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Agronomic and metabolomic response of melon (*Cucumis melo* L.) seedlings under the application of high indole-3-acetic acid concentrations

Respuesta agronómica y metabolómica de plántulas de melón (*Cucumis melo* L.) bajo la aplicación de altas concentraciones de ácido indol-3-acético

Oscar Sariñana-Aldaco[1]*, Mariana Isabel Garcia-Terrazas[1], Pablo Preciado-Rangel[2], Óscar Silva-Marrufo[3] and Eduardo Alberto Lara-Reimers[4]*

[1] Departamento de Horticultura, Universidad Autónoma Agraria Antonio Narro, Saltillo 25315, Coahuila, México; terrazas.mariana92@gmail.com
[2] Tecnológico Nacional de México, Instituto Tecnólogico de Torreón, Torreón 27170, Coahuila, México; pablo.pr@torreon.tecnm.mx
[3] Tecnológico Nacional de México, Instituto Tecnólogico del Valle del Guadiana, Durango 34371, Durango, México; oscar.sm@vguadiana.tecnm.mx
[4] Departamento Forestal, Universidad Autónoma Agraria Antonio Narro, Saltillo 25315, Coahuila, México.

ABSTRACT

The objective of the study was to evaluate the foliar application of high concentrations of indole-3-acetic acid (IAA) (0.0, 0.5, 1, 2 and 3 mM) on agronomic and metabolomic variables in melon seedlings, using a completely randomized design. The results indicate that the IAA at 0.5 mM improved the height by 20.98 %, and from 1 mM it decreased as the concentration increased. For total fresh and dry biomass, the control and the 0.5 mM concentration were the same, however, from 1 mM it decreased as the IAA concentration increased. For biomolecules, chlorophylls (*a* 25.60 %, *b* 51.31 % and total 32.50 %), flavonoids (13.13 %), antioxidant capacity (54.37 %) and proteins (26.38 %) increased as the IAA concentration increased. Ascorbic acid decreased with the application of IAA, and with 0.5, 1 and 2 mM the concentration of carotenoids increased by 11.76, 11.76 and 8.82 %, respectively, however, with 3 mM they began to decrease, but even so, they exceeded the control. From 1 mM of IAA, the agronomic characteristics of the seedlings began to decrease, therefore, it is advisable to apply concentrations lower than 0.5 mM to elucidate how they work.

Keywords: growth regulator; elicitor; signaling molecule; toxicity; metabolites.

RESUMEN

El objetivo fue evaluar la aplicación foliar de altas concentraciones de AIA (0.0, 0.5, 1, 2 y 3 mM) sobre variables agronómicas y metabolómicas en plántulas de melón, usando un diseño completamente al azar. Los resultados indican que el AIA a 0.5 mM mejoró la altura 20.98 %, y a partir de 1 mM diminuyó a medida que aumentaba la concentración. Para biomasa fresca y seca total, el control y la concentración de 0.5 mM fueron iguales, sin embargo, a partir de 1 mM la disminuyó a medida que aumentaba la concentración de AIA. Para biomoléculas, aumentaron clorofilas (*a* 25.60 %, *b* 51.31 % y total 32.50 %), flavonoides (13.13 %), capacidad antioxidante (54.37 %) y proteínas (26.38 %) a medida que aumenta la concentración de AIA. El ácido ascórbico disminuyó con la aplicación del AIA, y con 0.5, 1 y 2 mM incrementó la concentración de carotenoides en 11.76, 11.76 y 8.82 %, respectivamente, sin embargo, con 3 mM comenzaron a disminuir, pero, aun así, superaron al control. A partir de 1 mM de AIA comenzaron a disminuir las características agronómicas de las plántulas, por lo tanto, es recomendable aplicar concentraciones inferiores a 0.5 mM para dilucidar cómo funcionan.

Palabras clave: regulador de crecimiento; elicitor; molécula de señalización; toxicidad; metabolitos.

INTRODUCTION

Phytohormones (auxins, cytokinins, gibberellins, ethylene, abscisic acid, salicylic acid, benzoic acid and jasmonic acid) are natural compounds produced by plants that define a large part of development (Nehela *et al*., 2021; Zhao *et al*., 2021). They are synthesized at very low concentrations under normal conditions and can act at the synthesis site or be translocated to other organs to regulate different physiological and biochemical elicitation or inhibition processes (Fahad *et al*., 2015). In addition to this, they also function as signaling molecules, allowing plants to function during exposure to different types of stress (Rhaman *et al*., 2020). Under stress, whether biotic (insects, fungi, bacteria or viruses) or abiotic (salinity, drought, extreme temperatures and heavy metals), plants increase the synthesis of phytohormones, in order to carry out the signaling process and defense response occurs (Fahad *et al*., 2015; Ku *et al*., 2018).

Each phytohormone synthesized by the plant fulfills different functions and can act alone or together regulating different processes (Aerts *et al*., 2021). Auxins are the phytohormones of primary growth, be it root, stems, leaves, flowers and fruits (Chandra *et al*., 2018). Indole-3-acetic acid

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*Authors for correspondence: Oscar Sariñana Aldaco and Eduardo Alberto Lara Reimers
e-mail: oscarsarinana390@gmail.com; edybertolare@hotmail.com
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(IAA) is the most relevant auxin in terms of quantity and activity, which is synthesized from the aromatic amino acid tryptophan (Matilla, 2020). The synthesis is carried out in the apical meristems (apical buds), responsible for primary growth. For this reason, IAA controls processes such as cell division and elongation, participates in plant tropisms, acts in tissue differentiation, inhibits senescence, inhibits the sprouting of axillary buds (lateral meristems) and inhibits organ abscission (Chandra *et al*., 2018). Zinc (Zn) and boron (B) are related to IAA since its deficiency causes a lower synthesis of the phytohormone, which affects the processes in which it intervenes (Khan *et al*., 2022).

The IAA, when derived from a primary metabolite, turns into a secondary metabolite with important functions at the metabolomic level (Matilla, 2020). In this sense, metabolomics is one of the omic sciences that studies substances called metabolites, present in an organism, tissue or organ at a given stage of development and under particular environmental conditions (McLaughlin *et al*., 2023).

The melon (*Cucumis melo* L.) is a crop of economic importance that is produced worldwide, this being a source of carotenoids, vitamins, phenolic compounds, sugars, and minerals (Manchali *et al*., 2021). The main producing countries are China, Morocco and Spain (Mahmoodi *et al*., 2020). Due to the importance that melon represents worldwide, it is important to produce quality seedlings. Obtaining quality seedlings depends on their genetics, physiology and the environment in which they develop (Sariñana-Aldaco *et al*., 2021). Today their production is negatively affected by different environmental factors, which makes them prone to different infections by pathogens (Ruan *et al*., 2009). Given this, there are promising alternatives such as the exogenous application of elicitors such as phytohormones that allow improving the growth, yield and quality of vegetables. Elicitors are substances that, when applied to plants in a preventive manner, help to avoid or reduce damage from biotic or abiotic stressors, which is reflected in improvements in agronomic characteristics and greater synthesis of antioxidants (Caicedo-López *et al*., 2021).

The application of phytohormones in horticultural plants is a common practice, however, regarding the use of IAA, there are no specific data on the concentrations that can improve or harm their growth and development. In any case, for the application of these products, the concentration to be used, the environmental conditions and the biological model of study must be taken into account, since they are factors that influence the results (Montaño-Mata and Méndez-Natera, 2009; Caicedo-López *et al*., 2021). In addition to this, there are precedents that indicate that melon is a vegetable susceptible to high concentrations of IAA (Montaño-Mata and Méndez-Natera, 2009).

As already mentioned, there is no specific data on the use of IAA in vegetables. For this reason, to obtain approximate data on the concentrations that cause toxicity, an experiment was established in which high concentrations were tested in melon seedlings to evaluate the impact at the agronomic and metabolomic level. With this, the aim is to find the concentration with which the melon seedlings begin to become intoxicated, and from there, in subsequent experiments, try lower concentrations that allow us to improve the productivity.

MATERIAL AND METHODS

Plant material and experimental conditions

Expedition hybrid melon seeds (Harris Moran®, USA) were sown in 0.5 L styrofoam containers with a mixture of peat moss and perlite substrate (1:1 *v/v*). The experiment was established in a greenhouse covered with polyethylene in the Horticulture Departamente of the Antonio Narro Autonomous Agrarian University (Saltillo, Mexico). The average temperature was 29 °C and 50 to 60 % relative humidity.

The treatments applied were the following: IAA at four concentrations (0.5, 1, 2 and 3 mM) and an absolute control with deionized water, which were established based on the study by Vidyullatha and Topno (2022). The treatments were applied via the foliar route, a manual sprinkler was used, and the plants were sprayed to the point of dripping. The applications were every 10 days after the emergency, accumulating three applications during the experiment, which lasted 31 days. The applications were made between 6:00 p.m. and 7:00 p.m. Each IAA concentration was diluted in 10 mL of pure ethanol and subsequently made up to 500 mL with deionized water.

The irrigation system was manual, giving one irrigation per day at field capacity. The nutrition of the plants was carried out with the nutrient solution Steiner (1961) at 25 %.

Sampling and evaluations

Sampling and evaluation were carried out 24 h after the third application of the treatments (31 days after emergence). The sampling consisted of four seedlings per treatment to determine the concentration of different biomolecules. The sampling was carried out by removing all the leaves of the seedlings and freezing them with liquid nitrogen and then storing them in a deep freezer at –80 °C. The evaluations consisted of four seedlings per treatment that were chosen to measure height, leaf area, stem diameter and fresh and dry biomass.

Height and stem diameter were measured with a flexometer, and their results were expressed in cm and mm, respectively. Leaf area was measured using the AccuPAR model Lp-80 PAR/LAI ceptometer (Decagon Devices, Inc., Pullman, WA, USA), and its results were expressed in cm^2. The fresh and dry biomass was quantified with the help of a digital scale, and its results were reported in g and mg, respectively.

Biochemical analysis

For the determinations of the biomolecules in the leaves of the melon seedlings, the frozen tissue was lyophilized (Labconco Freezone 2.5 Plus Liter Freeze Dry System Model 7670021 Date 5-17-12new) and macerated to later carry out the extractions and quantifications according to the methodologies used.

The concentrations of foliar pigments were determined according to what was described by Wellburn (1994). For this, 15 mg of lyophilized tissue were mixed with 1.250 mL of 100 % methanol. The mixture was incubated for 24 h in the dark and the absorbance of the supernatant was evaluated in na ultraviolet-visible (Uv-Vis) spectrophotometer (Genesis 10s Uv-Vis, Thermo Scientific, Waltham, MA, USA) at 666, 653 and 470 nm. The pigments concentration was expressed in milligrams per gram of dry weight (mg g^{-1} DW), using the following formulas:

$$\text{Chlorophyll } a = [15.65(\text{abs}666) - 7.34(\text{abs}653)] \quad (1)$$

$$\text{Chlorophyll } b = [27.05(\text{abs}653) - 11.21(\text{abs}666)] \quad (2)$$

$$\text{Total chlorophyll} = \text{chlorophyll } a + \text{chlorophyll } b \quad (3)$$

$$\text{Carotenoids} = \frac{[(1000\text{abs}470) - 2.86(\text{chlorophyll } a) - 129.2(\text{chlorophyll } b)]}{221} \quad (4)$$

The extraction of soluble proteins and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) antioxidant capacity, 100 mg of lyophilized tissue were placed in a 2 mL tube and 2 mL of 0.1 M phosphate buffer (pH 7-7.2) were added. Then the mixture was homogenized with a vortexed for 20 s, sonicated for 5 min, and centrifuged at 12,500 rpm at 4 °C for 10 min. Finally, the supernatant was collected for analysis.

The soluble protein content was determined by the Bradford method (1976). In 2 mL tubes, 100 µL of the supernatant and 1 mL of Bradford reagent were added. Subsequently, it was left to rest for 5 min and the reading was taken in a Uv-Vis spectrophotometer at 595 nm. The results were reported in milligrams per gram of dry weight (mg g^{-1} DW).

The antioxidant capacity was determined by the method described by Re *et al*. (1999), which is based on the bleaching of the ABTS radical. The radical was obtained from the ratio of 7 mM ABTS with 2.45 mM potassium persulfate ($K_2S_2O_8$) (1:1 *v/v)* in the dark for 16 h, then diluted with 20 % ethanol until obtaining an absorbance of 0.7 at 754 nm. For quantification, 20 µL of the extract and 980 µL of the radical dilution were added to a 2 mL tube. Subsequently, the mixtures were shaken for 5 s and left to rest for 7 min in the dark. The reading was immediately carried out in a Uv-Vis spectrophotometer at 754 nm. The results were expressed in equivalents milligrams of ascorbic acid per gram of dry weight (mg AAE g^{-1} DW).

Total phenols were quantified by the method described by Singleton *et al*. (1999). The sample was extracted by placing 100 mg of lyophilized tissue in a 2 mL tube and adding 2 mL of extraction solution (water: acetone 1:1 *v/v*). The mixture was vortexed for 20 s, then sonicated for 5 min. Finally, the samples were centrifuged at 12,000 rpm for 10 min at 4 °C to obtain the supernatant and proceed to quantification. Quantification was performed in test tubes by adding 50 µL of the supernatant, 0.2 mL of 100 % Folin Ciocalteu reagent, 0.5 mL of 20 % Na_2CO_3, and 5 mL of distilled water. The mixture was allowed to settle for 30 min at 45 °C. Subsequently, the mixture was read at a wavelength of 750 nm in a Uv-Vis spectrophotometer. The results of phenols were expressed in equivalents milligrams of gallic acid per gram of dry weight (mg GAE g^{-1} DW).

The flavonoids were quantified following the methodology of Zhishen *et al*. (1999). Compounds were extracted by placing 100 mg of lyophilized tissue in 2 mL tubes and adding 2 mL of 100 % methanol. The mixture was homogenized by vortexing for 20 s, then sonicated for 5 min, and centrifuged at 4,000 rpm for 10 min at 4 °C to obtain the supernatant. For quantification, 250 µL of the supernatant were added to a test tube, followed by the addition of 75 µL of 5 % $NaNO_2$ and vortexed. After 5 min, 150 µL of 10 % $AlCl_3$ were added; Immediately, 0.5 mL of 1 M NaOH were added, plus 2.025 mL of distilled water. Finally, the absorbance at 510 nm was measured in a Uv-Vis spectrophotometer. The results were reported in equivalents milligrams of catechin per gram of dry weight (mg CE g^{-1} DW).

Ascorbic acid was quantified by high performance liquid chromatography (HPLC) with Uv detector (HPLC VARIAN 920LC), using the method of Nour *et al*. (2010). The extraction was similar to that of flavonoids. The obtained supernatant was filtered through 0.45 µm pore diameter membranes and transferred to vials for injection into the chromatograph. The mobile phases were 50 mM NaH_2PO_4 with a pH of 2.8 (Phase A) and 100 % acetonitrile (Phase B), with a ratio of 80:20, flow rate of 1.0 mL min^{-1}, and the column used was aquasil C-18 at 60 °C. The injection volume was 50 µL. Detection of the molecule was performed at 230 nm. Results were reported in milligrams per hundred grams of dry weight (mg 100 g^{-1} DW).

Experimental design and data analysis

A completely randomized design was used, considering eight replicates per treatment (four for biomolecule analysis and four for agronomic variables). An analysis of variance and Fisher's LSD test of means ($p \leq 0.05$) were performed. A Pearson correlation analysis was also performed between the agronomic variables and the biomolecules evaluated. Statistical procedures were performed using Infostat software (v2020) and GraphPad Prism 8 statistical software.

RESULTS

Seedling growth and biomass

The results show that the foliar application of IAA significantly affected the growth (Table 1) and biomass (Figure 1) of the melon seedlings. For height, the concentration of 0.5 mM IAA exceeded the control by 20.98 %, and the rest of the treatments were the same as the control. There were no statistically significant differences in stem diameter and leaf area.

Regarding fresh aerial biomass, it can be seen that the control and the 0.5 mM IAA concentration are statistically the same, however, the 1 mM concentration began to decrease as the IAA concentration increased, where the 3 mM treatment

Table 1. Effect of IAA on the growth of melon seedlings.
Tabla 1. Efecto de AIA en el crecimiento de plántulas de melón.

IAA (mM)	Height (cm)	Stem diameter (mm)	Leaf area (cm²)
AbC	6.67 ± 0.57 bc	0.48 ± 0.02 ab	16.47 ± 0.23 a
0.5	8.07 ± 0.15 a	0.52 ± 0.02 a	18.46 ± 0.86 a
1	7.23 ± 1.00 ab	0.50 ± 0.005 ab	17.84 ± 1.99 a
2	5.93 ± 0.75 c	0.47 ± 0.02 ab	15.70 ± 2.53 a
3	5.73 ± 0.20 c	0.46 ± 0.03 b	15.68 ± 2.18 a
CV (%)	9.35	5.80	10.62

Different letters within each column indicate significant difference between treatments (LSD, $p \leq 0.05$). $n = 4$; ± standard deviation (SD). AbC: absolute control; CV: coefficient of variation.

Letras diferentes dentro de cada columna indican diferencia significativa entre tratamientos (LSD, $p \leq 0.05$). $n = 4$; ± desviación estándar (SD). AbC: control absoluto; CV: coeficiente de variación.

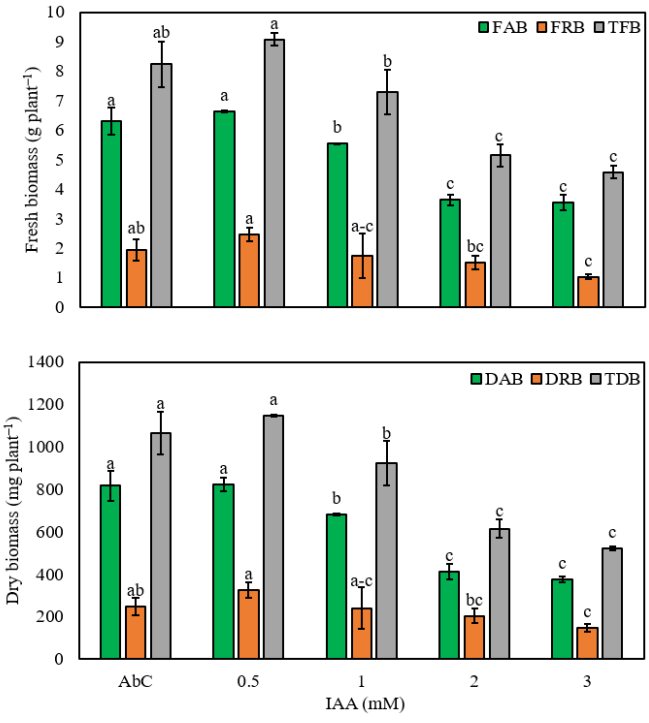

Figure 1. Effect of IAA on biomass accumulation in melon seedlings. Different letters indicate significant differences between treatments (LSD, $p \leq 0.05$). $n = 4$; ± SD. FAB: fresh aerial biomass; FRB: fresh root biomass; TFB: total fresh biomass; DAB: dry aerial biomass; DRB: dry root biomass; TDB: total dry biomass; AbC: absolute control.
Figura 1. Efecto de AIA en la acumulacion de biomasa en plántulas de melón. Letras diferentes indican diferencias significativas entre tratamientos (LSD, $p \leq 0.05$) $n = 4$; ± SD. FAB: biomasa aérea fresca; FRB: biomasa de raíz fresca; TFB: biomasa fresca total; DAB: biomasa aérea seca; DRB: biomasa de raíz seca; TDB: biomasa seca total; AbC: control absoluto.

was outperformed by the control by 77.46 %. Regarding the fresh root biomass, the treatments of 0.5, 1 and 2 mM of IAA were equal to the control, and the 3 mM showed an inferiority of 93.20 %, compared to the control. Regarding the total fresh biomass, the 0.5 and 1 mM IAA treatments were equal to the control, and the 2 and 3 mM were 60.31 and 79.91 % lower, respectively.

In aerial dry biomass, the control and the 0.5 mM IAA treatment were the same, and from the 1 mM it began to decline, with the 3 mM showing the least amount, being surpassed by the control in a 117.38 %. Root dry biomass was only negatively affected by the 3 mM IAA concentration, being 69.34 % minor than the control. The rest of the treatments were statistically equal to the control. In total dry biomass, the control and the 0.5 mM IAA treatment were the same, and decreasing considerably from the 1mM, where the 3 mM concentration of was the most affected as it was surpassed by the control by 103.92 %.

Foliar pigments of melon seedlings

The concentration of chlorophylls and carotenoids was affected by the different treatments applied (Figure 2).

In chlorophyll *a*, all the IAA treatments statistically outperformed the control, being the 3 mM the one that showed the best results, surpassing it by 25.60 %. For chlorophyll *b*, in the same way, all the concentrations with IAA surpassed the control, being the one with 3 mM the best, surpassing it by 51.31 %.

In total chlorophyll, all the concentrations with IAA surpassed the control, where once again the 3 mM was the one that showed the best results, surpassing it by 32.50 %. Regarding carotenoids, it can be seen that the 0.5, 1, and 2 mM IAA treatments were the best, surpassing the control by 11.76, 11.76, and 8.82 %, respectively. The 3 mM IAA concentration was statistically superior to the control, but inferior to the rest of the treatments.

Antioxidants, antioxidant capacity, and proteins of melon seedlings leaves

The results for these biochemical variables were highly varied (Figure 3). It can be seen that the IAA caused a decrease in ascorbic acid in all its concentrations, compared to the control. For total phenols there were no significant differences.

In flavonoids, it is observed that they increased as the IAA concentration increased, with the 3 mM showing the best results, exceeding the control by 13.13 %. Regarding antioxidant capacity, the 2 and 3 mM IAA treatments were the best, surpassing the control by 54.37 and 38.21 %, respectively. In soluble proteins, it can be seen that the 2 and 3 mM IAA concentrations were superior to the control, exceeding it by 24.86 and 26.38 %, respectively.

Correlation analysis

A Pearson correlation analysis was performed for agronomic and biochemical variables (Figure 4). In the agronomic variables it is observed that there is a positive correlation between total fresh biomass, dry aerial biomass and total dry biomass with fresh aerial biomass. There was also a positive correlation between aerial dry biomass and total dry biomass with total fresh biomass. Total dry biomass was positively correlated with aerial dry biomass.

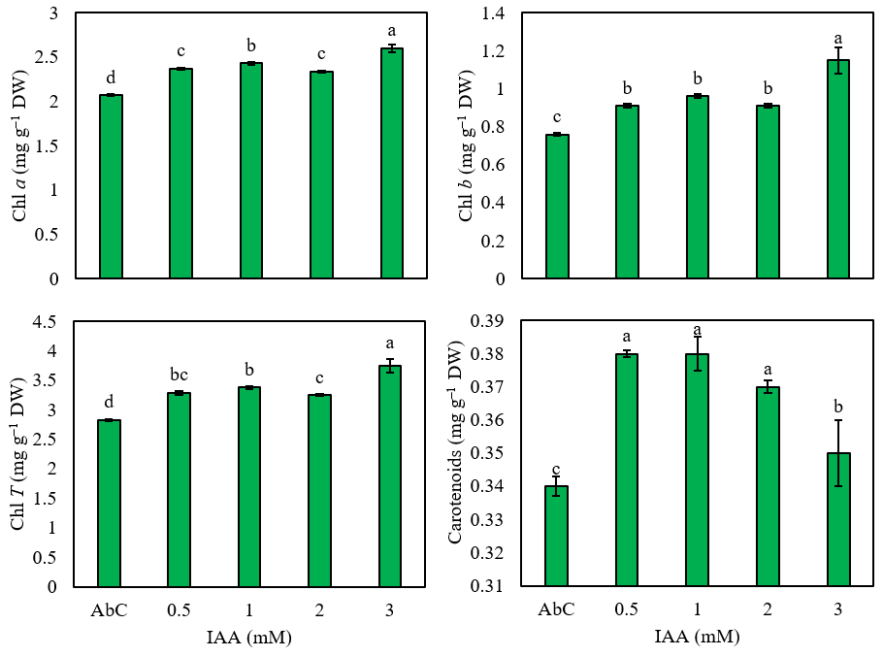

Figure 2. Effect of IAA on the concentration of foliar pigments in melon seedlings. Different letters indicate significant differences between treatments (LSD, $p \leq 0.05$). $n = 4$; ± SD. Chl *a*: chlorophyll *a*; Chl *b*: chlorophyll *b*; Chl *T*: total chlorophyll; DW: dry weight; AbC: absolute control.

Figura 2. Efecto del AIA en la concentración de pigmentos foliares en plantulas de melón. Letras diferentes indican diferencias significativas entre tratamientos (LSD, $p \leq 0.05$). $n = 4$; ± SD. Chl *a*: clorofila *a*; Chl *b*: clorofila *b*; Chl *T*: clorofila total; DW: peso seco; AbC: control absoluto.

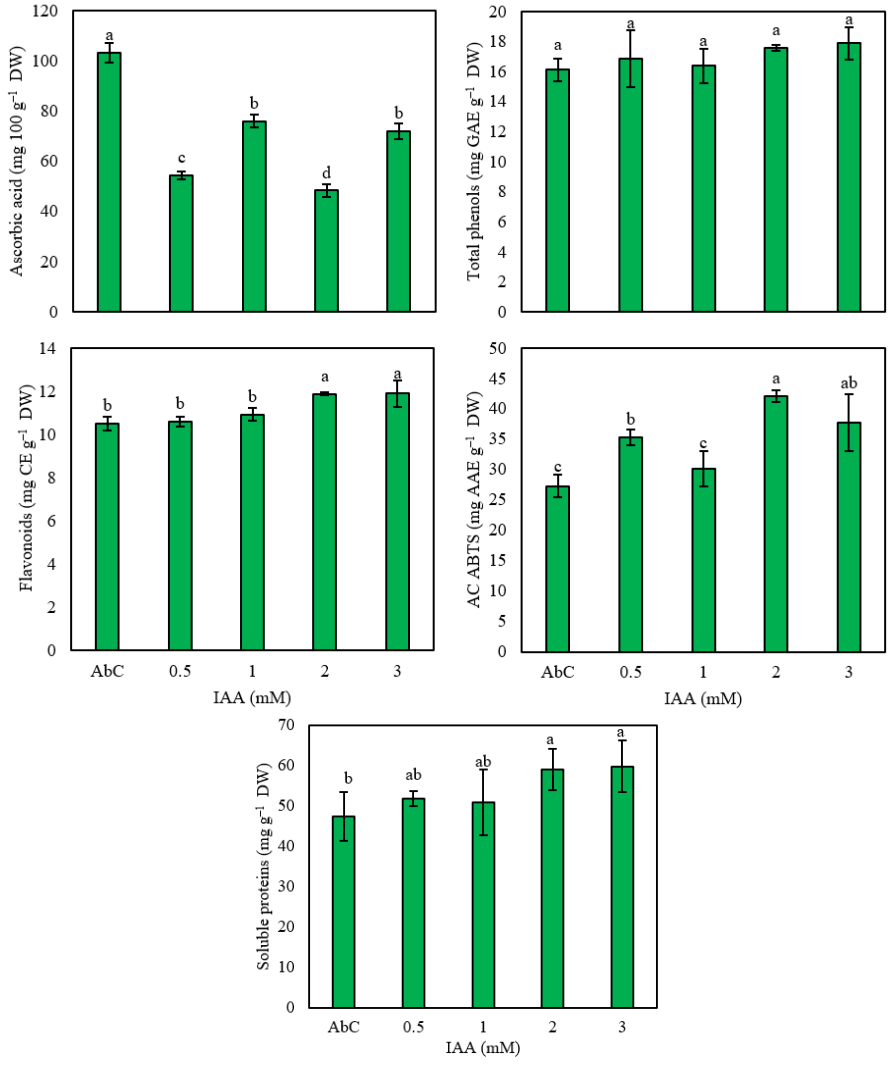

Figure 3. Effect of IAA on the concentration of antioxidants, antioxidant capacity, and proteins in the leaves of melon seedlings. Different letters indicate significant differences between treatments (LSD, $p \leq 0.05$). $n = 4$; ± SD. DW: dry weight; GAE: gallic acid equivalents; CE: catechin equivalents; AAE: ascorbic acid equivalents; AC: antioxidant capacity; AbC: absolute control.

Figura 3. Efecto del AIA en la concentarción de antioxidantes, capacidad antioxidante y proteínas en las hojas de plántulas de melón. Letras diferentes indican diferencias significativas entre tratamientos (LSD, $p \leq 0.05$). $n = 4$; ± SD. DW: peso seco; GAE: equivalentes de ácido gálico; CE: equivalentes de catequina; AAE: equivalentes de ácido ascórbico; AC: capacidad antioxidante; AbC: control absoluto.

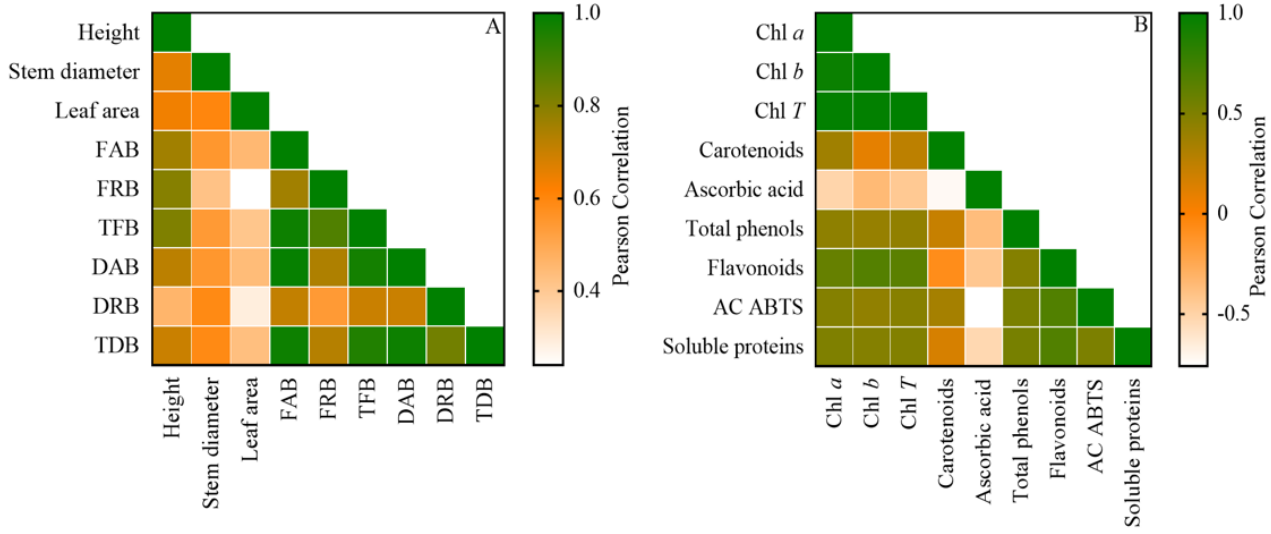

Figure 4. Pearson correlation matrix of agronomic (growth and biomass) (A) and biochemical (B) variables determined in leaves of melon seedlings treated with IAA. FAB: fresh aerial biomass; FRB: fresh root biomass; TFB: total fresh biomass; DAB: dry aerial biomass; DRB: dry root biomass; TDB: total dry biomass; Chl *a*: chlorophyll *a*; Chl *b*: chlorophyll *b*; Chl *T*: total chlorophyll; AC: antioxidant capacity.
Figura 4. Matriz de correlación de Pearson de variables agronómicas (crecimiento y biomasa) (A) y bioquímicas (B) determinadas en hojas de plántulas de melón tratadas con AIA. FAB: biomasa aérea fresca; FRB: biomasa de raíz fresca; TFB: biomasa fresca total; DAB: biomasa aérea seca; DRB: biomasa de ráiz seca; TDB: biomasa seca total; Chl *a*: clorofila *a*; Chl *b*: clorofila *b*; Chl *T*: clorofila total; AC: capacidad antioxidante.

Regarding the biochemical variables, it can be seen that chlorophyll *b* and total were positively correlated with chlorophyll *a*. Total chlorophyll was also positively correlated with chlorophyll *b*. Flavonoids were positively correlated with chlorophyll *a*, *b* and total. Antioxidant capacity and soluble proteins were also positively correlated with flavonoids. Ascorbic acid showed a negative correlation with the rest of the biomolecules.

DISCUSSION

Seedling growth and biomass

The production of quality seedlings depends on different factors (genetics, physiology and conditions for shoot and root development) (Giménez *et al*., 2008). However, in recent years the production of seedlings is affected by biotic and abiotic factors that negatively impact their quality (Ruan *et al*., 2009). These situations have been key in the development of different techniques that allow the best production of seedlings, which allows obtaining more profitable and quality productions. One of the techniques used is the use of elicitors such as phytohormones, which when applied to plants activate their metabolism and make them more resistant to subsequent factors that generate stress (Sariñana-Aldaco *et al*., 2020; Meena *et al*., 2022).

The results obtained for growth variables indicate that only in height there were significant differences with the concentration of 0.5 mM of IAA, which is possible thanks to the fact that IAA is involved in the primary growth of plants and its synthesis occurs mainly in the apical meristems (apical buds) (Salehi *et al*., 2014). From the 1 mM concentration, the height began to decrease, possibly due to the toxicity generated by the high concentrations of IAA applied. It is believed that in the variables of stem diameter and leaf area there were no differences because IAA is involved in primary growth and inhibits the development of lateral meristems that are in charge of secondary growth (Chandra *et al*., 2018).

Regarding the biomass, the concentration of 0.5 mM of IAA and the control were the same, and from the concentration of 1 mM it began to decline. This tells us about a possible intoxication by the IAA. When these types of substances are applied to elicit plants, it is very important to take into account different factors such as the biological study model, environmental conditions, and the concentrations of the elicitor to be used, since these elicitors show favorable results at low concentrations (Montaño-Mata and Mendez-Natera, 2009). Due to this, when these types of substances are used, it is advisable to review the literature on their use, and in the event that there is little existing information, it is recommended to carry out preliminary tests that allow obtaining information on the concentrations that improve the agronomic characteristics of the crops or in the case of this study of the concentrations that cause toxicity.

Similar results are reported by Gil-Rivero *et al*. (2016), who indicate that the lowest concentration of IAA (0.5 mg L^{-1}) was optimal in the in vitro propagation of papaya, and as the concentration rose, toxicity symptoms were observed wich negatively affected the propagation. In the same way, Montaño-Mata and Méndez-Natera (2009), mentioned that the yield of melon fruits was negatively affected as the IAA concentration rose up to 200 mg L^{-1}.

When an appropriate threshold for the organism is exceeded, negative responses are generated called toxicity (when it comes to biochemical compounds) or inhibition (physical factors such as temperature or irradiance) (Juárez-Maldonado *et al*., 2021). In the case of this study, for the height and biomass variables, toxicity was generated (from 1 mM of IAA), because IAA is a biochemical compound.

Foliar pigments of melon seedlings

The chlorophylls are one of the most important pigments that plants have, this because they control photosynthetic activity and are important in the production of photoassimilates (Zepka *et al*., 2019). In addition to this, plants also have accessory pigments such as carotenoids, which have photoprotective properties, and capture light in regions that are not covered by chlorophylls, which improves photosynthetic activity (Collini, 2019).

The results of the present investigation indicate that as the concentration of IAA in the seedlings increased, the concentration of chlorophylls increased. In carotenoids, all the treatments with IAA increased their concentration, compared to the control. However, with the concentration of 3 mM the carotenoids began to decrease. Li *et al*. (2020) mentioned that the application of IAA (30 and 60 mg L^{-1}) in *Cyphomandra betacea* seedlings increased the concentration of chlorophylls and carotenoids. However, by increasing the IAA concentration (90 and 120 mg L^{-1}), the concentration of the pigments began to decrease significantly.

The results obtained are the product of the IAA applications, since it is involved in the synthesis of pigments and in the accumulation of sugars such as glucose and fructose (Huan *et al*., 2021). All this is related to improvements in photosynthetic activity, however, applying high concentrations to plants can cause pigment oxidation as observed in carotenoids, which decreased significantly from 3 mM IAA concentration, compared to with the rest of the IAA treatments. This situation also helped to prevent oxidation of chlorophylls, as carotenoids are pigments involved in the detoxification of reactive oxygen species (ROS), where they tend to oxidize and thus protect chlorophylls to some extent, which is believed to have occurred in this study (Collini, 2019).

Antioxidants, antioxidant capacity, and proteins of melon seedling leaves

Antioxidants are compounds synthesized by plants in their different organs, characterized by delaying or preventing oxidative damage to other molecules, this by reducing ROS produced during stress (Hasanuzzaman *et al*., 2020).

The results of the present investigation show that flavonoids and antioxidant capacity were elevated with the highest IAA concentrations. Flavonoids are secondary metabolites responsible for the coloration of flowers, fruits and leaves.They are involved in pollination by attracting insects and have a photoprotective and antioxidant effect (Ferreyra *et al*., 2021). Gangwar *et al*. (2011) mentioned that the application of IAA at 10 µM in pea seedlings increased the concentration of reduced glutathione; important antioxidant in the defense system of plants. Possibly, this is because IAA at high concentrations can cause toxicity and consequently the production of ROS, which promote the synthesis of different antioxidants (Hasanuzzaman *et al*., 2020). In this way, Wang *et al*. (2022) mentioned that flavonoids can act as auxin transport inhibitors in high concentrations, which indicates that the higher the concentration of IAA in plants, the greater the accumulation of flavonoids.

Enzymes are proteins that are involved in different processes such as the antioxidant system, and in this study, it can be seen that increasing the IAA concentration increased the proteins, which may be related to the greater antioxidant capacity. Gong *et al*. (2019) indicated that in spinach seedlings, the activity of the superoxide dismutase enzyme was improved as the IAA concentration increased. It is very common that when some metabolite is found in excess, plants try to mitigate the stress that can be caused, and they do this by metabolizing it to a certain degree (Godoy *et al*., 2021). This may be a clear example of IAA, where in order to enter it into plant metabolism, specific transporters are required, which are proteins, which in this case is believed to be another explanation for the increase in proteins (Wang *et al*., 2022).

In the study it is also observed that the IAA applications decreased the concentration of ascorbic acid, in comparison to the control. The IAA has the ability to transform peroxidase enzymes in highly specific oxygenases that generate ROS, with oxidizing power, and one of the most important peroxidases in the antioxidant system of plants is ascorbate peroxidase, which is affected by IAA and does not fulfill its antioxidant function, but instead oxidizes ascorbic acid with the ROS produced (Rogozhin and Rogozhina, 2004).

Correlation analysis

The analysis of the agronomic variables showed a positive correlation between the variables of fresh biomass and dry biomass, which is evident when the conditions in which the experiment is carried out are homogeneous, being the case of this experiment. When conditions are heterogeneous, dry biomass is more accurate than fresh biomass to provide information on crop yield and productivity (González-Aguiar *et al*., 2018).

Regarding the biochemical variables, chlorophyll *a* and *b* showed a positive correlation. This because chlorophyll a is the main pigment of photosynthesis, traps light energy and emits high-energy electrons in the two photosystems, and in terms of chlorophyll b is the accessory pigment, which passes the trapped energy to chlorophyll a (Zepka *et al*., 2019). Flavonoids showed a positive correlation with chlorophylls, and this is possible thanks to the fact that flavonoids protect the photosynthetic apparatus from ultraviolet radiation (Zhou *et al*., 2016).

The antioxidant capacity and the proteins showed a positive correlation with the flavonoids, and this is due to the existing relationship in the defense system. Flavonoids are a very broad group of antioxidants, and some proteins such as enzymes, have high antioxidant power (Zhou *et al*., 2016; Leija-Martínez *et al*., 2018).

The IAA caused the oxidation of ascorbic acid and the increase of the rest of the biomolecules, thus explaining the negative correlation (Rogozhin and Rogozhina, 2004; Hasanuzzaman *et al*., 2020).

CONCLUSIONS

The results indicated that the use of IAA modified the agronomic and biochemical variables of the melon seedlings. It is concluded that the concentration of 0.5 mM of IAA is adequate to improve the agronomic characteristics of melon seedlings. In addition to this, it also improved the synthesis of chlorophyll *a*, *b*, total, carotenoids and antioxidant capacity, compared to the control. For this reason, it is advisable to handle IAA concentrations below 0.5 mM, which will possibly promote the production of vigorous seedlings, with improved photosynthetic activity and a better defense system. Otherwise, if concentrations higher than 0.5 mM of IAA are handled, the quality of the seedlings will decrease (less vigor), as observed in the present study.

CONFLICTS OF INTEREST

The authors declare that there are not conflicts of interest related to this article.

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