











# Effect of indole-3-acetic acid on growth, physiology and nutritional status of young arabica coffee plants

Wellington Abeldt Erlacher<sup>1</sup>, José Francisco Teixeira do Amaral<sup>2</sup>, José Augusto Teixeira do Amaral<sup>3</sup>,  
Bruno Fardim Christo<sup>4</sup>, Daniel Soares Ferreira<sup>5</sup>, Sandro Dan Tatagiba<sup>6</sup>, Brunno Cesar Pereira Rocha<sup>5</sup>,  
Marcelo Antonio Tomaz<sup>3</sup>, Wagner Nunes Rodrigues<sup>7</sup>, Fábio Luiz Partelli<sup>8</sup>

<sup>1</sup>Universidade Federal do Espírito Santo/UFES - Departamento de Agronomia, Domingos Martins, ES, Brasil

<sup>2</sup>Universidade Federal do Espírito Santo/UFES - Departamento de Engenharia Agrícola, Alegre, ES, Brasil

<sup>3</sup>Universidade Federal do Espírito Santo/UFES - Departamento de Agronomia, Alegre, ES, Brasil

<sup>4</sup>Universidade Federal de Santa Catarina/UFSC - Departamento de Administração, Florianópolis, SC, Brasil

<sup>5</sup>Universidade Federal de Viçosa - UFV - Departamento de Agronomia, Viçosa, MG, Brasil

<sup>6</sup>Instituto Federal Catarinense - Campus Videira, Videira, SC, Brasil

<sup>7</sup>Centro Universitário UNIFACIG, Manhuaçu, MG, Brasil

<sup>8</sup>Universidade Federal do Espírito Santo/UFES, Departamento de Ciências Agrárias e Biológicas/DCAB, São Mateus, ES, Brasil

Contact authors: wellington\_abldt@hotmail.com; jftamaral@yahoo.com.br; jata@cca.ufes.br; brunochristo@hotmail.com; danielccaufes@gmail.com; sandrodantatagiba@ifc.edu.br; brunno.rocha11@hotmail.com; marcelo.tomaz@ufes.br; wagner.nunes@sempre.unifacig.edu.br; partelli@yahoo.com.br

Received in June 21, 2022 and approved in November 17, 2022

## ABSTRACT

Coffee is one of the main agricultural commodities in the world. Thus, research aimed at reducing the productive risks of the crop has been increasingly encouraged, among which the use of plant hormones stands out. In addition, the objective of this work was to analyze the effect of the application of indole-3-acetic acid on the growth, nutrition and gas exchange of young *Coffea arabica* L plants. The experiment was carried out in the field in the city of Alegre, Espírito Santo, Brazil. The experimental design used was randomized blocks, testing the effect of the application of five doses of indole-3-acetic acid in young Arabica coffee plants, in four replications. The application of indole-3-acetic acid stimulates the growth rate of the stem diameter at a concentration of 60 mg L<sup>-1</sup>, as well as gas exchange in coffee plants, however it did not favor the increase in the substomatic concentration of CO<sub>2</sub> instantaneous and intrinsic efficiency in water use and instantaneous carboxylation efficiency. Although the application of EIA was not able to provide direct gains in coffee growth during the experimental period, a longer evaluation of the treatments would possibly provide promising results for the coffee crop. The multivariate analysis showed that higher doses of auxin have a high relationship with the macronutrients studied.

**Key words:** Gas exchange; auxin; hormonal balance; AIA; *Coffea arabica* L.

## 1 INTRODUCTION

Although there are several different species, 125 within the *Coffea* genus, reported by Krishnan et al. (2013) only two of the main ones are commercially cultivated, corresponding to 99% of world production. *C. arabica* L (Arabica coffee) is responsible for the largest amount of production with 64%, and the species *C. canephora* Pierre v. Conilon (Robusta coffee / Conilon) corresponds to 36% (Companhia Nacional de Abastecimento - CONAB, 2022). In addition to the fact that these two species differ in appearance and origin, they also differ widely in the quality and flavor of the drink (Bagyaraj et al., 2015; Davis et al., 2011)

Among the species of the *Coffea* genus, *C. arabica* L. presents an accentuated variation in vegetative growth and productive potential (Camargo, 2010), also known as coffee bienniality. Some factors have been attributed and studied to better understand this variation such as temperature, photoperiod, precipitation, competition for photoassimilates by fruits, hormonal balance in the plant, among others (Amaral et al., 2006; Barros et al., 1997; Ferreira et al., 2021a; Nazareno et al., 2003), but large gaps to solve this phenological event in coffee culture still remain.

To prevent the variation in vegetative and productive growth of coffee plants (bienniality), research using plant growth regulators have been increasingly applied (Hatanaka et al., 1991; Cavalcante Filho et al., 2018; Mwaniki et al., 2019; Muliassari et al., 2021). Growth regulators are synthetic or natural chemical compounds that can modulate and regulate the physiological aspects of plants (Ashikari et al., 2005; Pu et al., 2018). In a world where agriculture is increasingly vulnerable to climate change (Bunn et al., 2015; Martins et al., 2018; Moreira et al., 2020) and requiring about food demand (Food and Agriculture Organization of the United Nations - FAO, 2019), studying the action of these growth regulators in the development of coffee plants becomes of fundamental importance (Khan et al., 2020; Sasi et al., 2021; Buono, 2021)

The action of growth regulators on plant metabolism may have the ability to improve the modulation of its growth and development (Bacilieri et al., 2016; Santner et al., 2009). In coffee plants, studies have shown that the application of phytohormones such as giberililn, indole acetic acid, cytokinin and zeatin in controlled use is capable of maximizing plagiotropic branch growth, number of nodes and rosettes and greater fruit harvest. (Mukhtar et al., 2021; Waday et al., 2022; Bacilieri et al., 2016; Costa et al., 2009), improving the productivity.

Coffee growth and productivity may relate to the capacity of the growth regulator, indole-3-acetic acid (IAA), has in maximizing the photosynthetic capacity of plants; Huan et al., 2021; Miao et al., 2018; Zhao et al., 2017), improving the absorption of nutrients such as nitrogen and potassium (Liang et al., 2022) in plants under adverse stress (Huan et al., 2021).

Thus, considering the hypothesis that the isolated application of IAA can provide gains in photosynthetic, nutritional and growth capacity of young coffee plants, this study aimed to analyze the effect of indole-3-acetic acid application on growth, nutrition and gas exchange of young plants of *Coffea arabica* L.

## 2 MATERIAL AND METHODS

The experiment was executed in the field at the district of Celina, municipality of Alegre, Espírito Santo state, Brazil (geographic coordinates 20°46'S and 41°37'W). The region cultivated with the species *Coffea arabica* L. has an altitude of 750 m and annual accumulated precipitation greater than 1200 mm. The climate of the region, according to Köppen's climate classification (Alvares et al., 2013), is "Aw" type, with rainy summer and dry winter, presenting high climatic seasonality with seasons of low and high rainfall [from when to when] well defined.

To execute the experiment, arabica coffee plants of Catucaí 785/15 cultivar were used, newly implanted in a non-irrigated condition, in a spacing of 2.1 × 1.0 totaling 4760 plants per hectare. Before executing the experiment, the chemical attributes of the soil were determined (Table 1), correcting it, according to the recommendation proposed for arabic coffee in the state of Espírito Santo (Lani et al., 2007).

The experimental delineation used was in randomized blocks, and the isolated application of five concentrations of IAA (0 mg L<sup>-1</sup>; 0.1 mg L<sup>-1</sup>; 10 mg L<sup>-1</sup>; 20mg L<sup>-1</sup>; 60 mg L<sup>-1</sup>) was investigated in young Arabica coffee plants with four replications, and each experimental unit was composed of two useful plants. Before the application, the blank test was performed, determining 50 mL of syrup per plant.

For the preparation of the growth regulator solution (indole-3-acetic acid), the recommendation described by Quisen and Angelo (2008) and the concentrations proposed by Bacilieri et al. (2016) and Silva Filho et al. (2012). For that, a digital scale was used, in which a beaker was weighed and each

concentration of indole-3-acetic acid (C<sub>10</sub>H<sub>9</sub>NO<sub>2</sub>), 99% pure, was weighed. After this procedure, 3 drops of 1M NaOH were used for every 10 mg of IAA, totaling 18 drops of 1M NaOH.

After planting, the seedlings underwent an adaptation period of 30 days, and were subsequently subjected to treatments through the application of the growth regulator. For this, 50 mL of the solution containing the AIA were applied per plant at sunrise, using a manual backpack sprayer, seeking to reach the entire calculated volume of the solution in the coffee leaves. The adhering agent SILWET L-77 Ag – registered with the Ministry of Agriculture, Livestock and Supply under no. 02696 – was used in the preparation of syrup to facilitate the absorption of hormones by the plant.

Weekly, during the phenological phase, corresponding to the period of bean formation and vegetative growth (between January and April), for a period of 90 days, the length of the orthotropic branch (cm) and the plagiotropic branch (cm) and also the stem diameter (cm). To evaluate the growth rate of orthotropic (GROB) and plagiotropic (GRPB) and stem (GBSD) branches, we use the following equation 1:

$$GR = \frac{\Delta G}{\Delta T} \quad (1)$$

Where:

GR: Growth rate (cm day<sup>-1</sup>); ΔG: Variation of branch growth (cm); ΔT: Experiment Total time.

After 90 days of the experimental trial, gas exchange analyzes were performed on the third pair of leaves selected from the plagiotropic branch, which expanded after the treatments were applied, and the young leaves (sink leaves) were marked at the time of application. Only one sheet per repetition (this sheet that had been subjected to the application of the hormone) was used to analyze gas exchange.

Gas exchange analyses were performed using the infrared gas analyzer (IRGA Li-Cor 6400 XT), obtaining the net carbon assimilation rate (*A*, μmol m<sup>-2</sup> s<sup>-1</sup>); substomatic concentration of CO<sub>2</sub> (*C<sub>i</sub>*, μmol mol<sup>-1</sup>); stomatic conductance (*g<sub>s</sub>*, mmol m<sup>-2</sup> s<sup>-1</sup>); transpiration rate (*E*, mmol m<sup>-2</sup> s<sup>-1</sup>). From these variables, the instantaneous efficiency of water use (*A/E*, μmol mmol<sup>-1</sup>); estimative of intrinsic efficiency of water use (*A/g<sub>s</sub>*, μmol mol<sup>-1</sup>); and instantaneous efficiency of carboxylation (*A/C<sub>i</sub>*, μmol m<sup>-2</sup> s<sup>-1</sup>) were estimated.

**Table 1:** Soil chemical attributes.

Soil chemical attributes												
pH <sup>(1)</sup>	P <sup>(2)</sup>	K <sup>(2)</sup>	Na <sup>(2)</sup>	Ca <sup>(3)</sup>	Mg <sup>(3)</sup>	Al <sup>(3)</sup>	H+Al <sup>(4)</sup>	SB <sup>(5)</sup>	CTC <sup>(6)</sup>	t <sup>(7)</sup>	V <sup>(8)</sup>	m <sup>(9)</sup>
-----mg/dm <sup>3</sup> -----				-----Cmol <sub>c</sub> / dm <sup>3</sup> -----					-----%-----			
6.76	5.70	154.0	2.00	0.83	0.15	1.00	9.90	1.37	8.46	10.8	12.15	0.0

<sup>1/</sup> soil-water ratio 1:2.5; <sup>2/</sup> extracted by Mehlich-1; <sup>3/</sup> extracted by KCl; <sup>4/</sup> extracted by Calcium Acetate; <sup>5/</sup> sum of bases; <sup>6/</sup> CTC at pH 7.0; <sup>7/</sup> effective CTC; <sup>8/</sup> percentage of base saturation; <sup>9/</sup> percentage aluminum saturation.

At the end of the experimental test (120 days after planting and 90 days after application of the hormone), gas exchange evaluations were performed, selecting a day with clear sky, in the morning between 08:00h and 09:00h. Photosynthetically active radiation was standardized in artificial light saturated with 1000  $\mu\text{mol}$  of photons  $\text{m}^{-2} \text{s}^{-1}$  and  $\text{CO}_2$  at a concentration of 400 ppm in the chamber.

At the end of the experiment, 16 leaves were collected from each experimental unit, using the marked leaves, packed in paper bags, identified and delivered to the Laboratory of Mineral Nutrition of Plants, where they were dried in a greenhouse with forced air ventilation at 65 °C until constant weight is reached. Subsequently, the samples were individually ground in a Wiley mill to determine the foliar nutrient contents as recommended by Silva (2009).

The data were subjected to variance analysis by F test and, when significance was verified, the means were compared by Dunnett's test at a level of 5% probability, using software SAEG 9.1 (2007). To observe the existing correlation among the variables under study, the analysis of the Correlation of Person with t-test was executed to assess its significance at the level of 0.1% (\*\*\*) , 1% (\*\*) and 5% (\*) probability. Finally, aiming to group the treatments to obtain a joint information of the data, the multivariate analysis was performed by the main component method, using the scores of the first two main components,

which explains more than 70% of the total available variation, as recommended by Ferreira (2018). Multivariate analyses were performed with the aid of R software (R Core Team, 2021).

### 3 RESULTS

Among the variables investigated by analysis of variance (Table 2), 8 out of the 16 characteristics showed no significant difference for the concentrations of indole-3-acetic acid analyzed, being: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), growth rate of the plagiotropic branch (GRPB) and growth rate of the orthotropic branch (GROB).

Table 3 shows that the application of indole-3-acetic acid stimulated the growth rate of stem diameter (GRSD) at the concentration of 60  $\text{mg L}^{-1}$  (gs), and also the gas exchange of coffee plants (Table 3), providing greater stomatic conductance at concentrations of 20  $\text{mg L}^{-1}$  and 60  $\text{mg L}^{-1}$ . We also observed that the highest substomatic concentration of  $\text{CO}_2$  and the highest transpiration rate occurred in the lowest concentrations of IAA and higher net assimilation rate of  $\text{CO}_2$  at the concentration of 60  $\text{mg L}^{-1}$ . However, this higher transpiration did not rebound in lower instantaneous and intrinsic efficiency in water use and instantaneous efficiency of carboxylation, when compared to the control.

**Table 2:** Summary of the analysis of variance of the variables: net carbon assimilation rate (A), substomatic concentration of  $\text{CO}_2$  (Ci), stomatic conductance (gs), transpiration rate (E), instantaneous efficiency of water use (A/E), estimation of the intrinsic efficiency of water use (A/g<sub>s</sub>), instant efficiency of carboxylation (A/Ci), leaf contents of: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), and the growth rates of the plagiotropic (GRPB) and orthotropic (GROB) branches and stem diameter (GRSD) of young Arabica coffee plants subjected to application of different concentrations of indole-3-acetic acid.

MEAN SQUARE							
FV	GL	A	gs	Ci	E	A/E	A/g <sub>s</sub>
Block	3	1.308	0.000116	1083.12	0.0275	2.553	279.20
IAA	4	6.824*	0.01631*	15612.49*	0.4404*	26.19*	8605.78*
Error	12	1.516	0.000644	1168.15	0.0408	3.29	572.52
CV		13.78	9.92	19.92	22.12	17.08	17.54
MEAN SQUARE							
FV	GL	A/Ci	N	P	K	Ca	Mg
Block	3	0.00031	1.344	0.00476	3.4582	0.1382	0.3742
IAA	4	0.00256*	9.817 <sup>ns</sup>	0.00819 <sup>ns</sup>	6.4249 <sup>ns</sup>	1.6983 <sup>ns</sup>	0.1848 <sup>ns</sup>
Error	12	0.0045	15.216	0.0166	7.9868	7.0543	0.4640
CV		34.87	12.92	14.99	26.40	18.47	21.66
MEAN SQUARE							
FV	GL	S	GRPB	GROB	GRSD		
Block	3	0.0159	0.00037	0.0025	0.00052		
IAA	4	0.1862 <sup>ns</sup>	0.0020 <sup>ns</sup>	0.00035 <sup>ns</sup>	0.00193*		
Erro	12	0.0243	0.00072	0.001244	0.00033		
CV		16.00	9.40	11.25	34.99		

\* Significant F at 5% probability (P< 0,05) level, "ns" not significant.

The inverse was also observed in this study (Table 3), the lowest stomatic conductance at the concentration of 0.1 mg L<sup>-1</sup>, 10 mg L<sup>-1</sup> provided lower substomatic concentration of CO<sub>2</sub> (concentration of 0.1 mg L<sup>-1</sup>), allowing greater instantaneous and intrinsic efficiency in water use and instantaneous efficiency of carboxylation, at the lowest concentration of IAA (0.1 mg L<sup>-1</sup>), but did not reduce photosynthesis when compared with the control (Table 2).

It is noted via an initial analysis the absence of significant correlations between the variables phosphorus (P), magnesium (Mg) and calcium (Ca) with the other variables analyzed (Figure 1). Positive correlations greater than 90% among the variables *A/gse* *A/E* (97%), *A/Cie* *A/E* (93%), GRPB and *A/ci*(95%), *gse* *Ci* (92%), *gs* and *E* (92%), *A* and K

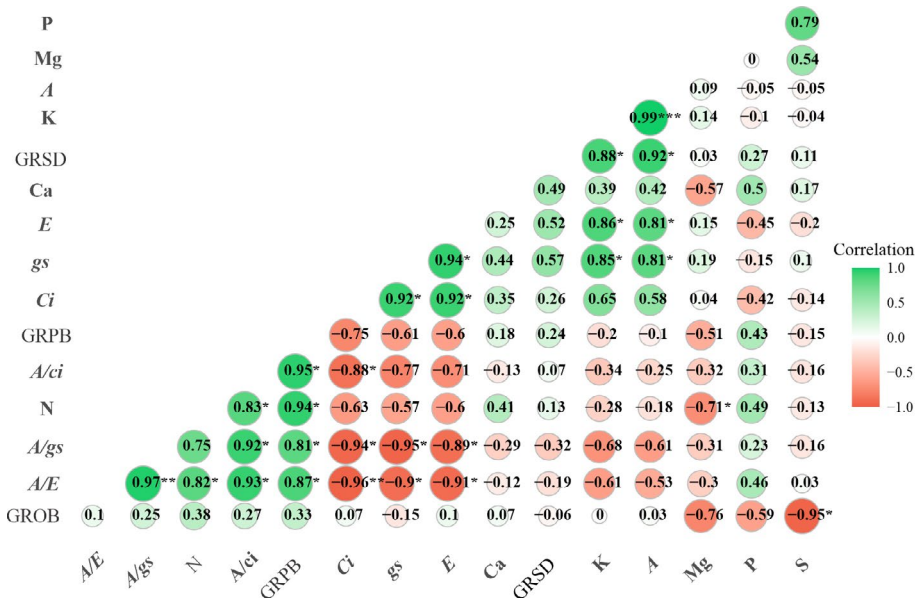
(99%) and *A* and GRSD (92%), as well as negative correlations higher than 90% among the variables *Ci* and *A/gs* (-94%), *Cie* and *A/E* (-96%), *gs* and *A/E* (-90%), *E* and *A/E* (-91%), *gs* and *A/gs*(-95%) and S and GROB (-95%).

In general, Figure 1 shows that, among the most accumulated macronutrients in coffee, potassium (K) presented the highest number of significant correlation with the other variables studied (correlations 85%, 86%, 88% and 99% with the variables *gs*, *E*, GRSD and *A*, respectively). Sulfur (S) showed a correlation of -95% and -15% with the growth rate of the orthotropic branch (GROB) and plagiotropic branch (GRPB), respectively, as well as a correlation of only 11% with the growth rate of the stem diameter (GRSD). Nitrogen (N) showed good correlation with GRPB (94%) and *A/E* (82%).

**Table 3:** Net assimilation rate of CO<sub>2</sub> (*A*, μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), stomatic conductance (*gs*, mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), substomatic concentration of CO<sub>2</sub> (*Ci*, μmol mol<sup>-1</sup>), transpiration rate (*E*, mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) instantaneous efficiency(*A/E*, μmol mmol<sup>-1</sup>)and intrinsic in water use (*A/gs*, μmol mol<sup>-1</sup>), instantaneous efficiency of carboxylation (*A/Ci*, μmol m<sup>-2</sup> s<sup>-1</sup>) and growth rate of stem diameter (GRSD, cm day<sup>-1</sup>), of young arabica coffee plants subjected to application of different concentrations of indole-3-acetic acid.

Concentration IAA	<i>A</i>	<i>gs</i>	<i>Ci</i>	<i>E</i>	<i>A/E</i>	<i>A/gs</i>	<i>A/Ci</i>	GRSD
0 mg L <sup>-1</sup>	8.33	0.066	153.10	0.824	10.17	127.04	0.055	0.0426
0.1 mg L <sup>-1</sup>	8.84 <sup>ns</sup>	0.043*	87.32*	0.622 <sup>ns</sup>	14.24*	203.30*	0.086*	0.0616
10 mg L <sup>-1</sup>	7.17 <sup>ns</sup>	0.045*	152.93 <sup>ns</sup>	0.606 <sup>ns</sup>	11.89 <sup>ns</sup>	161.57 <sup>ns</sup>	0.057 <sup>ns</sup>	0.02544
20 mg L <sup>-1</sup>	9.79 <sup>ns</sup>	0.103*	247.35*	1.345*	7.54 <sup>ns</sup>	95.24 <sup>ns</sup>	0.040 <sup>ns</sup>	0.0541
60 mg L <sup>-1</sup>	10.55*	0.111*	217.21*	1.171*	9.29 <sup>ns</sup>	94.97 <sup>ns</sup>	0.049 <sup>ns</sup>	0.0831*

\* significant in relation to the control (without Application of IAA), at 5% probability by Dunnett's test ns no significant.



**Figure 1:** Estimates of Pearson correlation of variables: net carbon assimilation rate (*A*), substomatic concentration of CO<sub>2</sub> (*Ci*), stomatic conductance (*gs*), transpiration rate (*E*), instantaneous efficiency of water use (*A/E*), estimation of the intrinsic efficiency of water use (*A/gs*), instant efficiency of carboxylation (*A/Ci*), leaf contents of: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), and the growth rates of the branches plagiotropic (GRPB), orthotropic (GROB) and stem diameter (GRSD) of young Arabica coffee plants subjected to application of different concentrations of indole-3-acetic acid. Significant by t test at 5% (\*), at 1% (\*\*) and at 0.1% (\*\*\*).

The analysis of main components in Figure 2 shows that, the first two components were able to explain 74.3% of the total data variation. This variation was able to provide the formation of three distinct groups of the treatments studied (Figure 2a), the first group was formed by the treatment referring to the control treatment ( $0 \text{ mg L}^{-1}$ ), the second by intermediate concentrations ( $0.1 \text{ mg L}^{-1}$  and  $10 \text{ mg L}^{-1}$ ) and the third group by the highest concentrations ( $20 \text{ mg L}^{-1}$  and  $60 \text{ mg L}^{-1}$ ).

The formation of the first group (Figure 2a) possibly occurred due to its good association with the variables sulfur (S) and magnesium (Mg), the second group with the variables net carbon assimilation rate (A), potassium (K), substomatic concentration of  $\text{CO}_2$  (Ci), transpiration rate (E), stomatic conductance (gs) and intermediate association with stem diameter growth rate (GRSD), calcium (Ca) and orthotropic branch growth rate (GROB). The third group presented a good association with the variables, estimation of the instantaneous efficiency of water use (A/E), estimation of the intrinsic efficiency of water use (A/gS), and instantaneous efficiency of carboxylation (A/Ci), nitrogen (N), phosphorus (P) and growth rate of the plagiotropic branch (GRPB).

#### 4 DISCUSSION

Some cultural practices are capable of maximizing biomass growth and accumulation in cultivated plants, and the use of indole-3-acetic acid may be included (Ferreira et al., 2021a; Huan et al., 2021; Li et al., 2019a; Ribeiro et al., 2019; Yao et al., 2019). In general, the application of IAA in plants stimulates growth due to the induction of cell division and stretching (Blum, 2009; Zou et al., 2018).

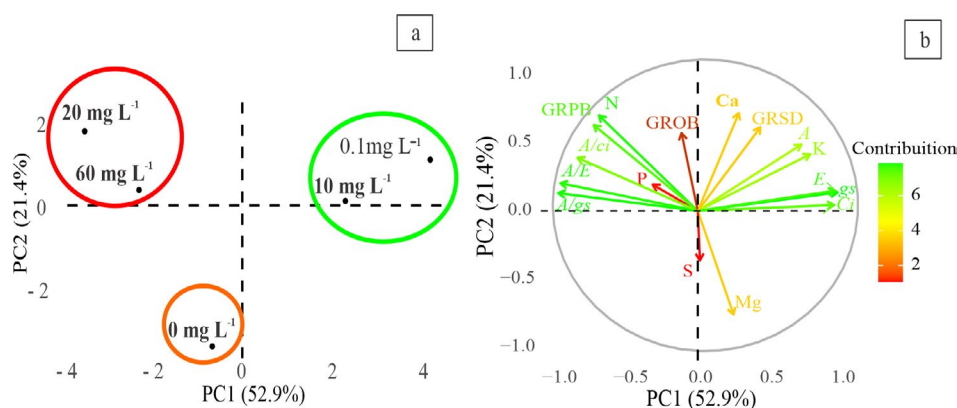
This study showed that the hormone application did not promote significant gains in the growth of the orthotropic and plagiotropic branches, as observed in the Dunnett's test (Table 2). This fact can be justified by using the concentration of exogenous auxin used in the

experiment (Huan et al., 2021). According to Wu et al. (2017), a low auxin concentration can promote an increase in plant growth, as observed in this study for stem diameter (Table 3). However, the use of high concentrations of auxin can provide significant gains or even inhibit plant growth (Liu et al.; 2018; Ye et al., 2019).

In work conducted by Huan et al. (2021) analyzing the effects of indole-3-acetic acid on the growth and absorption characteristics of selenium of *Cyphomandra betacea* seedlings, it was observed that at maximum concentrations of  $60 \text{ mg L}^{-1}$  auxin, it was able to provide gains in *Cyphomandra betacea* biomass. Although it was not possible to observe gains in biomass with the increase in IAA concentration in this study, the concentration of  $60 \text{ mg L}^{-1}$  provided the best responses to the net assimilation rate of  $\text{CO}_2$  (A) (Table 3), and was also well associated with growth variables (GRSD, GRPB, GRPB) when analyzed in a multivariate form (Figure 2).

As highlighted in the literature, the multivariate study of the set of variables can provide reliable biological information about the responses to the text treatments even when the set of variables does not show a significant univariate response (Barbosa et al., 2019; Barbosa et al., 2020; Ferreira, 2018; Ferreira et al., 2021b), making it feasible to keep these variables for more refined analyses.

Literature emphasized that the application of IAA can improve the photosynthetic efficiency of plants (Ahmad et al., 2001; Hayat et al., 2009; Huan et al., 2021; Miao et al., 2018; Zhao et al., 2017). Since photosynthesis is essential for plant development and growth, while the entire biomass production process depends on this activity, this increase in biomass is associated with high stomatic conductance, as well as greater fixation of  $\text{CO}_2$  (Blum, 2009). Likewise, we can assume that although the application of IAA could not provide direct gains in coffee growth during the trial period, a longer evaluation of the treatments would provide promising results for coffee culture.



**Figure 2:** Dispersion diagram (a) and contribution of variables (b) in relation to the first two main components related to the application of different concentrations of indole-3-acetic acid in young arabica coffee plants.

The interference of auxin in gas exchange was already reported in some studies. Ahmad et al. (2001), when they used different forms of auxin, investigated a higher rate of net assimilation and efficiency of carboxylation, ensuring greater vegetative growth and *Brassica juncea* Czern & Coss cv. species Varuna seeds. Similarly, auxin application promoted higher stomatic conductance, internal CO<sub>2</sub> concentration, efficiency in the use of water, transpiration rate and net photosynthetic rate, which favored the yield of *Cicer arietinum* L seeds (Hayat et al., 2009). However, answers regarding the effect of the application of auxin in coffee are still very restricted and need further investigation.

However, auxin can influence gas exchange, which consequently can affect the growth and development of this plant (Albacete et al., 2014). Thus, although the concentration 0.1 mg L<sup>-1</sup> presented liquid assimilation rate equal to the control the variable related responses of instantaneous efficiency ( $A/E$ ,  $\mu\text{mol mmol}^{-1}$ ) and intrinsic in the use of water ( $A/g_s$ ,  $\mu\text{mol mol}^{-1}$ ) and instantaneous efficiency of carboxylation, showing that the implementation of IAA can improve the growth modulation in coffee plants, even when plants are not subjected to environmental stresses.

In fact, the result obtained for the variable growth of branches was already expected, since the formation of branches is regulated by the complex interaction of plant hormones, including cytokinin and auxin (Taiz et al., 2017). This result corroborates with Bacilieri et al. (2016), in which the application of hormone consisting of auxin, cytokinin and gibberelins promoted an increase in the length of the plagiotropic branch of Arabica coffee.

As highlighted by Huan et al. (2021), the application of IAA in plants may affect the absorption of some nutrients. This statement was not directly observed in this study. However, it is possible to observe by the analysis of main components that the treatments related to the application of the different doses of IAA provided a differentiated association with micronutrients in coffee (Figure 2). In general, it is possible to highlight that the association of the group formed by the highest concentrations of IAA with nitrogen and phosphorus occurred due to the action that the IAA has in improving plant photosynthesis.

As highlighted by Li et al. (2019b) and Miao et al. (2018) gains in photosynthesis associated with IAA application occur due to the higher chlorophyll synthesis by the plant, since the addition of IAA regulates the gene expression of the plant involved in the synthesis of photosynthetic pigments, thus increasing the leaf contents of chlorophyll (Li et al., 2019b; Huan et al., 2021). Thus, for future work, the evaluation of photosynthetic tissues of plants may provide relevant information to understand the IAA response in the physiology and growth of coffee plants.

Nitrogen is one of the main constituent nutrients of the chlorophyll molecule (Taiz et al., 2017) and its demand

in the coffee plant is relatively high (Bohóquez et al., 2021; Machado et al., 2016; Partelli et al., 2007; Zabini et al., 2021), because Nitrogen, jointly with phosphorus, participates in the structures of proteins and other organic compounds important for plant growth (Marschner, 2012; Martinez et al., 2020; Taiz et al., 2017; Zabini et al., 2021).

Moreover, although no significant univariate effect of these nutrients was observed with the application of IAA, the multivariate behavior demonstrates that higher doses of auxin has high relation to these nutrients. We could observe that potassium had high correlation with gas exchange variables (Figure 1) and good association with intermediate Concentrations of IAA (Figure 2).

Potassium participates in the activation of several enzymes associated with respiration and photosynthesis (Marschner, 2012; Taiz et al., 2017). Since the intermediate concentrations (0.1 mg L<sup>-1</sup> and 10 mg L<sup>-1</sup>) presented the best results for the parameters of instantaneous and intrinsic efficiency in water use and instantaneous efficiency of carboxylation (Table 3), this result for potassium was expected.

We can also observe a correlation of 99% between the rate of liquid assimilation of CO<sub>2</sub> ( $A$ ) and potassium (K) (Figure 1). Potassium is one of the minerals that act fundamentally in the osmotic control of the stomatic opening, which can help in the control and stomatic closure, interfering in the efficiency of carboxylation in the auxin designs used (Marschner, 2012; Taiz et al., 2017). Since the control of closure and opening of stomates are the main mechanisms of control of gas exchange in terrestrial plants (Tatagiba et al., 2008).

Thus, the application of IAA promoted the growth of the stem diameter with direct effect on leaf gas exchanges. We assume that these alterations in gas exchange levels may have occurred by two paths. The first would be related to a possible reduction of the source and drain relationship in the plant, derived from the potential that IAA has in increasing the root surface of plants (Taiz et al., 2017), this development in the root system would provide an increase in the potential for water and nutrient absorption, which consequently could be associated with lower water and nutritional stresses by plants, which could provide an increase in gas exchanges (Ronchi et al., 2015; Taiz et al., 2017; Tatagiba et al., 2008).

The second path would be associated, as previously mentioned, to the improvements provided by auxins in the genic modulation of plants associated with the production of photosynthetic pigments (Huan et al., 2021). The increase of these photosynthetic pigments, associated with a greater efficiency of the root system (Taiz et al., 2017), may be associated with changes in gas exchange levels in plants supplemented with auxins. Thus, the application of auxin presents potential to reduce drastic falls in the vegetative growth of coffee as well as tends to improve the nutritional and physiological balance of coffee plants.

## 5 CONCLUSION

The application of indole-3-acetic acid stimulates the growth rate of the stem diameter at a concentration of 60 mg L<sup>-1</sup>, as well as gas exchange in coffee plants, however it did not favor the increase in the substomatic concentration of CO<sub>2</sub> instantaneous and intrinsic efficiency in water use and instantaneous carboxylation efficiency.

Although the application of EIA was not able to provide direct gains in coffee growth during the experimental period, a longer evaluation of the treatments would possibly provide promising results for the coffee crop.

The multivariate analysis showed that higher doses of auxin have a high relationship with the macronutrients studied.

## 7 ACKNOWLEDGMENTS

This study was funded in part by the National Council for Scientific and Technological Development - Brazil (CNPq), Coordination for the Improvement of Education - Brazil (CAPES) - Finance Code 001.

## 8 AUTHORS' CONTRIBUTION

WAE, BFC, DSF and BCPR wrote the manuscript, BFC, WAE, JATA carried out the experimentation, JFTA, MAT, WNR supervised the experiment and collaborated on the manuscript, SDT, FLP, JFTA revised and approved the final version of the work, SDT, WAE, BCPR and DSF conducted all statistical analyses.

## 9 REFERENCES

- AHMAD, A. et al. Photosynthetic efficiency of plants of *Brassica Juncea*, Treated with chlorosubstituted auxins. **Photosynthetica**, 39:565-568, 2001.
- ALBACETE, A. A. et al. Hormonal and metabolic regulation of source-sink relations under salinity and drought: From plant survival to crop yield stability. **Biotechnology Advances**, 32:12-30, 2014.
- ALVARES, C. A. et al. Köppen's climate classification map for Brazil. **Meteorologische Zeitschrift**, 22(6):711-728, 2013.
- AMARAL, J. A. T. et al. Crescimento vegetativo sazonal do cafeeiro e sua relação com fotoperíodo, frutificação, resistência estomática e fotossíntese. **Pesquisa Agropecuária Brasileira**, 41(3):377-384, 2006.
- ASHIKARI, M. et al. Cytokinin oxidase regulates rice grain production. **American Association for the Advancement of Science**, 309(5735):741-745, 2005.
- BACILIERI, F. S. et al. Efficacy of a plant growth regulator in the culture of coffee. **Bioscience Journal**, 32(2):346-353, 2016.
- BAGYARAJ, D. J. et al. Below ground microbial diversity as influenced by coffee agroforestry systems in the Western Ghats, India. **Agriculture, Ecosystems and Environment**, 202:198-202, 2015.
- BARBOSA, I. P. et al. Recommendation of *Coffea arabica* genotypes by factor analysis. **Euphytica**, 215(178):1-10, 2019.
- BARBOSA, I. P. et al. Sensory analysis of arabica coffee: cultivars of rust resistance with potential for the specialty coffee market. **Euphytica**, 216:165, 2020.
- BARROS, R. S. et al. Decline of vegetative growth in *Coffea arabica* L. in relation to leaf temperature, water potential and stomatal conductance. **Field Crops Research** 54(1):65-72, 1997.
- BLUMM, A. Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. **Field Crops Research**, 112:119-123, 2009.
- BOHÓQUEZ, C. A. A. et al. Kinetic parameters of nitrate absorption by adult coffee trees. **Frontiers in Sustainable Food Systems**, 21:677580, 2021.
- BUNN, C. et al. Bitter cup: Climate change profile of global production of Arabica and Robusta coffee. **Climatic Change**, 129:89-101, 2015.
- BUONO, D. D. Can biostimulants be used to mitigate the effect of anthropogenic climate change on agriculture? It is time to respond. **Science of The Total Environment**, 751(10):141763, 2021.
- CAMARGO, M. B. P. The impact of climatic variability and climate change on Arabic coffee crop in Brazil. **Bragantia**, 69(1):239-247, 2010.
- CAVALCANTI FILHO, P. F. M. et al. Effect of Growth Regulators in Production and Rooting of *Coffea arabica* L. Minicuttings. **American Journal of Plant Sciences**. 9(4):628-636, 2018.
- COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB. **Acompanhamento da safra brasileira 2022**. 9(3): 1-66. 2022. <<https://www.conab.gov.br/info-agro/safra/cafes/boletim-da-safra-de-caffe>>. Access in: January 2, 2023.
- COSTA, N. R. et al. Desempenho do cafeeiro Icatu vermelho sob ação de biorregulador aplicado em fases reprodutivas da cultura. **Agrarian**, 2(5):113-130, 2009.

- DAVIS, A. P. et al. Growing coffee: *Psilanthus* (Rubiaceae) subsumed on the basis of molecular and morphological data implications for the size, morphology, distribution and evolutionary history of *Coffea*. **Botanical Journal of the Linnean Society**, 167(4):357-377, 2011.
- DONG, C. G. et al. ADF Proteins Are involved in the control of flowering and regulate f-actin organization, cell expansion, and organ growth in arabidopsis. **The Plant Cell**, 13(6):1333-1346, 2001.
- FERREIRA, D. **Estatística multivariada**. 3.ed. Lavras: Editora UFLA. 2018. 642p.
- FERREIRA, D. S. et al. Biochemical and physiological changes in conilon coffee grown under different levels of irradiance. **Australian Journal of Crop Science**, 15(7):1074-1080, 2021a.
- FERREIRA, D. S. et al. Exploring the multivariate technique in the discrimination of *Coffea arabica* L. cultivars regarding the production and quality of grains under the effect of water management. **Euphytica**, 217:118, 2021b.
- FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS-FAO. **Food Outlook** - Biannual Report on Global Food Markets. Retrieved from. 2019. Available in: <<http://www.fao.org/documents/card/en/c/ca4526en>>. Access in: January 2, 2023.
- LIANG, T. et al. Topping Inhibited Potassium Uptake via Regulating Potassium Flux and Channel Gene Expression in Tobacco. **Agronomy**. 12(5): 1166, 2022.
- HATANAKA, T. et al. Effect of plant growth regulators on somatic embryogenesis in leaf cultures of *Coffea canephora*. **Plant Cell Reports**, 10:179-182, 1991.
- HAYAT, Q. et al. Auxin analogues and nitrogen metabolism, photosynthesis, and yield of chickpea. **Journal of Plant Nutrition**, 32:1469-1485, 2009.
- HUAN, Y. et al. Effects of indole acetic acid on the growth and selenium absorption characteristics of *Cyphomandrabetacea* seedlings. **Acta Physiologiae Plantarum**, 43(24):74, 2021.
- KHAN, N. et al. Crosstalk amongst phytohormones from planta and PGPR under biotic and abiotic stresses. **Plant Growth Regulation**, 90:89-203, 2020.
- KRISHNAN, S. et al. An assessment of the genetic integrity of ex situ germplasm collections of three endangered species of *Coffea* from Madagascar: Implications for the management of field germplasm collections. **Genetic Resources and Crop Evolution**, 60:1021-1036, 2013.
- LANI, J. A. et al. Cafeeiro. In: PREZOTTI LC, G. J. A. et al. **Manual de recomendação de calagem e adubação para o Estado do Espírito Santo (5ª aproximação)**. Vitoria: SEEA/INCAPER/CEDAGRO. p. 111-118, 2007.
- LI, H. et al. Effects of mutual grafting on cadmium accumulation characteristics of first post-generations of *Bidens pilosa* L. and *Galinsoga parviflora* Cav. **Environmental Science and Pollution Research**, 26: 33228-33235, 2019a.
- LI, J. et al. Effects of exogenous IAA in regulating photosynthetic capacity, carbohydrate metabolism and yield of *Zizania latifolia*. **Scientia Horticulturae**, 253(27): 276-285. 2019b.
- LIU, Q. et al. Effects of different rootstocks on cadmium accumulation of grafted *Cyphomandrabetacea* seedlings. **International Journal of Environmental Analytical Chemistry**, 99(12):1247-1254, 2018.
- MACHADO, L. S. et al. Efficiency and response of conilon coffee genotypes to nitrogen supply. **African Journal of Biotechnology**, 1535:1892-1898, 2016.
- MARSCHNER, H. **Mineral nutrition of higher plants**. San Diego: Academic Press, 1995. 889p.
- MARTINEZ, H. E. P. et al. Water deficit changes nitrate uptake and expression of some nitrogen related genes in coffee-plants (*Coffea arabica* L.). **Scientia Horticulturae**, 267:109254, 2020.
- MARTINS, L. D. et al. Carbon and water footprints in Brazilian coffee plantations - the spatial and temporal distribution. **Journal of Food and Agriculture**, 30:482-482, 2018.
- MIAO, Y. M. et al. Effects of IAA and GA<sub>3</sub> on melon growth and photosynthesis under low light stress. **Mol Plant Breeding**, 16:2335-2340, 2018.
- MOREIRA, T. R. et al. Quality determinants in coffee production. food engineering series. In: PEREIRA, L. L.; MOREIRA, T. R. **Global warming and the effects of climate change on coffee production**. Springer, Cham, 2021.
- MULIASARI, A. A. et al. Improvement generative growth of *Coffea arabica* L. using plant growth regulators and pruning. **E3S Web of Conferences**, 226:00003, 2021.
- MWANIKI, W. I. et al. Effects of genotype and plant growth regulators on callus induction in leaf cultures of *Coffea arabica* L. F1 hybrid. **African Journal of Biotechnology**, 18(31):1004-1015, 2019.



- NAZARENO, R. B. et al. Crescimento inicial do cafeeiro Rubi em resposta a doses de nitrogênio, fósforo e potássio e a regimes hídricos. **Pesquisa Agropecuária Brasileira**, 38(8):903-910, 2003.
- PARTELLI, F. L. et al. Diagnosis and recommendation integrated system norms, sufficiency range, and nutritional evaluation of arabian coffee in two sampling periods. **Journal of Plant Nutrition**, 30:1651-1667, 2007.
- PU, C. H. et al. Modified QuEChERS method for 24 plant growth regulators in grapes using LC-MS/MS. **Journal of Food and Drug Analysis**, 26(2):637-648, 2018.
- QUISEN, R. C.; ANGELO, P. C. S. **Manual de procedimentos do laboratório de cultura de tecidos da Embrapa Amazônia Ocidental**. (Embrapa Amazônia Ocidental. Documentos, 61). Manaus: Embrapa Amazônia Ocidental, 2008, 44p.
- R CORE TEAM. **R**. Language and environment for statistical computing. . R Foundation for Statistical. 2021. Version.string R version 4.0.0. Vienna, Austria. Available in: <<http://www.r-project.org>>. Access in: January 2, 2023.
- RIBEIRO, W. R. et al. Crescimento do cafeeiro conilon cultivar “ES 8122-Jequitibá” em função da fração de água transpirável no solo. **IRRIGA**, 24(3):512-526, 2019.
- RONCHI, C. P. et al. Respostas ecofisiológicas de cafeeiros submetidos ao déficit hídrico para concentração da florada no Cerrado de Minas Gerais. **Pesquisa Agropecuária Brasileira**, 50(1):24-32, 2015.
- SANTNER, A. et al. Plant hormones are versatile chemical regulators of plant growth. **Nature Chemical Biology**, 5(5):301-307, 2009.
- SASI, M. et al. Plant growth regulator induced mitigation of oxidative burst helps in the management of drought stress in rice (*Oryza sativa* L.). **Environmental and Experimental Botany**, 185:104413, 2021.
- SILVA, F. C. **Manual de análise química de solos, plantas e fertilizantes**. 2ª edição revisada e ampliada. Brasília: Embrapa, 2009. 627p.
- SILVA FILHO, M. J. et al. Efeito de bioestimulantes no cafeeiro. **Nucleus**, 9(2):275-280, 2012.
- TAIZ, L. et al. **Fisiologia e desenvolvimento vegetal**. 6 ed. 59 Porto Alegre: Artmed, 2017: 584p.
- TATAGIBA, S. D. et al. Variabilidade diurna e sazonal das trocas gasosas e do potencial de água das folhas de clones de Eucalyptus. **Engenharia na Agricultura**, 16(2):225-237, 2008.
- UNIVERSIDADE FEDERAL DE VIÇOSA - UFV. **Sistema para análises estatísticas e genéticas – SAEG**. Versão 9.1. Viçosa/MG: Fundação Arthur Bernardes, 2007.
- WADAEY, Y. A. et al. Isolation and characterization of plant growth-promoting rhizobacteria from coffee plantation soils and its influence on maize growth. **Applied and Environmental Soil Science**, Article ID 5115875, 2022.
- WU, H. T. et al. Effects of different concentrations of auxin on onion root tip growth. **Agri Tech Equipment**, 335:12-14, 2017.
- YAO, H. et al. Effects of mutual grafting on the cadmium accumulation characteristics of two ecotypes of *Solanum photeinocarpum*. **International Journal Phytoreme**, 21(5):503-508, 2019.
- YE, Q. H. et al. Effect of root soaked with auxins on the growth and chlorophyll fluorescence characteristics of Chinese olive seedlings. **Acta Botan Boreali Occidentalia**, 39:831-839, 2019.
- ZABINI, V. A. et al. Chemical analyses of flowers and leaves for nutritional diagnoses of coffee trees. **Ciência Rural**, 51(7):e2019079, 2021.
- ZHAO, S. H. et al. Effect of indoleacetic acid on photosynthesis of arsenic-stressed plants with different arsenic-accumulating ability. **Journal of Agro-Environment Science**, 36(4):1093-1101, 2017.
- ZOU, F. K. et al. Auxin which regulates plant growth and development. **Chinese Agricultural Science Bulletin**, 34: 34-40, 2018.