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Studies on the Rooting and the Consequent Plant Growth on the Stem Cuttings of *Thunbergia grandiflora* (Roxb. ex Rottl.) Roxb. 2-Effect of Indole-3-Butyric Acid

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Abstract: This study was conducted in a glasshouse of the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt. Two experiments were carried out during the two successive spring seasons of 2007 and 2008 using indole - 3 - butyric acid (IBA) in order to improve the rooting and the consequent plant growth on the stem cuttings of *Thunbergia grandiflora* (Roxb. ex Rottl.) Roxb. The basal ends of semihardwood stem cuttings (25 cm in length, defoliated, with 3 nodes) were dipped in IBA solutions at concentrations of 0.0 (control), 1500, 3000, 4500 and 6000 ppm for 30 seconds immediately before planting on 21st March in both growing seasons. Results recorded after four months from planting showed that the treatment of 6000 ppm IBA produced the highest values for the rooting percentage, root length, number of roots/plant and fresh and dry weights of roots/plant than did the other treatments. Also, IBA at concentration of 6000 ppm gave the highest positive effects on plant height, fresh and dry weights of branches/plant, number of leaves/plant and fresh and dry weights of leaves/plant, as compared to the other treatments. IBA at concentration of 6000 ppm, relative to the other concentrations, gave the highest content of the total soluble indoles in the cuttings bases after 30 days from planting, as well as the highest content of the total carbohydrates in the leaves after four months of the planting date. From the present findings, it can be recommended that for improving the rooting and the consequent plant growth on *Thunbergia grandiflora* semihardwood stem cuttings (25 cm in length, defoliated, with 3 nodes), the basal ends of the cuttings should be dipped in IBA solution at concentration of 6000 ppm for 30 seconds immediately before planting on 21st March.

Key words: *Thunbergia grandiflora* · Cuttings · IBA-indole-3-butyric acid

INTRODUCTION

Thunbergia grandiflora (Roxb. ex Rottl.) Roxb., Bengal clock vine or blue trumpet vine, is an important woody ornamental climber grown against fences, or on pergolas and arbours. It is very fast grower making dense coverings or, if allowed to hang, it makes thick curtains of beautiful pale to deep violet - blue flowers [1,2].

Roots that form on stem cuttings, directly from the stem or from the callus tissues at the bases of cuttings, are adventitious. Such roots are important in the propagation of plants by means of stem cuttings. The phenomenon of adventitious roots formation has been widely explored in connection with research on growth-promoting substances. Some adventitious roots develop from preformed dormant primordia that require an additional stimulus to resume growth [3, 4, 5].

Among the substances which exert important regulatory effects upon the reactions and metabolism of plants are those internally synthesized compounds called plant hormones which belong to three major groups viz., auxins, gibberellins and kinins. The most thoroughly studied group of plant hormones are the auxins. Some of the important roles of auxins include stimulation of cell division, cell enlargement, cell elongation, continued growth of callus, differentiation of tissues in callus, root formation on cuttings, stem elongation as well as synthesis of RNA, enzymes, protein and cell - wall components. The degree of auxin activity depends on its structural peculiarities and configuration [6, 7, 8, 9, 10].

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In cuttings that are naturally able to regenerate roots, applied auxins increase the number of developing adventitious roots [4]. It was reported that auxin levels are closely associated with rooting on stem cuttings [11,12]. As to the synthetic auxins, the compounds most widely employed with great practical importance for inducing root formation or initiation early and vigorous rooting on cuttings are α-naphthalene acetic acid (NAA) and indolebutyric acid (IBA). Also, the most effective treatment varies according to plant species [6,10]. In this respect, Hartmann and Kester [13] mentioned that IBA is used more frequently than NAA, since IBA has a higher activity, a broader range of effective concentrations without toxicity and it is effective in more plant species.

It was recorded that dipping the stem cuttings of several ornamental plants in IBA solution (1000-6000 ppm) improved the rooting and the vegetative growth on the cuttings e.g., Kumar *et al.* [14] on *Ficus elastica*; Gosh *et al.* [15] on *Punica granatum*; Bhattacharjee and Balakrishna [16,17] on *Hiptage madhablota*, *Ipomoea beraviensis*, *Clerodendrum splendens*, *Ipomoea tuberosa*, *Tecoma jasminoides*, *Thunbergia grandiflora* and *Vernonia elaegnifolia*; Souidan *et al.* [18] on *Ficus elastica*; El-Boraie [19] on *Jasminum sambac* and *Gardenia jasminoides*; Rowezak [20] on *Ficus retusa* and *Ficus benjamina*; Schoellhorn [21] on *Dichorisandra thyrsiflora* and *Cestrum elegans*; Sharma *et al.* [22] on *Gardenia lucida* and Sharma *et al.* [23] on *Acalypha wilkesiana*; Singh [24] on *Bougainvillea peruviana* and Hussein [25] on *Beaumontia grandiflora*.

A very little work concerning the effect of synthetic auxins on *Thunbergia grandiflora* plants was carried out. Therefore, the present study was undertaken to determine the extent of the improvement in the rooting and the vegetative growth on *Thunbergia grandiflora* stem cuttings by using indole -3- butyric acid (IBA) substance under the conditions of the early planting on 21st March which proved to be the most beneficial planting date for rooting the stem cuttings and improving the growth of this plant, based on the results of the first part of this study [26]

MATERIALS AND METHODS

The current investigation was conducted in a glasshouse at the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt. Two experiments were carried out during the two successive spring seasons of 2007 and 2008. Semihardwood stem cuttings (25 cm in length, defoliated, with 3 nodes) were taken on 21st March in both seasons. The basal ends of the cuttings were dipped in a dilute solution [0.0 (control), 1500, 3000, 4500 and 6000 ppm] of indole-3-butyric acid (IBA) for 30 seconds before setting them in the rooting medium. IBA (98%), Sigma- Germany, was primarily dissolved in 20% ethyl alcohol to allow it to dissolve in distilled water. Immediately after treatment, the cuttings were planted in 25-cm diameter plastic pots (4 cuttings/pot). The pots were filled with a mixture of peat moss and sand (3:1, v/v). The layout of the experiment was a randomized complete blocks design with three replicates for each treatment. Each replicate included five pots. In addition, eight spare cuttings (two pots)/replicate were used for the chemical analysis (and that were not included in the statistical analysis). The pots were placed on benches under a frame of wires covered with polyethylene sheets (110 cm high, 120 cm wide and 6.50 m long). The physical properties of the sand used in preparation of the potting mixture, as well as the chemical characteristics of the mixture are shown in Table 1. The plants were irrigated every 3 days.

Table 1: Physical properties of the sand used in the potting mixture, as well as the chemical characteristics of the peat moss + sand mixture during 2007 and 2008 seasons

Physical properties of the sand					
Texture	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Field capacity (%)
Sand	64.17	30.42	3.66	1.75	6.5

Chemical characteristics of the peat moss + sand mixture					
			Macro-nutrients content (%)		
Organic matter (%)	EC (dS/m)	pH	N	P	K
65.4	0.9	5.9	0.64	0.32	0.78

Fresh samples from the basal 2 cm of cuttings were taken before planting. Samples were chemically analyzed to determine total soluble indoles content (mg/g fresh matter) as described by Larsen *et al.* [27] and total soluble phenols (mg/g fresh matter) according to Swain and Hillis [28]. In addition, chemical analysis of dried samples of the basal 2 cm of cuttings was conducted before planting to determine total carbohydrates content (% of dry matter) as described by Dubois *et al.* [29]. The total nitrogen contents (% of dry matter) were determined according to Pregl [30] after digesting the dried samples using the method described by Piper [31]. Also, the C/N ratio was calculated.

After 30 days from planting (during root emergence), the chemical analyses of the basal 2 cm of cutting as fresh and dried samples were conducted to determine the same above mentioned chemical characteristics

Four months after planting date, the plants were dug out to record the following characteristics: rooting percentage, root length (cm), number of roots/plant, fresh and dry weights of roots/plant, plant height (cm), number of leaves/plant, fresh and dry weights of leaves (g)/plant and fresh and dry weights of branches (g)/plant. Also, chemical analysis of the dried leaves was conducted to determine total carbohydrates content (% of dry matter) according to Dubois *et al.* [29].

The data on the vegetative characteristics were subjected to analysis of variance and the significant differences between means were determined by using the "Least Significant Difference (L.S.D.)" test at the 5% level. The rooting percentage data were arcsine transformed and the transformed data were statistically analyzed [32].

RESULTS AND DISCUSSION

Rooting Percentage: The results recorded in the two seasons (Table 2) showed that treating *Thunbergia grandiflora* cuttings with IBA had a considerable effect on the rooting percentage. The untreated cuttings statistically gave the lowest rooting percentage, while the treated ones generally showed a steady increase in their rooting percentage as the IBA concentration was raised (1500, 3000 or 4500 ppm). Cuttings treated with IBA at 1500 or 3000 ppm showed a significant increase in the rooting percentage, as compared to the untreated cuttings. IBA at 4500 and 6000 ppm significantly increased the rooting percentage on cuttings as compared to the two lower IBA concentrations (1500 or 3000 ppm). However, IBA at 4500 and 6000 ppm statistically gave the same effect on the rooting percentage. Bilgrami *et al.* [8] attributed the stimulation of root formation and rooting percentage on cuttings treated with IBA to the activation of rhizocaline (a growth cofactor present in the plant tissues), besides the activation of cell division from which the roots are initiated. Auxin levels are closely associated with rooting on stem cuttings [11,12]. The general favourable effect of IBA on the rooting of *Thunbergia grandiflora* stem cuttings is similar to those found by Kumar *et al.* [14] on *Ficus elastica*; Gosh *et al.* [15] on *Punica granatum*; Bhattacharjee and Balakrishna [16,17] on different woody ornamental climbers (including *Hiptage madhablota*, *Ipomoea beraviensis*, *Clerodendrum splendens*, *Ipomoea tuberosa*, *Tecoma jasminoides*, *Thunbergia grandiflora* and *Vernonia elaegnifolia*); Panwar *et al.* [33]; Singh [34] and Singh [24] on *Bougainvillea peruviana* cv. Thimma; Rowezak [20] on *Ficus retusa* and *Ficus benjamina* and Hussein [25] on *Beaumontia grandiflora.*

Root Characters: Treating *Thunbergia grandiflora* stem cuttings with IBA showed a positive effect on the root parameters, viz. root length, number of roots/plant and the roots fresh and dry weights/plant (Table 2). However, in both seasons, cuttings treated with IBA gave significantly higher values for the different root characteristics (in most cases), compared to the untreated cuttings. The values recorded for the studied root parameters showed steady increases as the IBA concentration was raised up to 4500 ppm. For most of the studied root parameters, cuttings treated with IBA at 4500 ppm statistically gave higher values than the control, or the other two lower concentrations (1500 and 3000 ppm).

In both seasons, IBA at the highest level (6000 ppm) caused a significant increase in most of the studied characters, compared to the control or values obtained with IBA at 1500 and 3000 ppm. However, the two higher treatments (4500 and 6000 ppm) statistically gave similar effects in most cases. Similar positive effects have been reported by Hosni *et al.* [35] on *Bougainvillea x buttiana* 'Mrs Butt'; Rowezak [20] on *Ficus retusa* and *Ficus benjamina*; Singh [34] and Singh [24] on *Bougainvillea peruviana* cv. Thimma and Hussein [25] on *Beaumontia grandiflora* using IBA at 2000 - 5000 ppm. In this respect, Devlin [7]; Bilgrami *et al.* [8] and Jain [10] mentioned that auxins stimulate root initiation and numbers on cuttings. Esau [4] demonstrated that applied auxins increase the number of developing adventitious roots on cuttings that are naturally able to regenerate roots.

Table 2: Effect of IBA concentrations on the rooting percentage, root length, number of roots/plant, as well as the fresh and dry weights of roots/plant of *Thunbergia grandiflora* during 2007 and 2008 seasons.

IBA concentrations	*Rooting percentage	Root length (cm)	Number of roots/plant	Fresh weight of roots (g)/plant	Dry weight of roots (g)/plant
First season (2007)					
Control	40.0d	9.2	4.0	3.41	0.61
1500 ppm	51.7c	10.1	4.6	3.62	0.70
3000 ppm	65.0b	13.5	5.7	4.11	0.72
4500 ppm	75.0a	14.8	6.3	4.30	0.86
6000 ppm	75.0a	15.3	6.7	4.62	0.88
L.S.D. (0.05)	---	1.2	0.5	0.38	0.08
Second season (2008)					
Control	43.3c	8.4	3.8	3.69	0.59
1500 ppm	60.0b	9.5	5.3	4.12	0.70
3000 ppm	66.7b	11.3	5.4	4.53	0.86
4500 ppm	75.0a	15.6	7.3	4.69	1.03
6000 ppm	78.3a	14.9	7.7	4.73	0.95
L.S.D. (0.05)	---	1.4	0.4	0.43	0.06

* Within the columns for rooting percentage, means sharing one or more letters are insignificantly different at the 5% level, according to the "Least Significant Difference" test

Table 3: Effect of IBA concentrations on plant height, fresh and dry weights of stems/plant, number of leaves/plant, as well as the fresh and dry weights of leaves/plant of *Thunbergia grandiflora* during 2007 and 2008 seasons.

IBA concentrations	Plant height (cm)	Fresh weight of stems (g)/plant	Dry weight of stems (g)/plant	Number of leaves/plant	Fresh weight of leaves (g)/plant	Dry weight of leaves (g)/plant
First season (2007)						
Control	30.2	19.91	3.60	4.3	3.32	0.53
1500 ppm	34.6	23.53	3.81	4.8	3.86	0.69
3000 ppm	35.9	25.14	4.82	5.9	4.79	1.01
4500 ppm	36.4	27.96	5.94	6.2	5.30	1.01
6000 ppm	38.9	31.59	7.27	6.9	6.26	1.13
L.S.D. (0.05)	2.4	1.50	0.40	0.3	0.32	0.12
Second season (2008)						
Control	28.3	20.53	3.51	4.0	3.69	0.66
1500 ppm	30.8	22.35	4.20	5.2	4.30	0.73
3000 ppm	32.9	23.97	4.83	5.8	4.82	0.98
4500 ppm	34.6	26.58	5.08	6.5	5.11	1.18
6000 ppm	37.9	30.89	6.89	7.1	5.90	1.26
L.S.D. (0.05)	2.6	1.91	0.61	0.4	0.48	0.08

Vegetative Characters: Treating the cuttings with IBA markedly affected the plant vegetative growth (Table 3). In both seasons, IBA treatments gave significantly higher values for most of the studied vegetative characteristics (plant height, number of leaves/plant, as well as the fresh and dry weights of branches and leaves/plant), compared to the control cuttings. In general, raising the IBA concentration up to 6000 ppm caused steady increases in the different studied parameters. In both seasons, IBA at 6000 ppm gave significantly higher results than those of the lower concentrations (1500-4500 ppm). These increases in the vegetative growth due to IBA treatments are in agreement with the findings of Panwar *et al.* [33] on *Bougainvillea peruviana*, Schoellhorn [21] on *Dichorisandra thyrsiflora* and *Cestrum elegans*, Singh [34] on *Bougainvillea peruviana*, Sharma *et al.* [22] on *Gardenia lucida*, Sharma *et al.* [23] on *Acalypha wilkesiana* cv. Tahiti and Hussein [25] on *Beaumontia grandiflora*. They reported that IBA at concentrations of 1000-5000 ppm improved the plant vegetative growth. The promotive effect of IBA on the vegetative growth may be caused by the enhancement of rooting percentage and root growth on the treated cuttings, which leads to more uptake of water and nutrients from the growing medium, resulting in an increase in vegetative growth [36]. Also, some growth characteristics are promoted when certain growth factors called calines, present in each part of the plant, can be activated and be more effective in combination with auxins e.g., stem elongation is promoted by the combination of auxin and caulocaline. In this way auxin behaves like a coenzyme for the growth factor caline [8].

Chemical Composition at the Cuttings Bases

Total Carbohydrates Content, Total N Content and the C/N Ratio: Data recorded in the two seasons (Table 4) showed that after 30 days from planting, the total carbohydrates content at the bases of *Thunbergia grandiflora* cuttings treated with IBA was higher compared to the untreated control cuttings. These results are in harmony with those reported by Singh [37] on *Pyrostegia venusta*, Mitra and Bose [38] on litchi cuttings, El-Boraie [19] on *Jasminum sambac* and *Gardenia jasminoides*, and Hussein [25] on *Beaumontia grandiflora*. In contrast, the untreated cuttings bases had a higher total N contents compared to those received different IBA treatments. Thus, the C/N ratio at the bases of cuttings after 30 days from planting was lower in the untreated cuttings than those treated with the different IBA concentrations.

In cuttings bases received IBA treatments (which showed an improvement in rootability), the relatively high total carbohydrates content in the cuttings bases 30 days after planting, combined with a low total nitrogen content, are in agreement with the results obtained by YongKweon and KiSun [39] on white forsythia cuttings. Also, the relatively high C/N ratio in cuttings treated with IBA probably related to the high rooting percentage and root growth on the cuttings, compared to the untreated cuttings, as proposed by Rana and Chadha [40] on *Prunus* species, El-Boraie [19] on *Gardenia jasminoides* and Hussein [25] on *Beaumontia grandiflora*.

Generally, no specific trend was noted for the total carbohydrates content, total N content and C/N ratio in the basal parts of the cuttings at 30 days after planting, in response to raising the IBA concentrations from 1500 to 6000 ppm. Moreover, C/N ratio showed no apparent logical relation to rooting percentage on root growth. Similar results were found by El-Boraie [19] on *Jasminum sambac* and Hussein [25] on *Beaumontia grandiflora*.

The total carbohydrates and total N contents were lower in the cuttings bases at 30 days after planting, compared to values recorded before planting, regardless of IBA concentration. In contrast, the C/N ratio in the cuttings bases showed an opposite trend. Similar results were reported by Hussein [25] on *Beaumontia grandiflora*.

Total Soluble Indoles Content: Data in Table 4 showed that treating the bases of *Thunbergia grandiflora* stem cuttings with IBA increased the total soluble indoles content in the cuttings bases at 30 days after planting, compared to untreated control cuttings. In both seasons, control cuttings gave the lowest value after 30 days from planting, whereas raising the IBA concentration to 1500, 3000, 4500 or 6000 ppm caused a gradual increase in total soluble indoles content, which may explain the increase in the rooting ability on cuttings dipped in IBA. This was in agreement with that reported by YongKweon and KiSun [39] on white forsythia (*Abeliophyllum distichum*, Nakai); Hosni *et al.* [35] on *Bougainvillea x buttiana* 'Mrs Butt' and Hussein [25] on *Beaumontia grandiflora*.

The total soluble indoles content in the basal parts of the cuttings was higher before planting than at 30 days from planting, regardless of IBA concentration. This was proved by Hussein [25] on *Beaumontia grandiflora*.

Total Soluble Phenols Content: In both seasons, data presented in Table 4 showed that the total soluble phenols content in the cuttings bases was lower at 30 days from planting, than before planting. Also, this content was considerably affected by the IBA treatments. In both seasons, treated cuttings gave lower values at 30 days after planting than did the control. This was in harmony with that detected by Rowezak [20] on *Ficus retusa* and *Ficus benjamina* and Hussein [25] on *Beaumontia grandiflora*.

Leaf's Total Carbohydrates Content: Data in Table 4 showed that dipping the bases of *Thunbergia grandiflora* stem cuttings in an IBA solution prior to planting had a positive effect on the total carbohydrates content in the plant leaves, compared to the control cuttings which gave the lowest values in both seasons. Moreover, increasing the IBA concentration caused a steady increase in the total carbohydrates content, which reached its highest level with 6000 ppm. The enhancement of the synthesis and accumulation of carbohydrates in the resulting leaves after IBA treatments was confirmed by the results recorded by Darwesh [41] on *Ficus retusa*

Table 4: Effect of IBA concentrations on the total carbohydrates and total nitrogen contents, C/N ratio, total soluble indoles and total soluble phenols contents at the base of cuttings (before planting and after 30 days from planting), as well as the total carbohydrates content in the leaves of Thunbergia grandiflora during 2007 and 2008 seasons

IBA concentration	Chemical composition of basal part of cuttings: Total carbohydrates content(% of dry matter)	Total nitrogen content(% of dry matter)	C/N ratio	Total soluble indoles content(mg/g fresh matter)	Total soluble phenols content (mg/g fresh matter)	Total carbohydrates content in leaves (% of dry matter)
			First season (2007)			
Before planting	24.9	2.01	12.4	0.54	0.88	
After planting:						
Control	23.1	1.82	12.7	0.34	0.76	21.8
1500 ppm	24.6	1.60	15.4	0.38	0.73	22.1
3000 ppm	23.9	1.78	13.4	0.44	0.67	22.8
4500 ppm	24.8	1.65	15.0	0.45	0.64	24.6
6000 ppm	23.4	1.70	13.8	0.50	0.56	24.9
			Second season (2008)			
Before planting	25.3	2.19	11.6	0.49	0.79	
After planting:						
Control	22.8	1.95	11.7	0.31	0.62	20.5
1500 ppm	25.0	1.90	13.2	0.36	0.60	22.6
3000 ppm	23.6	1.88	12.6	0.38	0.59	22.8
4500 ppm	24.9	1.73	14.4	0.41	0.54	23.9
6000 ppm	23.6	1.82	13.0	0.48	0.50	24.5

"Hawaii" and Hussein [25] on *Beaumontia grandiflora*, who found that the total carbohydrates content in the leaves was increased steadily by raising the IBA concentration. The increase in the total carbohydrates content in the plant leaves due to IBA treatments may be attributed to the stimulation of plant vegetative growth, which in turn, increased the photosynthetic rate and accumulation of carbohydrates in the plant.

CONCLUSION AND RECOMMENDATION

Data of the present study indicated that it was possible to improve the rooting and the vegetative growth in *Thunbergia grandiflora* plants throughout dipping the basal ends of semihardwood stem cuttings in IBA solution at concentration of 6000 ppm for 30 seconds immediately before planting on 21st March.

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