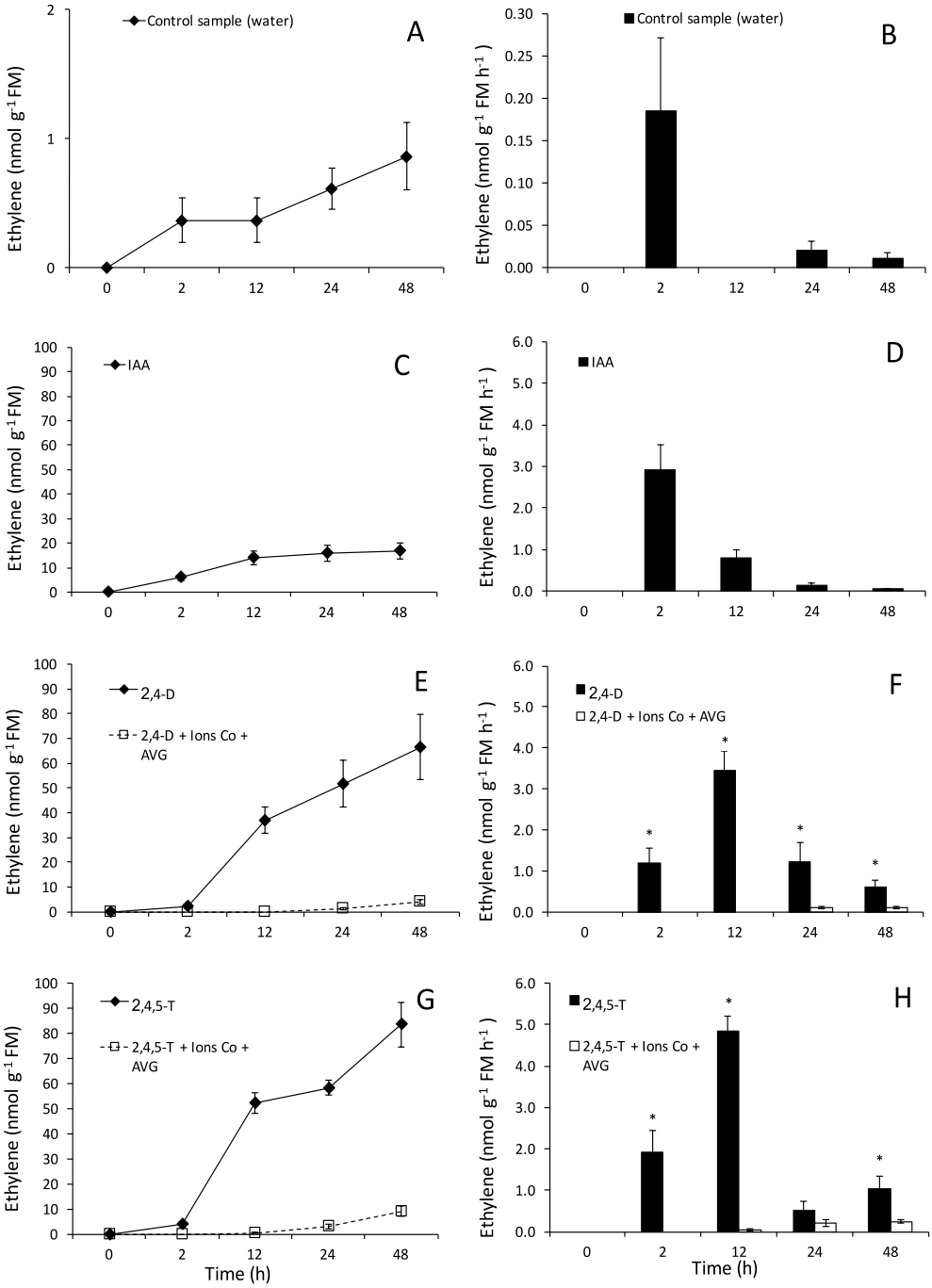
Bean plants, when treated with auxins, produced great quantities of ethylene, as opposed to maize plants, which did not produce the ethylene gas at minimum levels so that they could be detected by the methodology used in this study (Figure 1), according to what was shown by Kelley and Riechers (2007).

The total ethylene emanated and accumulated by bean plants treated with the synthetic auxins was about seven to nine times greater than what was emanated by plants treated with the natural auxin. A possible explanation would be that IAA is more quickly metabolized by plants than synthetic auxins (2,4-D and 2,4,5-T), mainly because of the action of cytochrome P450, which promotes hydroxylation of the auxin aromatic ring and by the action of the protein GH3, which conjugates IAA and amino acids (Grossmann 2010).

Apparently, the cytochrome P450 and the protein GH3 do not act degrading the synthetic auxins (Kelley and Riechers, 2007). Practically, in the control sample bean plants it was not possible to detect the production of ethylene. With the natural auxin, the peak of the production rate of ethylene occurred around 2 h after the treatment of bean plants; with the synthetic ones, this higher production rate of ethylene occurred around 12 h after the treatment. The explanation is due to the fact that the responses triggered by IAA occurred more quickly than the ones triggered by the synthetic auxins, since the IAA is, possibly, transported more quickly to the interior cell (Kerr et al. 2007). The production of ethylene by bean plants treated with auxins was inhibited by Co^{2+} and aminoethoxyvinylglycine (AVG).

ACC production

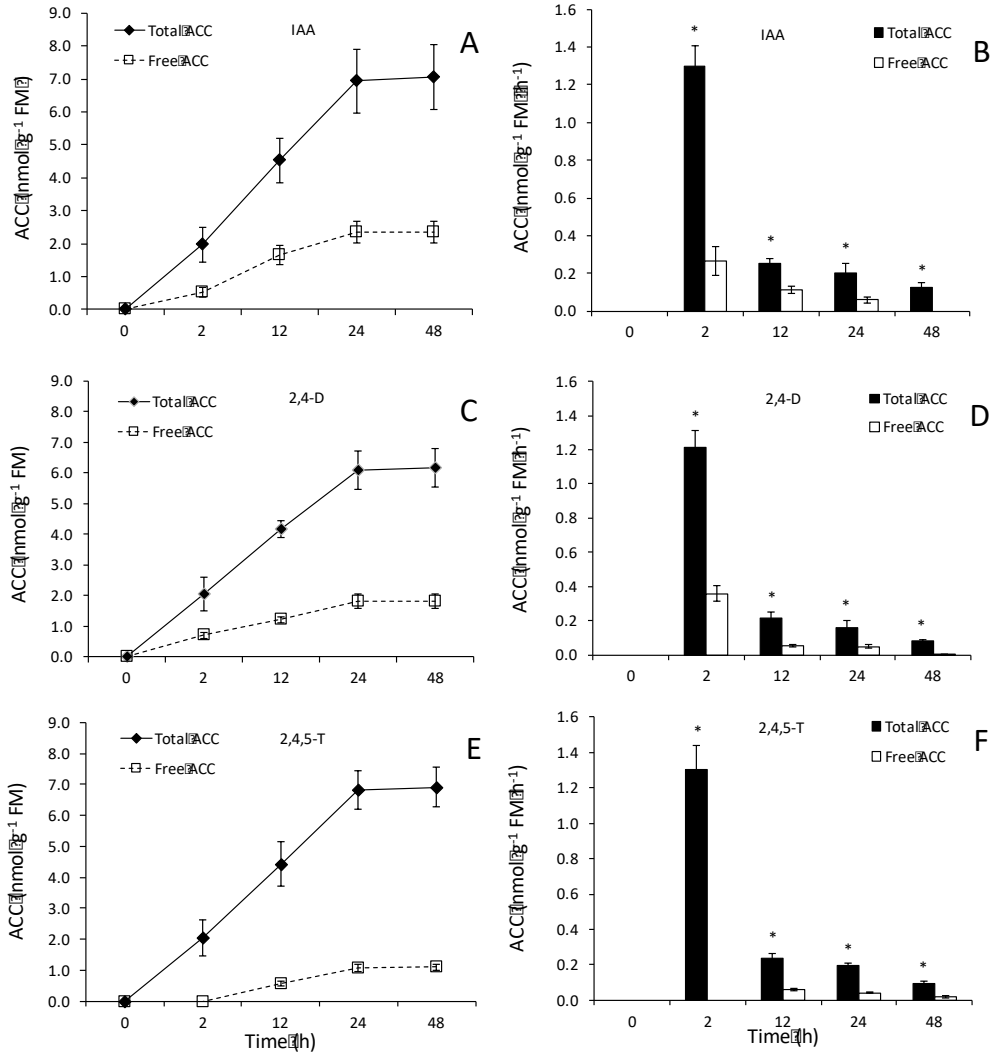
In maize plants, it was not possible to detect the production of ACC in response to auxins. In bean plants treated with auxins, the total ACC was similar in response to the three auxins (Figure 2). Bean plants treated with water (control sample) produced about 76% less total ACC than the ones treated with auxins. On the other hand, the free ACC was found in higher quantities in plants treated with IAA, compared to plants treated with 2,4-D and 2,4,5-T.



Bars with the averages and the standard error followed by an asterisk show differences by t de Student test at 5% probability.

Figure 1 Accumulation (left) and production rate (right) of ethylene in bean plants, two weeks after emergence, treated with: water (Control sample, A and B); IAA; IAA + AVG + Co^2 (C and D); 2,4-D; 2,4-D + AVG + Co^{2+} (E and F); 2,4,5-T; 2,4,5-T + AVG + Co^{2+} (G and H). Control sample bean plants plus AVG and Co^{2+} and the ones treated with IAA plus AVG and Co^{2+} did not produce detectable ethylene.

ACO activity was stimulated during a longer period of time in bean plants treated with 2,4-D and 2,4,5-T that the ones treated with IAA, due to the greater persistence of these auxins in the active form in bean plants (Kelley and Riechers, 2007). This is perhaps why a lower quantity of free ACC has been found in plants treated with the synthetic auxins than in the ones treated with IAA. In bean plants that were not treated with auxins, it was possible to observe a low ACC production (control sample plants produced on an average, during all evaluated periods, 0.71 nmol of ACC g^{-1} FM (free ACC) and 1.793 nmol of ACC g^{-1} FM (total ACC)). These data show that ACS seems to have been induced or stimulated both by the natural and the synthetic auxins.



Bars with the averages and the standard error followed by an asterisk show differences by t de Student test at 5% probability.

Figure 2 Accumulation (left) and production rate (right) of free and total ACC in bean plants, two weeks after emergence, treated with IAA (A and B); 2,4-D (C and D); 2,4,5-T (E and F). Control sample bean plants produced on an average, during all evaluated periods, 0.71 nmol of ACC g^{-1} FM (free ACC) and 1.793 nmol of ACC g^{-1} FM (total ACC). Maize plants did not produce ACC in response to auxins.

Photosynthetic pigments

Bean plants showed total chlorophyll and carotenoid loss, when treated with the synthetic auxins. However, the fall in the total chlorophyll levels was higher than the one in carotenoid levels, which leads to the yellowing of leaves; this is due to a higher structural stability of carotenoids (Table 1) (Gross 2012). The fall in the carotene concentrations (α and β carotene) was lower than the fall in xanthophylls (neoxanthin, violaxanthin, antheraxanthin and lutein) in bean plants (Table 1).

In bean plants, the absolute levels of all pigments, except for α-carotene, were lower in plants treated with the synthetic auxins (Grossmann et al. 2001); these falls were not observed when auxins were simultaneously provided with the inhibitors of ethylene production (Table 1).

Even sample control bean plants showed lower values of lutein, chlorophyll b and chlorophyll a than bean plants treated with AVG and Co^{2+} and with IAA together with AVG and Co^{2+}, emphasizing that bean plants from both treatments did not produce ethylene, whereas control sample plants produced it (Figure 1). Levels of the other pigments in beans presented statistically equal values, when compared to control sample plants with the plants treated with AVG and Co^{2+} and IAA plus AVG and Co^{2+}. This explains the role played by the ethylene produced in response to auxins in the senescence of bean plants.

On the other hand, in maize plants it was not possible to observe any statistical difference in the effects of the concentrations of photosynthetic pigments, between plants treated with auxins and control sample plants, except for antheraxanthin, which had the highest levels in maize plants treated with IAA (Table 2). Both in maize and bean plants, zeaxanthin was not detected, maybe due to the low luminosity of the place where plants were kept (Taiz and Zeiger, 2010), after the treatment with auxins.

Table 1 Concentrations of the photosynthetic pigments (μg g^{-1} MF), in bean plants, three days after being treated with water (control sample); IAA; 2,4-D; 2,4,5-T; AVG + Co^{2+}; IAA + AVG + Co^{2+}; 2,4-D + AVG + Co^{2+}; 2,4,5-T + AVG + Co^{2+}.

Treatment	Neoxanthin	Violaxanthin	Antheraxanthin	Lutein	Chlorophyll *b*	Chlorophyll *a*	α Carotene	β Carotene
Control sample	51.5 ± 6.3 a	58.6 ± 4.8 a	4.6 ±0.5 a	114.2 ± 11.6 c	255.2 ± 24.3 b	815.1 ± 74.6 b	3.5 ± 1.0 d	155.5 ± 19.3 a
IAA	34.1 ± 2.9 b	29.6 ± 1.6 b	6.2 ± 0.5 a	101.0 ± 7.4 c	192.7 ± 14.4 b	594.0 ± 40.0 b	3.4 ± 0.5 d	130.3 ± 12.8 b
2,4-D	13.9 ± 0.6 c	16.3 ± 1.5 b	2.4 ± 0.8 b	65.6 ± 4.0 d	31.1 ± 2.9 d	142.0 ± 12.1 d	11.0 ± 1.0 b	78.6 ± 5.8 c
2,4,5-T	23.7 ± 2.6 c	32.4 ± 3.1 b	2.0 ± 0.3 b	104.6 ± 8.4 c	53.8 ± 8.3 d	219.9 ± 29.9 d	16.5 ± 1.5 a	111.6 ± 8.2 b
AVG + Co^{2+}	52.9 ± 6.2 a	55.5 ± 7.5 a	6.8 ± 1.7 a	160.0 ± 11.9 a	290.4 ± 23.9 a	896.3 ± 72.8 a	5.9 ± 1.4 d	201.3 ± 15.0 a
IAA + AVG + Co^{2+}	54.5 ± 6.6 a	52.0 ± 7.0 a	8.1 ± 2.3 a	160.6 ± 18.2 a	299.7 ± 36.1 a	914.0 ± 111.5 a	4.3 ± 0.8 d	193.1 ± 22.6 a
2,4-D + AVG + Co^{2+}	30.2 ± 1.5 b	40.2 ± 1.9 a	2.4 ± 0.1 b	125.3 ± 4.5 b	107.4 ± 13.1 c	420.8 ± 42.7 c	8.4 ± 1.1 c	138.4 ± 12.1 b
2,4,5-T + AVG + Co^{2+}	19.8 ± 1.6 c	24.3 ± 1.6 b	1.6 ± 0.2 b	79.7 ± 4.5 d	95.7 ± 12.5 c	341.0 ± 36.7 c	4.2 ± 0.7 d	92.8 ± 5.8 c

Values of the averages plus or minus the standard error followed by the same letter do not differ among themselves, in the same column, at 5% level, by Scott-Knott test.

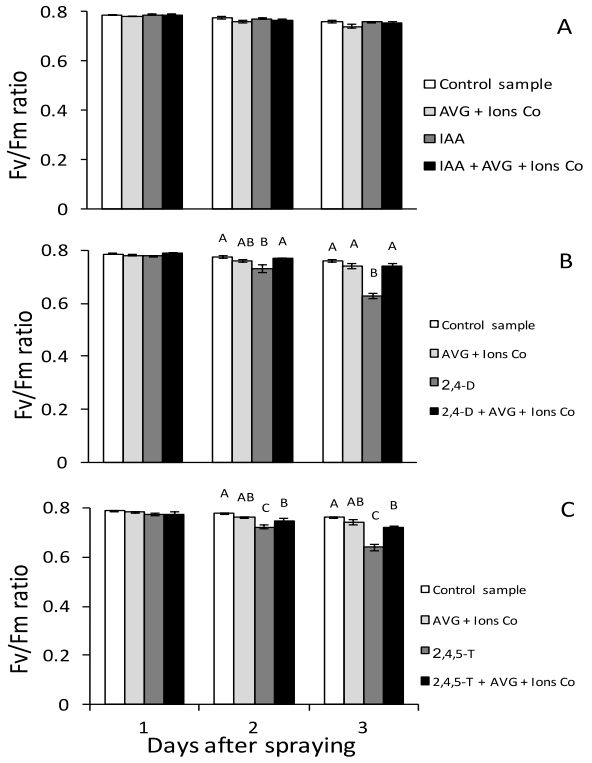
Table 2 Concentrations of the photosynthetic pigments (μg g^{-1} MF), in maize plants, three days after being treated with water (control sample); IAA; 2,4-D; 2,4,5-T; AVG + Co^{2+}; IAA + AVG + Co^{2+}; 2,4-D + AVG + Co^{2+}; 2,4,5-T + AVG + Co^{2+}.

Treatment	Neoxanthin	Violaxanthin	Antheraxanthin	Lutein	Chlorophyll *b*	Chlorophyll *a*	α Carotene	β Carotene
Control sample	29.6 ± 3.1	64.0 ± 4.0	5.6 ± 0.8 b	102.5 ± 10.4	185.1 ± 17.2	681.0 ± 55.3	0.5 ± 0.16	193.9 ± 11.6
IAA	48.3 ± 15.3	79.5 ± 21.9	9.5 ± 3.7 a	230.8 ± 63.4	292.3 ± 57.5	1150.2 ± 249.0	0.4 ± 0.24	274.4 ± 60.0
2,4-D	40.0 ± 9.0	66.2 ± 9.6	2.6 ± 0.5 b	127.6 ± 18.7	235.8 ± 25.0	930.4 ± 107.2	0.5 ± 0.14	196.1 ± 27.3
2,4,5-T	35.3 ± 7.4	62.9 ± 18.3	2.3 ± 0.4 b	130.2 ± 25.8	226.4 ± 36.4	857.4 ± 131.8	0.8 ± 0.38	197.3 ± 33.8
AVG + Co^{2+}	49.8 ± 1.8	73.0 ± 7.8	2.2 ± 0.7 b	144.3 ± 17.2	230.6 ± 23.7	902.7 ± 96.3	0.7 ± 0.29	235.1 ± 30.3
IAA + AVG + Co^{2+}	27.1 ± 1.2	52.1 ± 3.1	2.8 ± 1.1 b	95.5 ± 3.6	182.4 ± 13.3	678.9 ± 68.0	0.2 ± 0.07	171.8 ± 7.1
2,4-D + AVG + Co^{2+}	38.3 ± 6.8	64.3 ± 5.0	2.6 ± 0.7 b	123.3 ± 12.8	195.1 ± 29.8	780.5 ± 115.4	1.1 ± 0.63	197.9 ± 14.1
2,4,5-T + AVG + Co^{2+}	41.5 ± 11.5	70.4 ± 8.6	2.7 ± 0.6 b	119.6 ± 14.8	226.0 ± 45.7	821.3 ± 146.5	1.0 ± 0.38	194.7 ± 21.6

Values of the averages plus or minus the standard error followed by the same letter do not differ among themselves, in the same column, at 5% level, by Scott-Knott test.

Maximum quantum yield of the Photosystem II

Plants treated with IAA did not have reductions in the F_v/F_m ratio, possibly due to the fact that IAA was quickly metabolized by bean plants (Kelley and Riechers 2007). Bean plants had significant falls in the F_v/F_m ratio after the treatment with 2,4-D and 2,4,5-T (Figure 3). In bean plants treated with synthetic auxins together with AVG and Co^{2+}, the F_v/F_m fall was not observed, thus showing that the produced ethylene led to a fall in F_v/F_m.

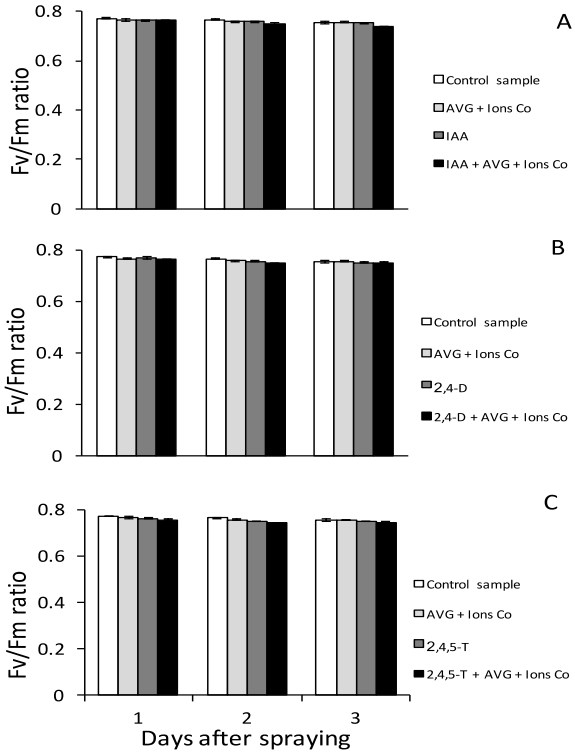


Bars with the averages and the standard error followed by the same letter, in each one of the days, do not differ among themselves, at 5% level, by Tukey's test.

Figure 3 F_v/F_m ratio followed for three days in bean plants treated with: water (Control sample); AVG + Co^{2+}; IAA; IAA + AVG + Co^{2+} (A); water; AVG + Co^{2+}; 2,4-D; 2,4-D + AVG + Co^{2+} (B); water; AVG + Co^{2+}; 2,4,5-T; 2,4,5-T + AVG + Co^{2+} (C).

The F_v/F_m ratio did not change significantly in maize plants treated with auxins, in relation to the control sample plants (Figure 4). These results show how the senescence caused by auxins is stimulated, mainly, by the formation of ethylene (Song 2014). Auxins, per se, would not be causing directly the senescence in dicots.

The levels of photosynthetic pigments (carotenoids and chlorophylls) reduced as the values of the F_v/F_m ratio were lower and with the increase in the ethylene emanated in response to auxins, mainly the synthetic auxins, which were the ones that most induced the production of ethylene in bean plants. These results showed the importance of these pigments in the effectiveness of non-photochemical quenching of light and the physiologic role of ethylene in response to auxins Grossmann et al. (2001).



Bars with the averages and the standard error, in each one of the days, do not differ among themselves, at 5% level, by Tukey's test.

Figure 4 F_v/F_m ratio followed for three days in maize plants treated with: water (Control sample); AVG + Co^{2+}; IAA; IAA + AVG + Co^{2+} (A); water; AVG + Co^{2+}; 2,4-D; 2,4-D + AVG + Co^{2+} (B); water; AVG + Co^{2+}; 2,4,5-T; 2,4,5-T + AVG + Co^{2+} (C).

Conclusions

Synthetic auxins appeared extremely toxic when provided at high concentrations, mainly to dicots; the biosynthesized ethylene in response to auxins is one of the main contributors to toxicity. Ethylene caused photosynthetic damages that could not be explained only by the effect of auxins. On the other hand, maize appeared practically insensitive to auxins, both the natural and the synthetic ones, proving to be unable to produce ethylene (detectable by the used technique) in response to the application of auxins.

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