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Effects of Immersion Time and Indole-3-Butyric Acid Dosage on the Quality of Black Pepper (*Piper nigrum* L.) Seedlings

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Abstract

Vegetative propagation via cuttings is the primary technique used in commercial black pepper plantations; however, a moderate capacity for adventitious rooting limits the production of high-quality seedlings. Consequently, the application of exogenous auxins has emerged as a promising strategy to overcome this issue, yet gaps remain regarding the optimal treatment method for cuttings. It is hypothesized that the genotype interacts with immersion time (exposure duration) and auxin dosage to enhance seedling quality. Despite this, these factors have not been evaluated in combination, leading to the improper use of auxins and suboptimal results, hindering the adoption of this technology by nursery operators. Therefore, this study aimed to evaluate the effects of indole-3-butyric acid (IBA) dosages and exposure times on 'Bragantina' black pepper seedlings. To this end, an experiment was conducted using a randomized block design in a factorial arrangement; treatments consisted of three immersion times (0—immediate contact, 3 h, and 6 h) and six IBA dosages (0, 750, 1500, 3000, 4000, and 5000 mg L^{-1}). At 120 days after planting, the seedlings were evaluated for growth, development, biomass, and quality characteristics. Strong modulation of shoot and root system development was observed in response to dosage and exposure time. Stem diameter and root length were directly proportional to IBA dosage, regardless of contact time. Exposure to IBA for 3 and 6 h resulted in increasing inhibition of leaf and shoot development as dosage increased, whereas immediate contact did not affect the aerial parts of the plant. Thus, the treatment involving immediate contact of the cuttings with a 3000 mg L^{-1} of IBA solution is more suitable, as it results in superior root development and seedling quality for the 'Bragantina' black pepper cultivar.

Keywords: auxins; black pepper; vegetative propagation; rhizogenesis

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1. Introduction

Black pepper (*Piper nigrum* L.) is a plant native to India and its fruits produce an important condiment with a spicy flavor, being one of the most used spices around the world [1,2]. Brazil stands out as the second largest producer of black pepper in the world, with 128,331 tons in 41,310 hectares of harvested area in the year 2022 [3]. Nationally, the State of Espírito Santo appears in first place in the ranking of main producers with approximately 76,533 tons in the year 2022, corresponding to almost 60% of Brazilian production [4].

The production of seedlings is one of the main steps related to the success of the crop. High-quality seedlings can improve transplant survival, promote vigorous seedling development, and bring uniformity and productivity [5]. For commercial planting, black pepper propagation is done asexually, with cuttings being the main propagation method in the main producing countries [6–8].

The initial development of seedlings is promoted by rhizogenesis, mediated by phytohormones, such as auxins [9]. Auxins are low-molecular-weight plant hormones responsible for plant growth and development and root initiation, with synthetic forms also available [10]. The main auxin produced by plants is indole acetic acid [11], but in inducing adventitious rhizogenesis of several plant species, indole-3-butyric acid (IBA) is the most used due to its availability and stability to light [12,13].

There is still a belief among nurserymen that the longer the cuttings are exposed to the auxin solution, the lower the dosage required to produce quality seedlings. Studies conducted for the 'Panniyur-1' genotype, or Bragantina as it is known in Brazil, indicate dosages of 500 mg L^{-1} for 12 h of IBA [14] and 1000 mg L^{-1} of IBA for 1 min [7]. For the Kottanadan genotype, the most recommended dosages were 1420.10 mg L^{-1} of IBA for 3 h [2] and 3000 to 4500 mg L^{-1} of IBA for 10 min [15]. In fact, when analyzing the previously mentioned works, the prolonged exposure time resulted in lower IBA dosages used for 'Bragantina' and 'Kottanadan'. However, different immersion times were not tested under the same study conditions. Furthermore, for these authors, although auxin is proven to be beneficial to the development of the black pepper plant, especially the root system, high doses can provide strong inhibition in the development of the aerial part, which may result in losses for commercialization. This can also happen when the auxin is exposed to cuttings for a long time, negatively affecting the quality of the seedlings.

Therefore, nurseries lack technical guidance, especially regarding the preparation of the product and the way in which auxins are applied. Furthermore, studies that evaluate the interaction between auxin dosage and cutting immersion time are scarce. Therefore, the objective of this study was to evaluate the effect of different doses of IBA and exposure times in inducing the growth, development and quality of 'Bragantina' black pepper seedlings.

2. Materials and Methods

The study was conducted at the Experimental Farm of the Capixaba Institute for Research, Technical Assistance and Rural Extension, located in the municipality of Linhares-ES, at geographic coordinates 19° 23′48′′ south latitude and 40° 03′42′′ west longitude, with an average altitude of 30 m and hot humid tropical climate conditions with rainy summer and dry winter, Aw, according to the Koppen classification [16]. The maximum and minimum temperatures and relative air humidity (Figure 1) every hour during the experimental period were obtained from the National Institute of Meteorology (INMET) [17] database, whose monitoring was carried out by an automatic meteorological station located in the municipality of Linhares-ES.

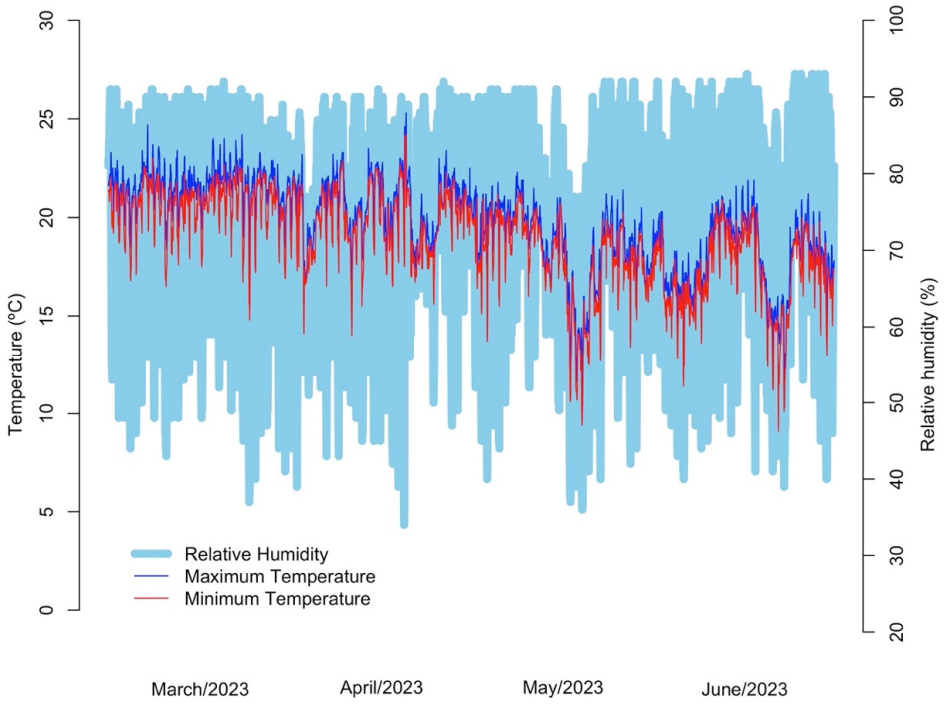


Figure 1. Maximum and minimum temperatures (°C) and relative air humidity (%) during the experimental period obtained with an automatic meteorological station located in the municipality of Linhares, Espírito Santo.

For the production of seedlings, 'Bragantina' black pepper cuttings were used, acquired from a clonal garden in an experimental nursery in the municipality of São Mateus-ES, which were collected from the median region of orthotropic branches, removed from the basal portion of mother plants. The cuttings were standardized, containing 2 nodes, and underwent phytosanitary processes, subjected to treatment with immersion in Carbomax® (Carbomax, Ribeirão Preto, Brazil) fungicide for approximately 10 min. Subsequently, each cutting had its base immersed in IBA for 0 h—immediate contact, 3 h or 6 h at concentrations of 0, 750, 1500, 3000, 4000 and 5000 mg L^{-1}. The solution was prepared with IBA ≥ 99% Sigma-Aldrich (Merck KGaA, Cotia, Brazil) according to Akshay et al. [7]. For the immersion treatment, a brief 5 s contact between the base of the cuttings and the IBA solution was performed at the 0 h mark. For the 3 and 6 h durations, the bases of the cuttings were kept in the IBA solution for the respective time periods.

The seedlings were arranged in an experimental design in randomized blocks in a factorial scheme (3 × 6). The first factor consisted of three different times of immersion of the seedlings in IBA (0 h, 3 h and 6 h of immersion) and the second factor consisted of 6 concentrations of IBA (0, 750, 1500, 3000, 4000 and 5000 mg L^{-1}). Thus, 4 replications (blocks) were evaluated, with 27 seedlings per treatment, totaling 1944 seedlings in the experimental field. Furthermore, the seedlings were kept in a nursery covered with a black shade cloth with 50% shading and irrigated by micro sprinklers with a flow rate of 70 $L.h^{-1}$ activated by timers every 2 min for a period of 30 s.

At the end of the experiment, 120 days after planting, the seedlings were evaluated for the growth and development characteristics of the aerial part: stem length (SL), obtained by measuring the stem to the apical bud using a graduated ruler, with the results expressed in cm; stem diameter (SD), determined in the collar region using a precision digital caliper and expressed in mm; number of leaves (NL), obtained by counting the total number of leaves of each seedling; leaf area (LA), obtained in cm^2, with the aid of the bench meter Type Scaner Area Meter LI-3100C (LI-COR, Lincoln, NE, USA); unit leaf area (ULA), measured

by the division between the leaf area and the number of leaves, with results expressed in cm^2; shoot length (SHL), measured by measuring the shoot using a graduated ruler, in cm; and shoot diameter (SHD), measured with the aid of a digital caliper, in mm.

The characteristics of the root system obtained were: root system length (RSL), measured with a graduated ruler, considering the greatest length of the main root, with the results expressed in cm; root volume (RV), obtained from the displacement of water in a graduated cylinder, by immersing the roots in 90 mL of water in a cylinder and measuring the displaced volume (due to the difference in volume, the direct answer is obtained by equivalence of units, considering 1 mL = 1 cm^3); root mass fraction (RMF), obtained according to Poorter et al. [18], based on dividing the dry mass of the root by the total dry mass, with results expressed in $g.g^{-1}$; and root fineness (RF), obtained by calculating the length of the root system divided by the root volume, expressed in cm^2.

The phytomass characteristics were also evaluated: shoot dry mass (SDM); root system dry mass (RSDM); and total dry mass (TDM). The SDM was obtained by cutting the stem in the seedling collection region, close to the substrate. To obtain the RSDM, the roots were washed with running water. For both characteristics, parts of the seedlings were placed in paper bags and dried in a forced air circulation oven at a temperature of 65 °C and subsequently weighed on a precision analytical balance, with the results expressed in g. The TDM was obtained by adding the SDM and the RSDM.

The quality of the seedlings was defined by the Dickson Quality Index (DQI), according to Dickson et al. [19] given by the following expression:

$$\mathrm{DQI} = \frac{SDM + RSDM}{\frac{SL}{SD} + \frac{SDM}{RSDM}}$$

For the statistical analyses, the means of the 27 seedlings from each treatment within each block were used, resulting in 4 means per treatment. The data were subjected to analysis of variance using the F test at 5% probability. The means of the characteristics in relation to the immersion time were subjected to the Scott–Knott mean grouping test ($p < 0.05$). The effects of IBA dosages on the evaluated characteristics were tested by regression analysis using the F test at 5% probability. When significant, models that best explained each effect were adjusted. The maximum points were obtained through the primary derivative of the regression equations. All statistical analysis and graphical representation were carried out with the help of R software [20], with the help of the ExpDes.pt [21] and FMSB [22] data packages.

3. Results

The different immersion times and IBA concentrations resulted in significant differences in the growth and development of 'Bragantina' black pepper plants (Tables A1 and A2, and Figure A1). The immersion time of 0 h proved to be statistically superior for the characteristics leaf area, number of leaves, and shoot diameter for the dosages of 1500, 3000, 4000 and 5000 mg L^{-1} of IBA applied (Table 1). For unit leaf area, time 0 h was significantly superior in dosages of 4000 and 5000 mg L^{-1} of IBA, yet there were no statistical differences between times 0, 3 and 6 h for dosages of 0, 750, and 1500 mg L^{-1} of IBA. For shoot length, the time of 0 h presented statistically superior means for the dosages of 750, 1500, 3000, 4000 and 5000 mg L^{-1} of IBA; however, for the dosage of 0 mg L^{-1} of IBA, the immersion time of 6 h was statistically superior (Table 1). When analyzing the regression curves, it is noted that for leaf area, unit leaf area, number of leaves, shoot length and shoot diameter, black pepper seedlings showed the same behavior, where there were no significant differences between the dosages at 0 h and decreasing linear adjustment as the IBA dosage was increased at immersion times of 3 and 6 h (Figure 2).

Table 1. Average values of leaf area, unit leaf area, number of leaves, shoot length and shoot diameter of 'Bragantina' black pepper seedlings in three different immersion times (0, 3 and 6 h) and six dosages of indole-3-butyric acid (IBA) (0, 750, 1500, 3000, 4000 and 5000 mg L^{-1}).

Times/Dosages	0	750	1500	3000	4000	5000
	Leaf Area (cm^2)					
0	66.39 a	91.70 a	88.01 a	87.76 a	80.02 a	87.92 a
3	63.86 a	82.90 a	60.91 b	34.06 b	10.36 b	2.38 b
6	75.12 a	57.49 b	18.11 c	8.72 b	1.34 b	0.00 b
	Unit Leaf Area (cm^2)					
0	30.25 a	34.93 a	29.18 a	34.88 a	33.27 a	39.04 a
3	28.57 a	37.39 a	33.67 a	22.85 a	12.78 b	9.47 b
6	29.47 a	27.93 a	36.95 a	8.03 b	6.06 b	0.00 b
	Number of Leaves					
0	2.15 a	2.62 a	3.11 a	2.49 a	2.46 a	2.25 a
3	2.36 a	2.27 a	1.82 b	1.65 b	0.41 b	0.17 b
6	2.61 a	2.03 a	0.55 c	0.33 c	0.05 b	0.00 b
	Shoot Length (cm)					
0	5.61 b	8.33 a	7.72 a	7.57 a	7.04 a	6.45 a
3	5.52 b	6.06 b	4.89 b	4.11 b	1.71 b	1.14 b
6	8.19 a	5.46 b	1.45 c	1.43 c	0.56 c	0.00 b
	Shoot Diameter (mm)					
0	3.24 a	3.54 a	3.75 a	3.66 a	3.34 a	2.58 a
3	3.14 a	3.45 a	2.61 b	2.06 b	1.06 b	0.96 b
6	3.56 a	3.00 a	0.95 c	0.94 c	0.45 b	0.00 c

Means followed by the same letter in the columns do not significantly differ from each other using the Scott–Knott cluster test at 5% probability.

In Table 2, it is possible to observe the comparison of the averages of the analyzed characteristics in relation to the immersion time of the black pepper cuttings in the IBA. It is verified that for the characteristics root system length (RSL), root volume (RV) and root mass fraction (RMF), they presented higher averages in the immersion times of 3 and 6 h. For root fineness (RF), shoot dry mass (SDM) and total dry mass (TDM), the highest averages were found at 0 h immersion time. For the other characteristics, no statistical differences were identified between the pile immersion times in the IBA.

Table 2. Average values for stem length (SL), stem diameter (SD), root system length (RSL), root volume (RV), root mass fraction (RMF) and root fineness (RF), shoot dry mass (SDM), root system dry mass (RSDM), total dry mass (TDM) and Dickson Quality Index (DQI) of 'Bragantina' black pepper seedlings submitted to indole-3-butyric acid (IBA) and three immersion times (0, 3 and 6 h).

Times	SL	SD	RSL	RV	RMF	RF	SDM	RSDM	TDM	DQI
0	7.31 a	6.97 a	10.97 b	1.73 b	0.14 b	7.2 a	1.71 a	0.30 a	2.01 a	0.3 a
3	7.22 a	7.1 a	13.69 a	2.73 a	0.22 a	5.79 b	1.41 b	0.41 a	1.82 b	0.4 a
6	7.46 a	6.99 a	12.70 a	2.45 a	0.21 a	5.24 b	1.34 b	0.37 a	1.71 b	0.37 a

Means followed by the same letter in the columns do not significantly differ from each other using the Scott–Knott cluster test at 5% probability.

For stem length, an average of 7.33 cm was observed, statistically equal for all IBA dosages applied (Figure 3A). The stem diameter had an increasing linear effect in relation to the increase in IBA doses (Figure 3B). The length of the root showed an increasing linear behavior as the IBA dosage increased (Figure 3C). The root volume had a quadratic behavior and a maximum point of 2.98 cm^3 at a dosage of 3658.33 mg L^{-1} of IBA, with R^2 of 0.9621 (Figure 3D). For the root mass fraction, a quadratic effect was observed with a maximum

point of 0.23 g.g^{-1} at a dosage of 3313.84 mg L^{-1} of IBA and R^2 of 0.9666 (Figure 3E). Root fineness presented an average of 6.07 cm^2, the same for all doses of IBA applied (Figure 3F).

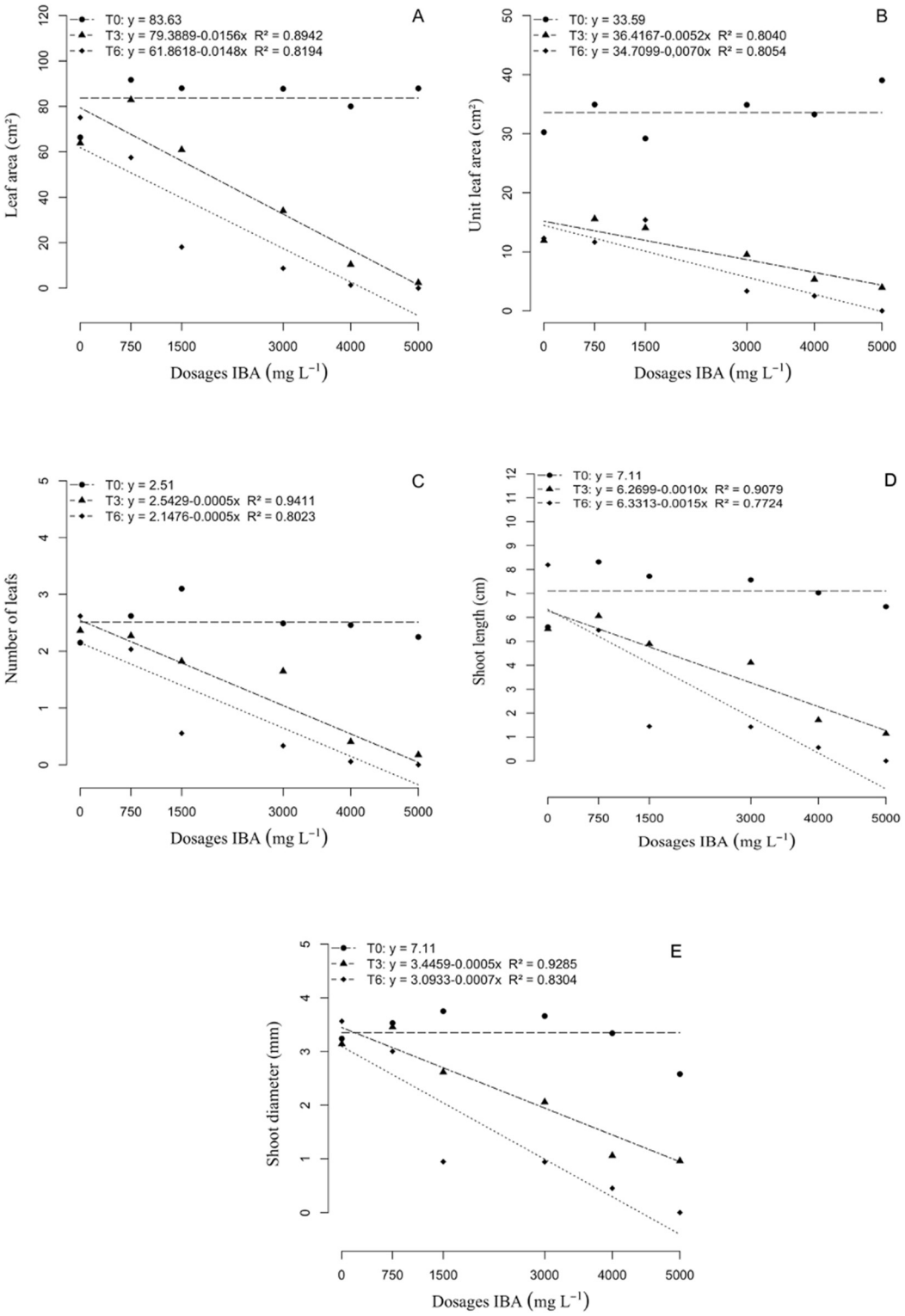


Figure 2. Effect of different dosages of indole-3-butyric acid (IBA) and immersion time (0, 3 and 6 h) on leaf area (**A**), unit leaf area (**B**), number of leaves (**C**), shoot length (**D**) and shoot diameter (**E**) of 'Bragantina' black pepper seedlings.

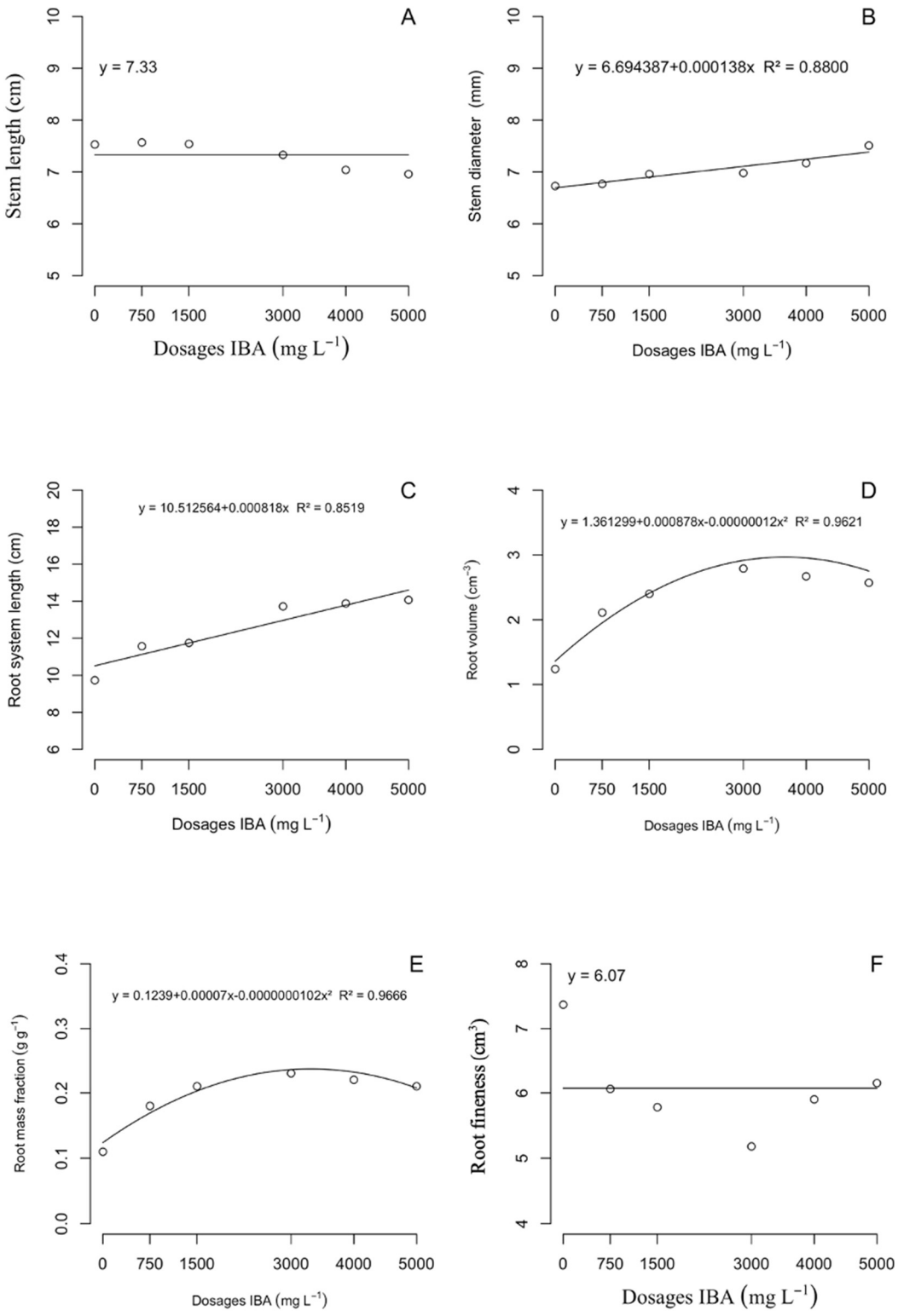


Figure 3. Effect of different dosages of indole-3-butyric acid (IBA) on stem length (**A**), stem diameter (**B**), root system length (**C**), root volume (**D**), root mass fraction (**E**) and root fineness (**F**) of 'Bragantina' black pepper seedlings.

The adjusted equations for the characteristics related to the accumulation of phytomass and quality of black pepper seedlings can be seen in Figure 4. The dry mass of the aerial part presented an average of 1.49 g in all IBA dosages applied; hence, there is no relation between dosages applied and dry mass of the aerial part. The dry mass of the root system showed a

quadratic adjustment with a maximum point of 0.44 g at the IBA dose of 2991.07 mg L^{-1} and R^2 of 0.9286. The total dry mass presented an average of 1.84 g in all IBA doses to which the cuttings were subjected. The Dickson Quality Index showed quadratic behavior, with a maximum point of 0.44 at a dose of 3077.12 mg L^{-1} of IBA and R^2 of 0.9371.

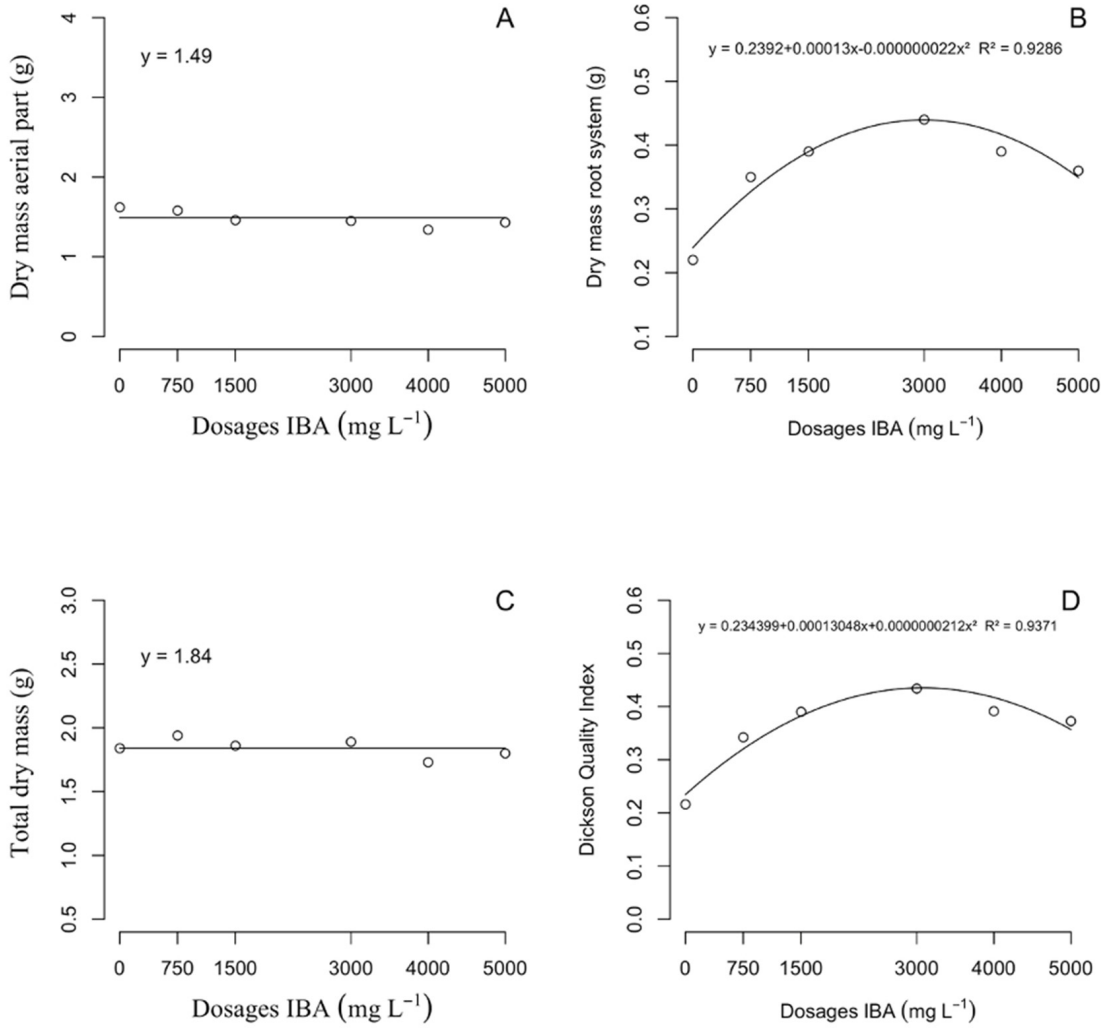


Figure 4. Effect of different dosages of indole-3-butyric acid (IBA) on the dry mass of the aerial part (**A**), dry mass of the root system (**B**), total dry mass (**C**) and Dickson Quality Index (**D**) of 'Bragantina' black pepper seedlings.

4. Discussion

The growth of the aerial part and leaves of black pepper was negatively affected as the cuttings were exposed to increased dosage and prolonged exposure (3 h and 6 h) to IBA. However, the different dosages of IBA applied to the cuttings did not affect the leaf area, unit leaf area and number of leaves when they were treated at 0 h immersion time (immediate contact). This fact may be related to the hormonal imbalance generated by high doses of IBA and prolonged exposure time. This is because the relationship between cytokinin and auxin plays a role in plant growth, and these two hormones have antagonistic and synergistic interactions [23]. Auxin has a regulatory function of apical dominance and the amounts of cytokinin in the stem of plants. This occurs because the expression of oxidase/dehydrogenase is induced, suppressing the expression of ATP/ADP, which are used by the enzyme isopentenyl transferase (IPT), fundamental for the biosynthesis of cytokinin [24]. Thus, high amounts of auxin, which is found in treatments with the highest

dosages of IBA, may suggest a reduction in cytokinin levels in the plant, impairing the growth of the aerial part.

Furthermore, auxin plays a crucial role in the expression of genes linked to strigolactone biosynthesis [25]. Strigolactones are plant hormones responsible for modulating the structure of plants, being linked to the plant's secondary growth, such as shoots and lateral branches [26]. Thus, despite the governance of auxin apical dominance through the inhibition of cytokinin, this does not occur in lateral shoots, as strigolactones act in regulating the transcription of the TCP BRANCHED1 (BRC1) gene responsible for suppressing the elongation of lateral shoots [27,28]. In line with this, the output of auxin from lateral shoots contributes to their development [26]. However, in our studies, the growth and development of the shoots was harmed by the longer exposure times and higher dosages of IBA to which the cuttings were subjected. This fact may be related to excessive amounts of auxin preventing both the main growth and the lateral shoots. This was not observed in the treatment with lower exposure of cuttings to IBA.

Regardless of not demonstrating significant statistical differences for dry mass of the root system, prolonged exposure of cuttings to IBA (3 and 6 h) had a significantly greater effect on root length and root volume than immediate contact (0 h). Furthermore, the use of IBA in black pepper cuttings provided significant gains in the root system with constant growth in length in relation to the applied dosage. However, at large doses there was a decrease in root volume, root mass fraction and dry mass of the root system.

In fact, auxin is the main plant hormone responsible for initial root development [11]. For the rhizogenesis process, in many species, large amounts of auxin are required, which are accumulated in specific cells [29]. In cuttings, in some plant species, endogenous auxin accumulates in the basal part, inducing the formation of adventitious roots [30]. However, in other species, such as black pepper, the amount of auxin produced by the plants is not sufficient for the satisfactory formation of roots, requiring the application of phytoregulators exogenously. Furthermore, the rooting response with the use of exogenous auxin can be variable within the same species, as is the case with black pepper [2,7,14,15].

It is believed that auxin levels in plant species are linked to proteins of the GH3 (Gretchen Hagen 3) family, which modulate auxin homeostasis. High auxin levels trigger increased GH3 expression, resulting in auxin degradation or storage [31]. Thus, the difference between genotypes with high versus low rooting ease is tied to GH3 protein formation and concentration [12]. However, it is worth noting that auxin is not the sole factor responsible for the high root counts observed in plants; factors such as environmental conditions, nutrient availability, substrate, and the application of other phytohormones play a fundamental role in rhizogenesis [11].

Although increasing IBA dosages negatively influenced the leaf count and leaf area of 'Bragantina' black pepper seedlings, this condition did not adversely affect stem growth and development, nor the shoot and total biomass accumulation capacity of the seedlings. It is worth highlighting that, according to Melo et al. [32], a larger leaf area indicates better functioning of the plant's photosynthetic apparatus—consequently reflecting greater stem length and diameter growth as well as biomass accumulation—a pattern not observed in our study. This indicates that, despite the inhibition of leaf development, the seedlings maintained their capacity to produce photoassimilates, without interfering with overall seedling growth and development.

It is noted that the excessive amount of IBA harmed the quality gain of the seedlings, inferred by the Dickson Quality Index, where the 'Bragantina' black pepper seedlings showed higher quality at the dosage of 3077.12 mg L^{-1} of IBA. However, no statistical differences were identified for the Dickson Quality Index in relation to the pile immersion time. It should be noted that the higher the Dickson Quality Index, the greater the robustness

of the seedlings and the better their ability to adapt to field conditions, which favors their development and increases their chances of survival [33].

In the literature, studies show that the ideal dosage and time of exposure of cuttings to auxin phytoregulators are variable factors depending on the black pepper genotype, although these factors have not previously been evaluated together. Ambrozim et al. [14], evaluating the 'Bragantina' genotype, found that a dosage of 500 mg L^{-1} of IBA, with the cuttings immersed for 12 h in IBA, provided better quality of the seedlings. Oliveira et al. [2] found that immersion for 3 h in 1420.10 mg L^{-1} of IBA provided an improvement in the characteristics of seedlings of the 'Kottanadan' genotype. Akshay et al. [7] recommend immersing 'Panniyur-1' cuttings for 1 min in IBA at 1000 mg L^{-1}. For Coradini et al. [15] immersion for 10 min in the combination of 3000 to 4500 mg L^{-1} of IBA with 300 µM sodium nitroprusside was ideal for the 'Kottanadan' genotype. In all these studies, gains in the induction of rhizogenesis and seedling quality with the use of IBA were evident. However, according to the authors, it was found that large dosages applied provided strong inhibition of the development of the aerial part of the seedlings, which could result in losses for commercialization.

Under the conditions of our studies, the dosage of 2991.07 mg L^{-1} of IBA was the one that presented the highest dry mass of the root system, this dosage being very close to the dosages of 3077.12 mg L^{-1} of IBA, where the seedlings presented the highest Dickson Quality Index, a characteristic used to define which seedlings have the highest and best quality. Furthermore, despite the gains obtained in the root system in black pepper seedlings under prolonged exposure to IBA (3 and 6 h), the characteristics of the aerial part were negatively impacted. Also, the quality of the seedlings was not affected by the time of exposure of the cuttings to IBA. Thus, in summary, as it provides better development, growth and greater quality of seedlings of the 'Bragantina' genotype, and ease of application in the nursery, we recommend a dosage of 3000 mg L^{-1} of IBA applied to cuttings immersed for 0 h (immediate contact). Regarding duration, we recommend applying IBA to the cuttings for 0 h (immediate contact); although this does not yield significant gains in seedling quality compared to 3 or 6 h treatments, it is recommended primarily because it is simpler and requires less time for seedling production, while also avoiding the negative effects of prolonged exposure on shoot and biomass development.

5. Conclusions

- Immersion times of 3 and 6 h provided greater root development, as demonstrated by the highest values of root system length, root volume and root mass fraction, but were detrimental to the development of leaf area, number of leaves, and shoot diameter for dosages of 1500, 3000, 4000 and 5000 mg L^{-1} indole-3-butyric acid;
- The application of IBA with a time of immersion of 0 h (immediate contact) allows gains in root fineness, dry mass of the area and total dry mass;
- The length of time the cuttings were immersed in the IBA did not affect the quality of the seedlings;
- A dosage of approximately 3000 mg L^{-1} of indole-3-butyric acid (IBA) provides greater dry mass of the root system and quality of 'Bragantina' black pepper seedlings.

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Abbreviations

The following abbreviations are used in this manuscript:

SL	Stem length
SD	Stem diameter
NL	Number of leaves
LA	Leaf area
ULA	Unit leaf area
SHL	Shoot length
SHD	Shoot diameter
RSL	Root system length
RV	Root volume
RMF	Root mass fraction
RF	Root fineness
SDM	Shoot dry mass
RSDM	Root system dry mass
TDM	Total dry mass
DQI	Dickson Quality Index
IBA	Indole-3-butyric acid

Appendix A

Table A1. Summary of the analysis of variance in factorial scheme with the source of variation (SV), degrees of freedom (DF) and sum of squares for the leaf area, unit leaf area, number of leaves, shoot length and shoot diameter of 'Bragantina' black pepper seedlings, in three different immersion times (0, 3 and 6h) and six dosages of indole-3-butyric acid (IBA) (0, 750, 1500, 3000, 4000 and 5000 mg L^{-1}).

SV	Sum of Squares					
	DF	Leaf Area	Unit Leaf Area	Number of Leaves	Shoot Length	Shoot Diameter
Block	3	237 ns	637.3 *	1.023 ns	3.41 ns	0.392 ns
Time	2	41,390 ***	2937.1 ***	31.215 ***	237.57 ***	42.594 ***
Dose	5	23,178 ***	3650.2 ***	26.195 ***	168.41 ***	45.865 ***
Time × Dose	10	19,289 ***	3754.4 ***	18.462 ***	144.66 ***	21.571 ***
Residue	51	17,017	3842.5	14.529	140.29	20.253
CV (%)		35.85	34.36	32.73	35.86	26.8

Coefficient of variation (CV). Significance levels: *** $p < 0.001$, * $p < 0.05$, ns = not significant.

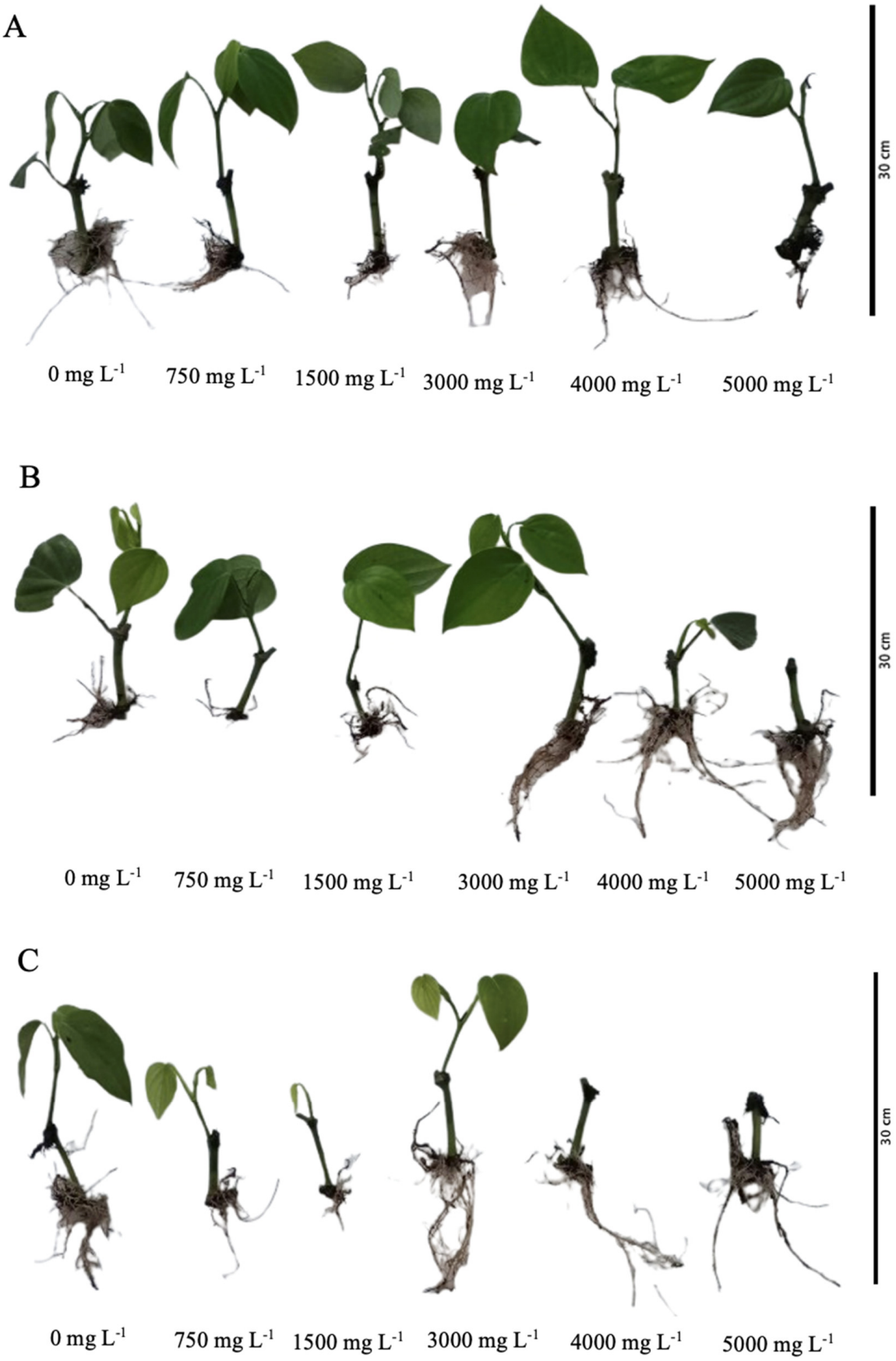


Figure A1. 'Bragantina' black pepper seedlings subjected to immersion times of 0 h (**A**), 3 h (**B**), and 6 h (**C**) at IBA concentrations of 0, 750, 1500, 3000, 4000, and 5000 mg L^{-1}.

Table A2. Summary of the analysis of variance in factorial scheme with the source of variation (SV), degrees of freedom (DF) and sum of squares for the stem length (SL), stem diameter (SD), root system length (RSL), root volume (RV), root mass fraction (RMF), root fineness (RF), shoot dry mass (SDM), root system dry mass (RSDM), total dry mass (TDM) and Dickson Quality Index (DQI) of 'Bragantina' black pepper seedlings, in three different immersion times (0, 3 and 6 h) and six dosages of indole-3-butyric acid (IBA) (0, 750, 1500, 3000, 4000 and 5000 mg L^{-1}).

SV	Sum of Squares										
	DF	SL	SD	RSL	RV	RMF	RF	SDM	RSDM	TDM	DQI
Block	3	9.904 **	1.2945 ns	152.19 ***	0.127 ns	0.00357 ns	41.70 ns	0.1926 ns	0.03812 ns	0.3793 ns	0.02583 ns
Time	2	0.720 ns	0.2508 ns	90.74 **	12.843 ***	0.09146 ***	49.24 *	1.8627 ***	0.14280 ns	1.1045 *	0.13745 ns
Dose	5	4.353 ns	4.9340 ns	178.91 **	19.611 ***	0.11171 ***	31.12 ns	0.6454 ns	0.34327 *	0.3066 ns	0.34217 *
Time × Dose	10	5.879 ns	3.7239 ns	84.35 ns	9.708 ns	0.05126 ns	63.97 ns	1.3447 ns	0.16669 ns	1.3740 ns	0.16994 ns
Residue	51	27.793	18.3204 ns	334.03	32.436	0.16954	254.82	5.0748	1.19452	8.6480	1.16923
CV (%)		10.06	8.54	20.55	34.65	29.6	36.78	21.21	42.4	22.28	42.33

Coefficient of variation (CV). Significance levels: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns = not significant.

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