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Growth Regulator Indole-3-Butyric Acid on Rooting Potential of *Actinidia deliciosa* Rootstock and *Actinidia arguta* Female Scion Species Stem Cuttings

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Abstract: A study was conducted to access the effectiveness of exogenous application of indole-3-butyric acid (IBA) on adventitious root formation in kiwifruit semi-hardwood stem cuttings (SCs) from *Actinidia deliciosa* rootstock and *Actinidia arguta* female scion. Treatment comprised IBA concentrations of 0, 10, 100, 1000, 10,000 and 100,000 ppm. Parallel experiments for *A. deliciosa* and *A. arguta*'s treatment were arranged in a randomised complete block design, with 12 replications. In *A. deliciosa*, treatments had significant ($p \leq 0.05$) effects in rooting percentage, number of roots, root length, size of callus formation and callus percentage, except for dry root mass. In *A. arguta*, treatments showed significant ($p \leq 0.05$) effects in rooting percentage, number of roots, root length and dry root mass. No callus formation was observed in *A. arguta*. Relative to the control, in *A. deliciosa*, the highest (42%) rooting percentage and lengthy (0.301 cm) roots, were observed at 10,000 ppm IBA concentration, whereas the most (0.295) number of roots were produced at 100,000 ppm IBA concentration. Calli percentage (94%) was highest at 100 ppm IBA, while the size of callus formation was the biggest (2.8) at IBA concentration of 100,000 ppm, when compared to the control. In *A. arguta*, the highest (100%) rooting percentage was achieved at the control (0 ppm), 100 ppm and 10,000 ppm IBA concentrations, whereas the greatest (0.9815) number of roots were observed at the IBA concentration of 10 ppm. Lengthy (1.0839 cm) roots were achieved at IBA concentration of 100 ppm, whereas the greatest (0.1061 g) dry root mass was attained at IBA concentration of 10,000 ppm. In conclusion, the use of growth regulator IBA was effective for root formation in SCs of *A. deliciosa* rootstock. In *A. arguta* female scion, IBA application improved the quality of rooting (more and longer roots). IBA application showed its potential in stimulating root development at 10,000 ppm IBA.

Keywords: asexual propagation; adventitious root; exogenous IBA; plant growth regulator; root initiation; root formation; propagation; stimulatory effects

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1. Introduction

The genus *Actinidia*, which is native to China and is commonly known as the kiwifruit plant, is a valuable fruit crop throughout the world [1]. It is one of the most commercially available fruits on the international market, with more than 4,348,011 metric tonnes of global kiwifruit production from an area of 268,788 ha, of which China is the leading producer [2]. Kiwifruit is a dioecious fruit crop grown and consumed due to its exceptionally high levels of vitamins (C, E and folate) and other nutrients, such as dietary fibre and potassium, all of

which are nutritionally relevant. It also contains a variety of bioactive components, such as a wide range of antioxidants, phytonutrients, and enzymes, all of which act to support healthy function and metabolism [3].

Several vegetative propagation techniques are used for the commercial cultivation of the kiwifruit plants including grafting, layering and in vitro 'clonal' propagation [4]. Generally, propagating kiwifruit plants involves employing the scion variety onto a seedling rootstock [5]. However, grafting is labour-intensive, and can lead to compatibility issues between rootstock and the scion [6]. On the other hand, in vitro propagation can produce multiple plants in short tenure [4,7], but the technique is costly, requiring skilled labour and specialized laboratory facilities [8]. Kiwifruit is generally vegetatively propagated through the use of hardwood cuttings in order to retain the same morpho-physiological and genetic characteristics of the donor plants, ensuring uniformity [9]. This method of propagation is the most convenient and cost-effective technique of clonal regeneration for most horticultural crops such as fruits, nuts and ornamentals [10–12]. Vegetative propagation can be beneficial in the commercialization of kiwifruit in meeting the significantly increasing demand for planting material. However, despite significant advancements in vegetative propagation by stem cuttings (SCs), insufficient rooting efficiency remains an economic hiccup for the horticultural industry. The SCs of kiwifruit were said to be recalcitrant to rooting, especially when propagated by hardwood SCs [13]. The success of vegetative propagation relies on the protrusion of adventitious rooting, which is influenced by several factors, such as cutting type and levels of hormonal treatments, also known as plant growth regulators (PGRs) [13]. Kiwifruit propagation by SCs with the application of PGRs is one of the most common practices [14,15]. Auxin is one of the most important PGRs, and plays a central role in stimulating the formation and development of adventitious roots [14,16]. The adventitious root process and the physiological stages of rooting are correlated with changes in endogenous auxin concentration, which is known to trigger adventitious root formation. Indole-3-acetic acid (IAA) is the most common natural form of auxin. A high rooting rate is usually associated with a high endogenous auxin concentration at the beginning of the rooting process [15]. The use of exogenous indole-3-butyric acid (IBA) is a common plant regulator in the initiation and proliferation of adventitious roots [17]. Based on their effectiveness in stimulating adventitious roots, IBA has been utilized to stimulate rooting formation in many difficult-to-root plant species, including *Actinidia* SCs [14,15].

Stem cuttings for use in vegetative propagation can be characterized into hardwood, semi-hardwood and softwood types [18]. The choice of SC is selected according to the appearance of the plant, although SCs from healthy and vigorous plants are mostly preferred [9]. Hardwood cuttings, also known as dormant, leafless cuttings, are obtained from the mature stem of the previous growth season. These cuttings are prepared during late fall, winter or early spring, and are stored for prolonged duration, retaining high adventitious potential [19]. Most hardwood cuttings were documented to induce excessive callus development and to root poorly [20,21]. Hardwood cuttings of various length from 10 to 76 cm long have been successfully used for kiwifruit cuttings [22]. The hardwood SCs of *A. deliciosa* cultivars have been reported to produce the best rooting percentage and number of roots per cutting with the IBA application of 4000–8000 ppm [23,24]. Bhushan and Gupta [25] have reported that *A. deliciosa* hardwood cuttings of 15 and 20 cm length provided the best rooting performance when IBA was applied at 3500 ppm. ErcİşlİĭI et al. [22] attained best rooting performance with hardwood SCs collected in February as compared to January, at IBA application of 6000 ppm. Again, Ercisli et al. [26] reported the best (40 and 42%) rooting percentage in SCs of *A. deliciosa* 'Hayward' when treated with IBA concentrations of 4000 and 6000 ppm, respectively. On the other hand, Peticilă et al. [27] reported that *A. arguta* hardwood SCs of 15 and 20 cm length gave the best rooting performance

in terms of rooting percentage (41.8%) and number of roots (8.5) at the IBA application of 2000 ppm.

Semi-hardwood cuttings, on the other hand, are obtained from woody and evergreen plant species generally taken during the summer season from new shoots. The cuttings vary in length, from 7.5 to 20 cm long [9,28]. A study by Pratima and Rana [29] reported that semi-hardwood SCs of kiwifruit resulted in higher rooting performance in the cuttings taken in the month of July, as compared to the months of June and August at IBA application of 5000 ppm. Rana et al. [30] reported that cuttings prepared during the active growth stage (July–August) gave better results than those prepared during the dormancy stage (January). Similarly, for Choudhary et al. [12], among different cutting types tested, semi-hardwood SCs of different cultivars performed better as compared to hardwood SCs with respect to parameters such as number of primary roots, number of secondary roots, total root length and fresh and dry weight of roots per cutting at IBA application of 4000 ppm. Erturk et al. [28] observed that *A. deliciosa* semi-hardwood SCs have higher rooting performance than hardwood SCs treated with IBA of 4000 ppm. In a study on the effect of different levels of IBA on the rooting of semi-hardwood SCs of kiwifruit, the best results have been obtained with the IBA application of 10,000 ppm IBA [15]. The higher rooting potential of semi-hardwood SCs has been attributed to the endogenous auxin in the tender vegetative growth of semi-hardwood SCs [9]. On the other hand, softwood cuttings, ranging from 7.5 to 12.5 cm in length, are taken from mid-summer to early autumn, and exclude the very soft shoot tips which occur above the leaf axillary buds because they are often low in stored carbohydrates and commonly contain unwanted flower buds [9,13,27]. The objective of the present study was to evaluate the potential responses of semi-hardwood stem cuttings of *A. deliciosa* rootstock and *A. arguta* female scion plant treated at concentrations straddling low, medium and high levels of PGR.

2. Materials and Methods

2.1. Description of the Study Site

Experiments for *A. deliciosa* rootstock and *A. arguta* female scion semi-hardwood SCs were carried out in Politsi, Westfalia Nursery (23°65′36″ S, 30°21′67″ E), Tzaneen, Limpopo Province of South Africa, under greenhouse conditions from winter (May–July) to spring (August–October). The temperature at the nursery location varies between 25 °C (minimum night temperature) and 27 °C (maximum day temperature) during summer (November–January) under natural photoperiod (12/12 h, day/night). Irrigation in the greenhouse comprised a mist irrigation system, installed to supply water in the form of mist every 8 min for 10–15 s.

2.2. Preparation of Plant Material

Semi-hardwood SCs measuring 10–16 cm long, 0.1–0.5 cm diameter, with 3–4 nodal buds were excised at sunrise in spring (October) from 1-year-old canes developed from 14-year-old mature *A. deliciosa* rootstock and *A. arguta* female scion plants, using sterile secateurs. For the sterilization process, secateurs were surface sterilized for 5 min in 70% commercial bleach (JIK®) solution containing 2.45% active sodium hypochlorite (NaOCl) and rinsed a few times with distilled water (dH_2O), followed by 70% ethanol for 1 min prior to excising each SC, to maintain sterility and avoiding contamination [15]. The 14-year-old mature *A. deliciosa* rootstock and *A. arguta* female scion kiwifruit plants under commercial production are cultivated under field conditions at Nooyenskopje Farm situated at Magoebaskloof (23°53′13″ S, 29°56′13″ E), Tzaneen, in Limpopo Province of SA. The excised SCs were classified as *A. deliciosa* rootstock and *A. arguta* female scion, and each plant species were placed in 200 L plastic beakers filled with distilled water to avoid

dehydration, and transported to the University of Limpopo. Upon arrival, all the SCs were removed from the plastic beaker, wrapped with a moistened paper towels, and then sealed in zip-lock transparent plastic bags and stored in a refrigerator at 5 °C for a 10-day-cold stratification pre-treatment period, prior to planting.

2.3. Preparation of Plant Growth Regulators

Indole-3-butyric acid was acquired from Sigma-Aldrich (Merck Life Science Pty Ltd., Modderfontein, South Africa). The required stock solutions were prepared according to Atak and Yalçın [13]. To prepare a 1 mg/mL stock solution, 100 mg of IBA was added to a 100 mL volumetric flask. Thereafter, 3–5 mL of 70% ethanol was added to the volumetric flask to dissolve the PGR powder. Once completely dissolved, the solution was brought to volume with distilled water through continuous stirring to keep the material in solution. Thereafter, the stock solution was used as required.

2.4. Treatment of Stem Cuttings

The prepared SCs were disinfected at the base (5 cm) using 25% commercial bleach (JIK®) solution containing 0.875% active NaOCl with 2 drops of Tween 20 for 10 min and then rinsed 3 times with dH_2O to remove traces of the sterilant solution. Prior to PGR-SCs treatment, the apical region were slant-cut toward the last apical nodal bud, whereas the basal part of the SCs were cut horizontally just below the last bottom nodal bud, as described by Wilson et al. [9], to obtain SCs of 2–3 nodal buds. Prior to rooting hormone treatments, a 2 cm long incision was made at each SC's base to enhance the rooting process [30]. Afterwards, at the base of the SCs, a longitudinal lesion was made, with approximately 10% of the total length of the SCs. The basal 5 cm portion of all the tested *Actinidia* species SCs were immersed for 5 s in the prepared IBA concentrations (0, 10, 100, 1000, 10,000 and 100,000 ppm) and then planted 5 cm deep in 24-hole seedling trays, filled with a mixture of pine bark, vermiculite and perlite, at a 2:1:1 (v/v) ratio. Seedling trays with SCs were then irrigated to field capacity and later placed on a suspended mesh-wire to avoid interference of roots with the ground and minimize contamination. The SCs were maintained under natural photoperiods for 14 weeks. The glasshouse day and night ambient temperatures averaged 26 °C and 16 °C, respectively, with maximum temperatures controlled by opening the roof of the glasshouse. Relative humidity was maintained between 70 and 80%. Two weeks after SCs were established, each tray was fertilised with Multifeed P Efekto ® fertiliser (Nulandies, Johannesburg, South Africa), to provide a total of 0.70 mg Nitrogen (N), 0.64 mg Potassium (K), 0.64 mg Phosphorus (P), 1.21 mg Magnesium (Mg), 1.5 mg Iron (Fe), 0.15 mg Copper (Cu), 0.7 mg Zinc (Zn), 2 mg Boron (B), 6 mg Manganese (Mn) and 0.14 mg Molybdenum (Mo) per ml water.

2.5. Treatments and Research Design

The treatments in each trial comprised IBA concentration at 0, 10, 100, 1000, 10,000 and 100,000 ppm, with zero (0 ppm) constituting the untreated SCs (control) [31]. In both the *A. deliciosa* rootstock and *A. arguta* female scion SCs, treatments were arranged in a randomized complete block design (RCBD), with 12 replications (n = 72). Each replication contained 4 SCs of each test plant species. Blocking was carried out for shading by the greenhouse walls in the morning.

2.6. Data Collection

A 103 days, after the treatment application, the plant variables were determined. The established SCs were gently uprooted from the seedling trays without damaging the roots, then rinsed under running tap water to remove excess media particles and blotted dry with a paper towel. The number of roots was manually counted, and the root length (cm) was

measured using a meter stick. Thereafter, roots were severed from the SCs, and fresh root mass (g) was determined. Measured roots were then oven-dried at 90 °C until a constant mass was reached, then the dry mass (g) of roots was determined using an analytical balance (Sartorius). The size of callus formation at the base of the SCs was assessed using a point rating scale, as follows: 1 = none, 2 = low, 3 = medium, 4 = extensive, 5 = very extensive [32].

The rooting and callus percentage were calculated using the formula [13]

$$\text{Rooting percentage} = \frac{\textbf{rooted stem cuttings}}{\textbf{total stem cuttings}} \times 100$$

$$\text{Callus percentage} = \frac{\textbf{callused stem cuttings}}{\textbf{total stem cuttings}} \times 100$$

2.7. Data Analysis

Prior to analysis of variance (ANOVA) of the data using SAS software (SAS Institute, Inc., 2010) [33], treatments (0, 10, 100, 1000, 10,000 and 100,000 ppm IBA) in both *A. deliciosa* and *A. arguta* experiments were expressed as exponentials [10^0, 10^1, 10^2, 10^3, 10^4, 10^5 and 10^6 (%)] and transformed using $\log_{10}10^x$, where $\log_{10}10^x = 1$ [31], resulting in an *x*-axis of 0, 1, 2, 3, 4, 5 and 6 [34], expressed for rooting percentage, callus percentage and size of callus formation. Transformation was carried out to homogenize the interval between the concentrations. Significant ($p \leq 0.05$) treatment means were separated using Fischer's Least Significant Difference test and then subjected to lines of the best fit. Generally, when the variables versus increasing treatment levels exhibited positive quadratic relations, the relationships were modelled through the regression curve estimates from the quadratic equation ($Y = b_2x^2 + b_1x + c$) [35] to derive the optimum values using the relation $x = -b_1/2b_2$ [31].

3. Results

The tested kiwifruit SCs from *A. deliciosa* rootstock and *A. arguta* female scion plants, developed well and formed roots, some with calluses, depending on the applied IBA (ppm).

3.1. Actinidia deliciosa Rootstock Stem Cuttings

Plant growth regulator IBA had high significant ($p \leq 0.05$) effects on rooting percentage, number of roots and root length (cm), as indicated by the asterisk (*) (Table 1). On the other hand, as shown in Table 1, there were no significant ($p \leq 0.05$) effects on dry root mass (g). Treatment with IBA contributed 26, 28, 20 and 11% to the total treatment variation (TTV) in rooting percentage, number of roots, root length (cm) and dry root mass (g), respectively (Table 1). Significant effects were also observed on callus percentage and size of callus formation in *A. deliciosa* rootstock SCs (Table 2). The treatments contributed 29 and 5% to the TTV in callus percentage and size of callus formation, respectively (Table 2).

Relative to the untreated control (6%), IBA concentrations of 10, 100, 1000, 10,000 and 100,000 ppm, significantly increased rooting percentage by 10, 6, 23, 42 and 31%, respectively, but the effects of the respective IBA at 10, 1000, 10,000 and 100,000 ppm did not differ from each other (Table 3). Similarly, IBA concentrations at 0, 10, 100, 1000 and 100,000 ppm did not differ significantly from each other (Table 3). The highest rooting percentage (42%) was achieved at IBA concentration of 10,000 ppm IBA (Figure 1e).

Table 1. Mean sum of squares (SS) for rooting percentage (RT%), number of formed roots (NRs), root length (RTL) and dry root mass (DRM) stem cuttings of *Actinidia deliciosa* rootstock plants.

Source	DF	RT %		NRs		RTL		DRM	
		SS	%	SS	%	SS	%	SS	%
Actinidia deliciosa									
Replication	11	5.0075	11	0.24138	8	0.29518	11	0.00147	17
Treatment	5	11.2296	26 *	0.80712	28 *	0.55064	20 *	0.00099	11 ^{ns}
Error	55	27.3583	63	1.83424	64	1.90489	69	0.00622	72
Total	71	43.5954	100	2.88275	100	2.75071	100	0.00869	100

% = (SS/Total) × 100, * denotes significance at $p \leq 0.05$, and ^{ns} denotes not significant at $p \leq 0.05$.

Table 2. Mean sum of squares (SS) for callus percentage and size of callus formation of stem cuttings of *Actinidia deliciosa* rootstock plants in response to IBA concentrations in vivo.

Source	DF	Callus Percentage		Size of Callus Formation	
		SS	%	SS	%
Replication	11	3984.4	9	48.6970	80
Treatment	5	12,890.6	29 *	2.7439	5 *
Error	55	28,046.9	62	9.2040	15
Total	71	44,921.9	100	60.6450	100

% = (SS/Total) × 100, * denotes significance at $p \leq 0.05$.

Table 3. Responses of rooting percentage, number of formed roots, root length and dry root mass of stem cuttings, ± standard error of a mean (SEM), of *Actinidia deliciosa* rootstock plants to IBA concentrations.

IBA	Rooting Percentage	Number of Roots	Root Length (cm)	Dry Root Mass (g)
(ppm)	Variable ^{y}	Variable ^{y}	Variable ^{y}	Variable ^{y}
		Actinidia deliciosa		
0	6 ^{b} ± 3.26	0.0677 ^{bc} ± 0.20	0.0785 ^{b} ± 0.23	2.47×10^{-3} ^{ab} ± 0.00
10	10 ^{ab} ± 3.72	0.0688 ^{bc} ± 0.10	0.0643 ^{b} ± 0.08	3.60×10^{-4} ^{b} ± 0.00
100	6 ^{b} ± 3.26	0.0496 ^{c} ± 0.08	0.0613 ^{b} ± 0.14	0.0000 ^{b} ± 0.00
1000	23 ^{ab} ± 4.82	0.2085 ^{ab} ± 0.19	0.1615 ^{ab} ± 0.16	8.30×10^{-3} ^{ab} ± 0.00
10,000	42 ^{a} ± 9.89	0.2932 ^{a} ± 0.37	0.3008 ^{a} ± 0.50	9.06×10^{-3} ^{a} ± 0.01
100,000	31 ^{ab} ± 9.30	0.2951 ^{a} ± 0.37	0.2034 ^{ab} ± 0.28	7.02×10^{-3} ^{ab} ± 0.01

^{y} Column means with the same letter were not significantly ($p \leq 0.0.5$) different, according to Fischer's Least Significant Difference Test. The letters a, b and c indicate significant differences between means.

The IBA concentrations of 10, 100, 1000, 10,000 and 100,000 ppm significantly increased the number of roots, by 0.0688, 0.0496, 0.2085, 0.2932 and 0.2951, respectively, when compared to the untreated control (0.0677) (Table 3). The IBA concentrations at 0, 10 and 1000 did not differ significantly from each (Table 3). Similarly, IBA concentrations at 1000, 10,000 and 100,000 ppm did not differ significantly from each other (Table 3). Again, IBA concentrations at 0, 10 and 100 ppm did not differ significantly from each other (Table 3). The highest number of roots (0.295) was attained at IBA concentration of 100,000 ppm IBA (Figure 1f).

Relative to the untreated control (0.0785 cm), IBA concentrations of 10, 100, 1000, 10,000 and 100,000 ppm significantly increased root length, by 0.0643, 0.0613, 0.1615, 0.3008 and 0.2034 cm, respectively, but the effects of the respective IBA at 10, 100, 1000 and 100,000 ppm did not differ from each other (Table 3). Similarly, IBA concentrations at 1000, 10,000 and

100,000 ppm did not differ significantly from each other (Table 3). The longest root length (0.301 cm) was measured at the IBA of 10,000 ppm (Figure 1e).

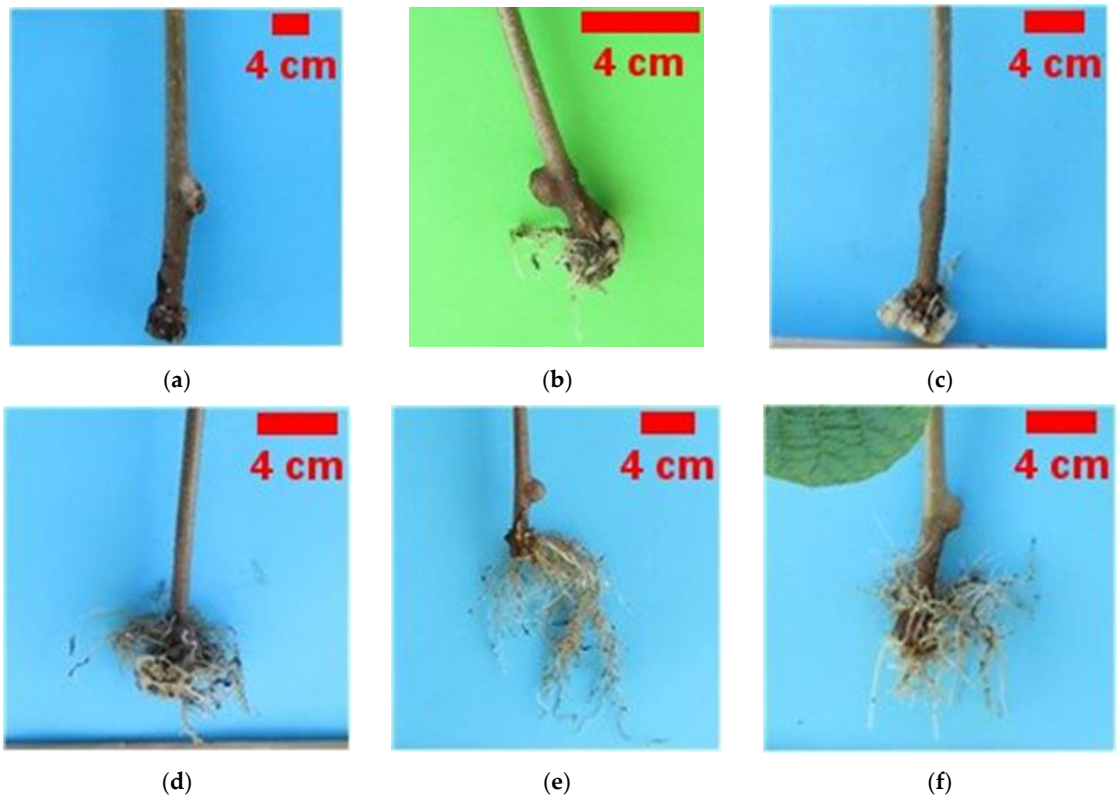


Figure 1. Representation of root formation in *Actinidia deliciosa* stems cuttings in response to different IBA concentrations: (**a**) 0 ppm; (**b**) 10 ppm; (**c**) 100 ppm; (**d**) 1000 ppm; (**e**) 10,000 ppm and (**f**) 100,000 ppm.

The IBA concentrations of 10, 100, 1000, 10,000 and 100,000 ppm significantly increased the dry root mass, by 0.000360, 0.0000, 0.00830, 0.00906 and 0.00702 g, respectively, when compared to the untreated control (0.00247 g) (Table 3). The IBA concentrations at 0, 10, 100, 1000 and 100,000 ppm did not differ significantly from each (Table 3). Similarly, IBA concentrations at 0, 1000, 10,000 and 100,000 ppm did not differ significantly from each other (Table 3). The highest dry root mass (0.00906 g) was attained at IBA concentration of 10,000 ppm IBA (Figure 1e).

The IBA concentrations of 10, 100, 1000, 10,000 and 100,000 ppm significantly increased the callus percentage, by 90, 94, 77, 69 and 58%, respectively, when compared to the untreated control (94%) (Table 4). The IBA concentration at 0, 10, 100, 1000 and 10,000 ppm did not differ significantly from each (Table 4). Similarly, IBA concentrations at 1000, 10,000 and 100,000 ppm did not differ significantly from each other (Table 4). The highest callus percentage (94%) was attained at IBA concentration of 100 ppm IBA (Figure 2b).

Relative to the untreated control (2.6), IBA concentrations of 10, 100, 1000, 10,000 and 100,000 ppm significantly increased size of callus formation, by 2.4, 2.4, 2.2, 2.5 and 2.8, respectively, but the effects of the respective IBA at 10, 100, 1000 and 10,000 ppm did not differ from each other (Table 4). Similarly, IBA concentrations at 0, 10,000 and 100,000 ppm did not differ significantly from each other (Table 4). Again, IBA concentrations at 0, 10, 100 and 10,000 ppm did not differ significantly from each other (Table 4). The greatest size of callus formation (2.8) was measured at the medium IBA of 100,000 ppm (Figure 2c).

Table 4. Responses of callus percentage and size of callus formation of stem cuttings, ± standard error of a mean (SEM), of *Actinidia deliciosa* rootstock plants to IBA concentrations in vivo.

IBA	Callus Percentage	Size of Callus Formation
(ppm)	Variable [y]	Variable
0	94 [a] ± 3.26	2.6 [ab] ± 0.25
10	90 [a] ± 3.72	2.4 [bc] ± 0.21
100	94 [a] ± 3.26	2.4 [bc] ± 0.19
1000	77 [ab] ± 4.82	2.2 [c] ± 0.27
10,000	69 [ab] ± 9.30	2.5 [abc] ± 0.27
100,000	58 [b] ± 9.89	2.8 [a] ± 0.39

[y] Column means with the same letter were not significantly ($p \leq 0.0.5$) different, according to Fischer's Least Significant Difference Test. The letters a, b and c indicate significant differences between means.

(**a**) (**b**) (**c**)

Figure 2. Representation of callus formation of stem cuttings of *Actinidia deliciosa* rootstock plants in response to IBA concentrations: (**a**) 0 ppm; (**b**) 100 ppm and (**c**) 100,000 ppm after 103 days; showing callus (yellow arrow) and root (red arrow) formation after 103 days.

3.2. Actinidia Arguta Female Stem Cuttings

Plant growth regulator IBA had significant ($p \leq 0.05$) effects on rooting percentage, number of roots, root length (cm), and dry root mass (g), as shown by the symbol * (Table 5). The treatment IBA contributed 18, 31, 38, and 43% to the TTV, in rooting percentage, number of roots, root length (cm) and dry root mass (g), respectively (Table 5).

Table 5. Mean sum of squares (SS) for rooting percentage (RT%), number of formed roots (NRs), root length (RTL) and dry root mass (DRM) stem cuttings of *Actinidia arguta* female plants.

Source	DF	RT %		NRs		RTL		DRM	
		SS	%	SS	%	SS	%	SS	%
Actinidia arguta female									
Replication	11	0.05735	9	0.12700	14	0.19646	12	0.00787	12
Treatment	5	0.10345	18 *	0.29423	31 *	0.60664	38 *	0.02995	43 *
Error	55	0.42682	73	0.51500	55	0.81200	50	0.03117	45
Total	71	0.58763	100	0.93624	100	1.61510	100	0.06898	100

% = (SS/Total) × 100, * denotes significance at $p \leq 0.05$.

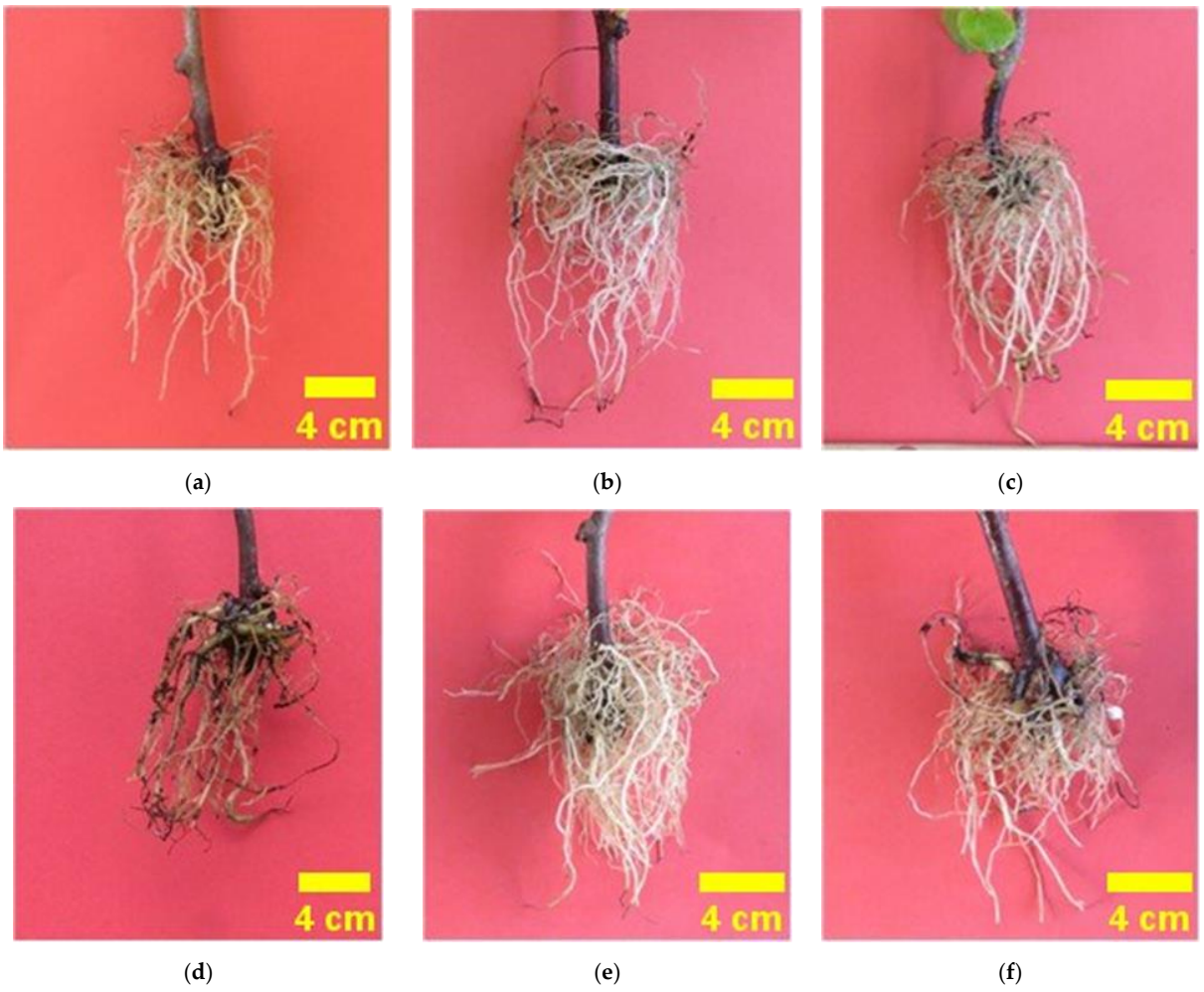
Relative to the untreated control (100%), IBA concentrations of 10, 100, 1000, 10,000 and 100,000 ppm, significantly increased rooting percentage by 91.67, 100, 83.33, 100 and 91.67%,

respectively, but the effects of the respective IBA at 0, 10, 100, 10,000 and 100,000 ppm did not differ from each other (Table 6). Similarly, IBA concentrations at 10, 1000 and 100,000 ppm did not differ significantly from each other, as illustrated by the same letter b (Table 6). The highest rooting percentage (100%) was achieved at IBA concentration of 0, 100 and 10,000 ppm IBA (Figure 3a,c,e).

Table 6. Responses of rooting %, number of roots, root length and dry root mass of stem cuttings, ± standard error of a mean (SEM), of *Actinidia arguta* female plants to IBA concentrations.

IBA	Rooting Percentage	Number of Roots	Root Length (cm)	Dry Root Mass (g)
(ppm)	Variable [y]	Variable [y]	Variable [y]	Variable [y]
		Actinidia arguta female		
0	100.00 [a] ± 0.00	0.9433 [ab] ± 0.39	0.9707 [b] ± 0.42	0.0810 [b] ± 0.01
10	91.67 [ab] ± 3.55	0.9815 [a] ± 0.46	0.9980 [ab] ± 0.56	0.0747 [bc] ± 0.01
100	100.00 [a] ± 0.00	0.9722 [a] ± 0.31	1.0839 [a] ± 0.51	0.0874 [ab] ± 0.01
1000	83.33 [b] ± 7.11	0.7922 [c] ± 0.70	0.8216 [c] ± 0.85	0.0417 [d] ± 0.01
10,000	100.00 [a] ± 0.00	0.8882 [b] ± 0.29	1.0100 [ab] ± 0.76	0.1062 [a] ± 0.04
100,000	91.67 [ab] ± 4.70	0.9319 [ab] ± 0.48	0.8500 [c] ± 0.61	0.0601 [cd] ± 0.02

[y] Column means with the same letter were not significantly ($p \leq 0.05$) different, according to Fischer's Least Significant Difference Test. The letters a, b, c and d indicate significant differences between means.



(**a**) (**b**) (**c**)

(**d**) (**e**) (**f**)

Figure 3. Representation of root formation in response to different IBA concentrations: (**a**) 0 ppm; (**b**) 10 ppm; (**c**) 100 ppm; (**d**) 1000 ppm; (**e**) 10,000 ppm and (**f**) 100,000 ppm in *Actinidia arguta* female stem cuttings after 103 days.

The IBA concentrations of 10, 100, 1000, 10,000 and 100,000 ppm significantly increased the number of roots, by 0.9815, 0.9722, 0.7922, 0.8882 and 0.9319, respectively, when compared to the untreated control (0.9433) (Table 6). The IBA concentrations at 0, 10, 100, and 100,000 ppm did not differ significantly from each (Table 6). Similarly, IBA concentrations at 0, 10,000 and 100,000 ppm did not differ significantly from each other (Table 6). The highest number of roots (0.9815) was attained at IBA concentration of 10 ppm IBA (Figure 3b).

Relative to the untreated control (0.9707 cm), IBA concentrations of 10, 100, 1000, 10,000 and 100,000 ppm significantly increased root length, by 0.9980, 1.0839, 0.8216, 1.0100 and 0.8500 cm, respectively, but the effects of the respective IBA at 0, 10 and 10,000 ppm did not differ from each other (Table 6). Similarly, IBA concentrations at 10, 100 and 10,000 ppm did not differ significantly from each other (Table 6). The longest root length (1.0839 cm) was measured at the IBA of 100 ppm (Figure 3c).

The IBA concentrations of 10, 100, 1000, 10,000 and 100,000 ppm significantly increased the dry root mass, by 0.0747, 0.0874, 0.0417, 0.1062 and 0.0601 g, respectively, when compared to the untreated control (0.0810 g) (Table 6). The IBA concentrations at 0, 10 and 100 ppm did not differ significantly from each other (Table 6). Similarly, IBA concentrations at 10 and 100,000 ppm did not differ significantly from each other, as illustrated by the same letter c (Table 6). Again, IBA concentrations at 100 and 10,000 ppm did not differ significantly from each other (Table 6). The highest dry root mass (0.1062 g) was attained at IBA concentration of 10,000 ppm IBA (Figure 3e).

4. Discussion

Root initiation and development in the two tested Kiwifruit cultivars demonstrated different responses to the root growth promoter IBA. In *A. arguta* female scion, IBA application improved the quality of rooting (more and longer roots). The IBA concentration of 10,000 ppm was found to be more beneficial in adventitious root induction in SCs of both *A. deliciosa* rootstock and *A. arguta* female scion, as compared to other concentrations, improving rooting percentage, number of roots, root length (cm), dry root mass (g), callus percentage and size of callus, suggesting that the growth regulator concentrations of 10,000 ppm may be important for the root performance of *Actinidia* plant species. Root growth variables of the tested *Actinidia* plant species measured in this study, namely rooting percentage, number of roots, root length (cm), dry root mass (g), callus percentage and size of callus have been found to be significantly improved with the application of growth regulator IBA, except for dry root mass in *A. deliciosa* SCs, with no significant effects. In *A. arguta* female SCs, significant effects were observed in all variables. However, no regenerative response in terms of callus formation were observed in *A. arguta* female SCs.

Actinidia deliciosa rootstock semi-hardwood SCs recorded the highest rooting percentage (42%) at the IBA application of 10,000 ppm and at lower (10 to 1000 ppm) IBA concentrations, rooting percentage was very low (10 to 23%). The dependency of *A. deliciosa* rootstock SCs to IBA was evident in this study. Whereas *A. arguta* female scion semi-hardwood SCs attained the highest rooting percentage (100%) at the IBA application of 0, 100 and 10,000 ppm. The *A. arguta* stem cuttings rooted irrespective of the presence or absence of IBA, although treatment with high concentration (100 and 10,000 ppm) improved morphological appearance, showing better rooting response with respect to the quality and the number of the roots. Rathore [32], for *A. arguta* SCs, showed rooting-independency to growth regulator IBA. However, the application of IBA improved the morphological appearance, showing better rooting response with respect to the quality and the number of the roots. The independency of *A. arguta* with respect to IBA for root formation was also documented in a study on the effect of growth stimulant on *A. arguta* shoot cuttings [36]. However, for other measured variables such as the root number, root length and dry root

mass, significant effects of IBA concentrations were observed, but without any regenerative response in terms of callus formation on the basal-cut end of the *A. arguta* female semi-hardwood SCs. The observed high rooting percentage in *A. deliciosa* rootstock and *A. arguta* female scion SCs could be due to the stimulatory effects of exogenous auxins stimulating adventitious root production in these SCs [37]. The variation in rooting percentage of *A. deliciosa* rootstock and *A. arguta* female scion seems to be due to the physiological nature of cuttings. The higher rooting potential of semi-hardwood SCs has been attributed to the endogenous auxin in the tender vegetative growth of semi-hardwood SCs [9]. Haissig [38] observed that, depending on the endogenous level of plant growth-regulating substance, exogenous application of auxin may be promotive, ineffective, or even inhibitory for the rooting of SCs.

At lower (100 ppm) IBA application, *A. deliciosa* rootstock semi-hardwood SCs recorded the highest callus percentage (94%). The biggest (2.8) callus measured was observed at the highest IBA application of 100,000 ppm. Notably, *A. deliciosa* semi-hardwood SCs tend to be recalcitrant to developing normal roots without the existence of calli formation at the basal cut-end of the SCs. Miri-nargesi and Sedaghathoor [39] experienced similar observation of 100% calli percentage in *A. deliciosa* SCs at IBA concentration of 4000 ppm. Stem cuttings, particularly of hardwood plants, commonly develop stronger callogenesis, which is followed by root formation at the physiological base of the cutting. These are greatly encouraged by the application of auxins such as IBA [9]. On the other hand, *A. arguta* female SCs showed good quality rooting response, without callus development. Atak and Yalçın [13] reported that better rooting response is achievable under conditions that do not favour callogenesis formation, probably because dense calluses have the tendency to hinder normal root emergence, leading to rooting failure.

Actinidia deliciosa rootstock semi-hardwood SCs recorded the greatest number of roots produced (0.295) at the IBA application of 10,000 ppm, whereas *A. arguta* female scion semi-hardwood SCs showed no difficulties in the number of root production and development. The cultivar obtained the greatest number of roots (0.9815) at the IBA application of 10 ppm IBA. In general, the number of roots in *A. arguta* semi-hardwood SCs was found to be superior to *A. deliciosa* semi-hardwood SCs. Biasi et al. [24] and Choudhary et al. [17] similarly reported the difference in the number of roots among *A. deliciosa* kiwifruit cultivars. Thangamani et al. [25] reported that *A. deliciosa* semi-hardwood SCs taken in July had better rooting ability in terms of the number of roots. The higher number of roots in semi-hardwood SCs could be associated with the auxin action, which triggered carbohydrate and nitrogenous material breakdown and translocation at the base of the cuttings, resulting in faster cell elongation and cell division in a favourable environment [40]. Rahman et al. [41] observed that *A. deliciosa* semi-hardwood SCs with the highest number of roots were found in SCs planted in February, rather than in January. Irshad et al. [42] also reported that *A. deliciosa* semi-hardwood SCs planted in February produced more roots. The variation in rooting might be due to the climatic conditions during the time of planting. Low temperature in winter disfavours the formation of adventitious roots, whereas in summer, the temperature, rainfall and humidity are conducive to the formation of adventitious roots [14].

Actinidia deliciosa rootstock semi-hardwood SCs recorded the highest root length (0.301 cm) at the IBA application of 10,000 ppm, whereas *A. arguta* female scion stem semi-hardwood stem cuttings obtained the highest root length (1.0839 cm) at the IBA application of 100 ppm IBA. Thangamani et al. [25] observed that *A. deliciosa* semi-hardwood SCs taken in the month of July had higher root length (23.06 cm) than hardwood (7.25 cm) and softwood (7.32 cm) SCs treated with IBA of 300 ppm. The increase in length of root may be due to the successful rooting of IBA-treated cuttings [43]. Alam et al. [44] reported that

IBA promoted cell elongation, which helped to increase root length. Wilson [9] highlighted the fact that the length of the roots increased as a result of the ability of the roots to absorb water and nutrients in sufficient quantities. Hence, the increase in root length could also be due to the allocation of more nutrients for root formation.

Actinidia deliciosa rootstock semi-hardwood SCs recorded the highest dry root mass (9.06×10^{-3} g) at the IBA application of 10,000 ppm, whereas *A. arguta* female scion semi-hardwood SCs attained the highest dry root mass (0.1062 g) at the IBA application of 10,000 ppm. Pratima and Rana [29] reported that the best rooting performance in terms of dry root mass (3.9) in SCs treated with 5000 ppm were taken in the month of July, as compared to the months of June and August. Rana et al. [30] reported that cuttings prepared during the active growth stage (July–August) gave better results than those prepared during the dormancy stage (January) [23]. Auxins such as IBA, when applied in higher concentration, resulted in higher dry root mass [16]. The increase in dry root mass is due to more and longer roots [44].

The formation of adventitious roots is a crucial step in the propagation of SCs of economically important horticultural and woody species [45]. Generally, the root formation potential of SCs is regulated by various factors such as plant species, cutting type (softwood, semi-hardwood and hardwood) [46], season, wounding [47] and auxins [9,37]. It was evident from this study that the two kiwifruit plant species had a significant effect on various root characteristics, such as rooting percentage, number of roots, root length and dry root mass. In general, all the root characteristics for SCs with *A. arguta* were found to be significantly superior to those of the *A. deliciosa* plant species. Biasi et al. [24] have also reported the difference in the rooting performance among different cultivars of kiwifruit. In their study, cultivars Allison and Abbott had significantly higher rooting performance than Hayward, Bruno and Tomuri. Similarly, Choudhary et al. [17] reported that the cultivar Kens Red had significantly higher rooting performance that other tested cultivars. Another of the most important factors for successful rooting of SCs is the suitable type of cuttings [9]. In kiwifruit, most studies were conducted on the rooting performance of hardwood stem cuttings [22,24]. Consequently, there is limited information available on the propagation of *A. deliciosa* and *A. arguta* semi-hardwood SCs [14]. Choudhary et al. [12] reported that the *A. deliciosa* semi-hardwood cuttings have performed better than the hardwood cuttings at IBA application of 4000 ppm. Similarly, Erturk et al. [28] observed that *A. deliciosa* semi-hardwood SCs have higher rooting performance than hardwood cuttings treated with IBA of 4000 ppm. Generally, the semi-hardwood cuttings of kiwifruit are known to have higher rooting success than the hardwood cuttings [14,28]. The season in which the cuttings are harvested also plays an important role in the rooting performance of *Actinidia* species, which is strongly related to the endogenous auxin content. Thangamani et al. [25] disclosed that the SCs taken in July had better rooting performance in terms of the number of main roots. The higher number of roots in semi-hardwood SCs could be associated with the number of endogenous auxins, the carbohydrate availability, and other rooting co-factors. Irshad et al. [42] reported that cuttings with the highest number of roots were found in SCs planted in February, whereas SCs planted in January had the lowest number of roots. According to Valenta [48], the percentage of rooted cuttings was higher in cuttings prepared in the summer than in those prepared in the winter. The variation in rooting performance might be due to the climatic conditions during the time of planting. The temperature in winter is low, which does not favour the formation of roots, whereas in summer, the temperature, rainfall and humidity are conducive to the formation of roots [14]. Various researchers have emphasized the importance of application of wounding in the propagation of SCs, in order to facilitate and promote adventitious root formation [49]. During the process of clonal propagation, which is the basis of propagation in numerous horticultural crops, wounding

plays a key role in cell reprogramming and adventitious root initiation. The initial trials of mechanical wounding on cuttings are manually performed using sharp blades, typically together with the application of exogenous auxins [50]. The first application of slicing to tissue on herbaceous and woody SCs resulted in improvements in root percentages and root number, compared to uninjured controls [47]. Similarly, Mackenzie et al. [49] attained an increase in percentage of adventitious formation in clonal propagation experiments with wounded cuttings of the M.26 apple rootstock. Orellana et al. [51] also suggested that exposing the phloem proximities is one of the most relevant aspects for a positive effect on rooting response.

The adventitious root formation requires the presence of the auxin source in a specific cell type. These cells synthesized IPA through the TAA1 pathway, which further converted to IAA and influenced the action of YUCCA 2. Cells on the periphery of the cluster divide faster, and possess auxin canalization through PIN action, forming an auxin gradient, which, in turn, leads to epigenetic modification with the formation of different cell types. Endogenous auxin also plays a primary role in the rooting step as part of the plant propagation procedure [4]. Namely, application through the root is considered the simplest means of auxin canalization, and the root can form only after the formation of a sieve element vessel. Application of exogenous auxin can induce root primordia in the already-formed xylem pole "pericycle" cell. There are two key factors for successful rooting: a high rate of auxin biosynthesis in new shoots and a high rate of auxin flux from these shoots, through the vessel [4].

5. Conclusions

In this study, it was established that SCs of different *Actinidia* spp. (rootstock, and female scions) require concentration of IBA for effective root establishment and development. Stem cuttings derived from *A. deliciosa* rootstocks, showed dependency of IBA treatment for effective rooting percentage, number and length of roots (10 000 ppm IBA). At lower IBA concentrations, callogenesis was evident. On the other hand, *A. arguta* female scion SCs, achieved 100% rooting percentage without IBA treatment (0 IBA), However, the addition of IBA improved morphological appearance, showing better rooting response with respect to the quality and the number of the roots with no evidence of callogenesis. The information generated on the propagation of these *Actinidia* spp. will serve as a basis for the researchers in developing new rootstocks and scions for the quality production of plant materials.

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