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Stimulatory Effect of Indole – 3 – Butyric Acid and Rooting Media on Adventitious Rooting in *Epipremnum Aureum* 'Marble Queen' Stem Cuttings

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ABSTRACT

Epipremnum aureum 'Marble Queen' has high demand in the floriculture industry and is propagated commercially using single nodal stem cuttings. In the floriculture industry, they are exported as rooted and unrooted cuttings. Two experiments were conducted to determine the rooting performance of 'Marble Queen' cuttings for both rooted and unrooted export purposes. Experiment one tested the rooting performances of unrooted cuttings after an export simulation (48-hour cold storage at 21°C). In experiment two, the effect of rooting media and IBA was evaluated to produce rooted cuttings. Two different rooting media (coir dust and oasis) and four different concentrations of IBA (0 mg·L^{-1}, 250 mg·L^{-1}, 500 mg·L^{-1}, and 1000 mg·L^{-1}) were tested. Rooting media showed the most significant effects on the rooting of 'Marble Queen' cuttings. Coir dust medium reported the highest number of roots per cutting, root-to-shoot ratio, and average root diameter. Minimum days to bud and root initiation and a higher number of leaf buds were observed in the oasis medium. Early rooting and a higher number of roots were observed with the application of 500 mg·L^{-1} IBA. The use of an appropriate rooting medium and optimum concentration of IBA would help in the rapid propagation of 'Marble Queen' cuttings. Applying 500 mg·L^{-1} IBA in the oasis medium was more efficient in early rooting and shoot growth of 'Marble Queen' cuttings. Coir dust medium, along with 500 mg·L^{-1} IBA, was most suitable for the efficient growth and development of 'Marble Queen' cuttings.

1. Introduction

Epipremnum aureum (Linden ex André) G.S. Bunting is one of the most popular tropical ornamental plants used as a hanging basket crop (Meshram & Srivastava, 2015). The plant is also known as Devil's Ivy because of its ability to stay green under dark conditions (Meshram & Srivastava, 2014). Five varieties of *E. aureum* are commercially cultivated, including *E. aureum* 'Golden Pothos,' *E. aureum* 'Marble Queen,' *E. aureum* 'Neon,' *E. aureum* 'Jade Pothos,' and *E. aureum* 'N Joy' (Guan et al., 2019). The plant is largely grown as an indoor plant (Meshram & Srivastava, 2015). Due to their tolerance to low light levels, *E. aureum* is widely used in totems, hanging baskets, or dish gardens in the interior-scaping (Meshram & Srivastava, 2015). They are hardy vines that require very little care.

Plant propagation is crucial in horticulture as it allows rapid multiplication of plants while maintaining the desirable traits of the mother plants and lowers the plants' bearing ages. Depending on the species, various methods are used to optimize a nursery production system or even to address a particular propagation challenge (Roberto & Colombo, 2020). Ornamental and foliage plants can be propagated using one of three techniques, i.e., asexual (vegetative methods), sexual (using seed), and micro-propagation (using tissue culture). Nevertheless, most ornamental plants are multiplied vegetatively, using stems, suckers, leaf cuttings, bulbs, corms, and tubers (Kumar et al., 2021).

Cane, leaf-bud, leaf, stem, and tip cuttings are the most common types used for the vegetative propagation of foliage plants (Parthiban et al., 2016). However, stem cuttings are one of the cheapest, simplest, and least time–consuming methods of plant multiplication, commonly used in the commercial production of *E. aureum* plantlets (Netam et al., 2018). The young independent plants produced from stem cuttings are genetically identical to the parent, offering significant starting resources for generating marketable or completed plants. *E. aureum* can be propagated easily in soil or water through stem cuttings by growing on moss sticks as a climber or allowing it to trail down (Meshram & Srivastava, 2015). It is in high demand in floriculture and is propagated commercially using single-nodal stem cuttings. The floriculture industry exports them as either rooted or unrooted cuttings under controlled environmental conditions.

The induction of adventitious roots is a crucial step during plant propagation through vegetative methods. When propagating plants from stem cuttings, rooting hormones (RHs) are often used as growth regulators to increase the rooting rate, adventitious root development, and the quantity and quality of roots (Massoumi et al., 2017). Even though endogenous auxins are produced during plant organogenesis (Cavallari et al., 2021), indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) are the most widely used synthetic growth hormones (Elmongy et al., 2018). Numerous studies have demonstrated that IBA is more effective in promoting adventitious root growth than Indole – 3 – acetic acid (IAA). This effect has been attributed to its higher tissue and solution stability, increased resistance to photodegradation, biological inactivation, and adhesion to the shoots (Sarropoulou et al., 2023).

A suitable rooting medium ensures adequate aeration, drainage, and faster root growth. Plant height, number of leaves, quantity and diameter of florets per spike, and weight of the cuttings are key variables significantly influenced by the growth medium. Plant growth, development, and flowering are influenced by the right balance of nutrients and environmental conditions (Nemati et al., 2021). Although different potting materials are available, including cocopeat, vermicompost, peat moss, sand, and soil, the choice of the growth medium is based on the analysis of the physical and chemical characteristics of the material (Kumar et al., 2019).

Even though *E. aureum* is in high demand in the floriculture industry and is propagated commercially using single nodal stem cuttings; those cuttings often show poor rooting. This may be attributed to the poor quality of the growing medium and improper use of hormones. Therefore, this experiment was conducted to optimize the rooting of *E. aureum* cuttings using an appropriate rooting medium and an optimum concentration of IBA.

2. Methods

2.1. Setting Up and Managing the Experiment

The experiment was conducted under the net house conditions at Mike Flora (Pvt.) Ltd., Rambukkana, Sri Lanka (N 7º 18' 59.004" E 80º 23' 26.015"). The company is situated in the Wet Zone Mid-country (WM3) in Sri Lanka, 112 m above sea level, with minimum and maximum temperatures of 19 ºC and 28 ºC, respectively. The laboratory analyses were conducted at the research laboratory of the Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka (N 7º 15' 43.92" E 80º 35' 2.76"). *Epipremnum aureum* ‘Marble Queen’ was selected as the experimental plant material in the study.

The experiment was established as a completely randomized design (CRD) with two factors: rooting medium and IBA concentration. There were two levels for rooting medium, *i.e.,* floral foam (oasis) and coir dust. There were four levels for IBA concentrations, *i.e.,* 0 $mg \cdot L^{-1}$, 250 $mg \cdot L^{-1}$, 500 $mg \cdot L^{-1}$, and 1,000 $mg \cdot L^{-1}$. Two experiments were conducted to determine the effect of rooting medium and IBA on the rooting performance of the 'Marble Queen' cuttings. The first experiment tested rooting performances of unrooted cuttings after an export simulation (48 hours of cold storage at 21 ºC). The second experiment evaluated the effect of rooting medium and IBA to produce rooted cuttings. All treatment combinations were replicated ten times, and each replicate consisted of three cuttings.

A 2,000 mL stock solution of 1,000 $mg \cdot L^{-1}$ IBA solution was prepared by dissolving 2 g of commercially available IBA hormone (Sun Root by Sunbeam Seeds, Kundasale, Sri Lanka) in distilled water. 500 mL of 250 $mg \cdot L^{-1}$ and 500 $mg \cdot L^{-1}$ IBA solutions were prepared by diluting 250 mL and 125 mL of the stock solution with distilled water, respectively.
The large blocks of floral foam (oasis) were cut into cubes with a dimension of 3 cm × 3 cm × 3 cm (*i.e.,* 27 cm^3). The coir dust was preconditioned by heating up to 100 ºC for three hours. Then, this sterilized coir dust was filled into the net pots of 1.5 cm × 1.5 cm × 1.5 cm.

Stem cuttings of 'Marble Queen' were collected from the mother stock maintained at the company premises. Healthy stems (runners) with more than seven mature leaves were harvested to obtain 2 – 3 cm long cuttings. Single nodal stem cuttings with one leaf were used as the propagation material (Fig. 1).

Figure 1. Single Nodal Stem Cutting of ‘Marble Queen’

One batch of cuttings was subjected to an export simulation where cold storage was provided at 21 ºC for a period of 48 hours. After 48 hours, those cuttings were treated with different IBA concentrations and tested under the two growth media (1^{st} experiment). The second batch of cuttings was directly evaluated under different IBA concentrations and growth media (2^{nd} experiment).

The IBA-treated cuttings were propagated in an oasis or coir dust medium (Fig. 2). Three cuttings of ‘Marble Queen’ were planted in each oasis piece or net pot filled with coir dust. Oasis pieces were put into plastic cups filled with water. Net pots filled with coir dust were placed in sterilized trays. Then, both samples were kept inside a propagator, which provided

70% shade, a temperature of 28 ºC - 30 ºC, and a relative humidity of 60 – 70%. The cuttings were watered once every two days. The propagation period was eight weeks.

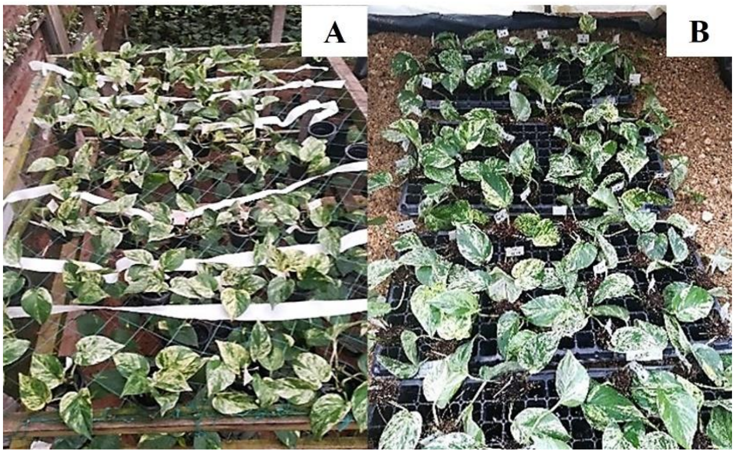


Figure 2 Propagation of 'Marble Queen' Cuttings in Oasis Medium (A) and in Coir Dust Medium (B)

2.2. Data collection

Data collection was started ten days after the establishment of the experiment and was continued in two-day intervals to check for root and bud initiation. The days required for first bud initiation and root initiation were recorded. Shoot fresh and dry weights, root fresh and dry weights, number of roots, and new buds were also recorded eight weeks after establishment. The number of roots and new buds were counted in all the replicates in each treatment combination. Three replicates from each treatment combination were selected to measure shoots' and roots' fresh and dry weights, total root length, and average root diameter. Weight measurements were made using a digital balance (Model: AJ 3200E, Shinko Denshi Co. Ltd., Tokyo, Japan). Dry weights were taken after oven-drying plant samples at 70 ºC for 24 hours. Root length and average root diameter of a plant were measured using three replicates per treatment combination using a root scanner (EPSON Perfection V700 Photo Scanner, Model: J221A, in Canada) and WinRhizo software (Regent Instruments Inc., Canada).

2.3. Statistical analysis

The parametric data were subjected to analysis of variance (ANOVA) using SAS software (SAS version 9.0, SAS Institute., Cary, NC, USA). The significant difference between treatment means was evaluated using Duncan's new multiple range test at a significance of $P \leq 0.05$. Non-parametric data were analysed using the Chi-square test at the significance level of $P \leq 0.05$. All graphs were prepared in MS Excel (2016).

3. Results and Discussion

3.1. Results

The rooting medium significantly affected days to bud initiation, root initiation, number of roots, and root–to–shoot ratio on a fresh weight basis ($P \leq 0.001$). However, the rooting medium did not affect the number of buds and root–to–shoot ratio on a dry weight basis ($P \geq 0.05$). The IBA concentration affected ($P \leq 0.05$) on days to root initiation, number of roots, and root length (Table 1). The interaction effect of rooting medium and IBA concentration significantly affected root length and diameter (Table 1).

Table 1. Summary of the chi-square and analysis of variance (ANOVA) for the measured variables in the study

Parameter	Rooting medium (RM)	IBA concentration	RM × IBA
Days to bud initiation	***	NS	NS
Days to root initiation	***	**	NS
Number of roots	***	***	NS
Number of buds	NS	NS	NS
Root length	**	***	**
Root diameter	**	NS	*
Root-to-shoot fresh weight ratio	***	NS	NS
Root-to-shoot dry weight ratio	NS	NS	NS

3.1.1. Days to Bud Initiation

The number of days required for bud initiation was extended in the coir dust medium in both experiments and all IBA concentrations. In experiment one, the highest number of days to bud initiation was observed in the coir dust medium with 250 mg·L^{-1} IBA (17 days), which was significantly higher than that in the oasis medium with the same concentration of IBA (Fig. 3). The lowest number of days to bud initiation was observed in the oasis medium combined with 1,000 mg·L^{-1} IBA application (11 days) which was significantly lower than that of the coir dust medium with the same IBA concentration (Fig. 3). In experiment two, the highest number of days to bud initiation was observed in the coir dust medium with 0 mg·L^{-1} IBA (19 days) which was significantly higher (P≤0.001) than that in the oasis medium with the same concentration of IBA. The lowest number of days to bud initiation (11 days) was observed in the oasis medium combined with 250 mg·L^{-1} IBA application (Fig. 3).

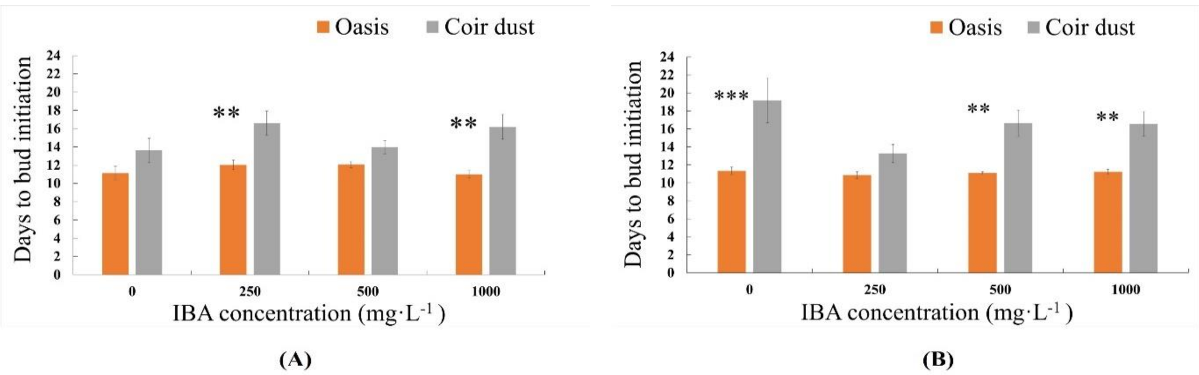
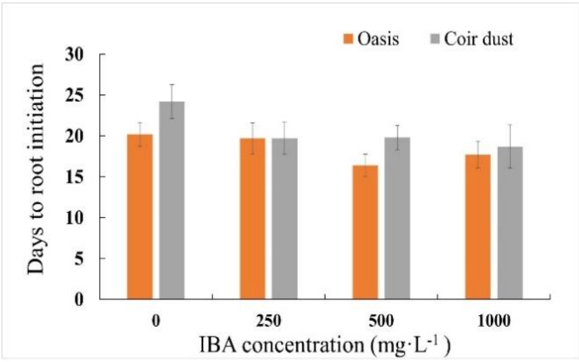


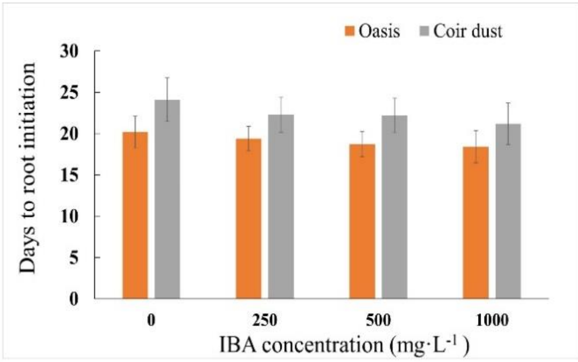
Figure 3 Effect of rooting medium and IBA concentration on the days to bud initiation of 'Marble Queen' cuttings in experiments one (A) and two (B). Experimental data represent means ± standard error with n = 10. '***' significantly different at P≤0.001, '**' significantly different at P≤0.01 and '*' are significantly different at P≤0.05 according to Chi-square test.

3.1.2. Days to Root Initiation

The number of days required for root initiation was more extended in the coir dust medium than that in the oasis medium in both experiments and across all IBA concentrations (Fig. 4). In experiment one, the root initiation was fastest in the oasis medium coupled with the 250 mg·L^{-1} IBA application (*i.e.*, after 16 days of establishment), which was eight days earlier than the latest rooted cuttings in coir dust medium with 0 mg·L^{-1} IBA application (Fig. 4). In experiment two, the root initiation was fastest in oasis medium with 1,000 mg·L^{-1} IBA application (18 days) while that was slowest (24 days) in the coir dust medium with 0 mg·L^{-1} IBA (Fig. 4).



(A)

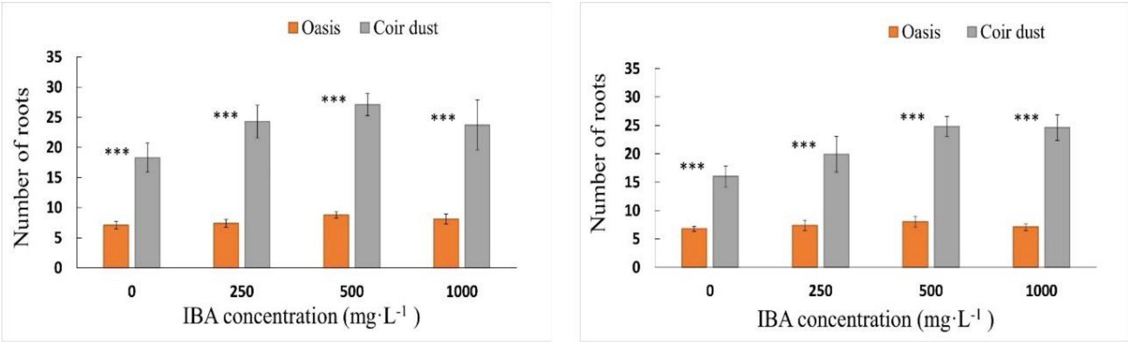


(B)

Figure 4. Effect of rooting medium and IBA concentration on the days to root initiation of 'Marble Queen' cuttings in experiments one (A) and two (B). Experimental data represent means ± standard error with n = 10.

3.1.3. Number of Roots

The average number of roots developed per cutting differed significantly ($P \leq 0.001$) between coir dust and oasis media. The coir dust medium had a significantly higher number of roots across all concentrations of IBA in both experiments. In experiment one, the highest number of roots per cutting (27) was observed in the coir dust medium with 500 mg·L^{-1} IBA application, which was nearly four times higher than the lowest number of roots (7) observed in the oasis medium with 0 mg·L^{-1} IBA application (Fig. 5). In experiment two, the highest number of roots per cutting (25) was observed in the coir dust medium with 500 mg·L^{-1} IBA. In comparison, the lowest number of roots per cutting (7) was observed in the oasis medium combined with 0 mg·L^{-1} IBA application (Fig. 5). For all the treatments, the maximum and minimum number of roots per cutting was recorded in 500 mg·L^{-1} IBA and 0 mg·L^{-1} IBA, respectively.

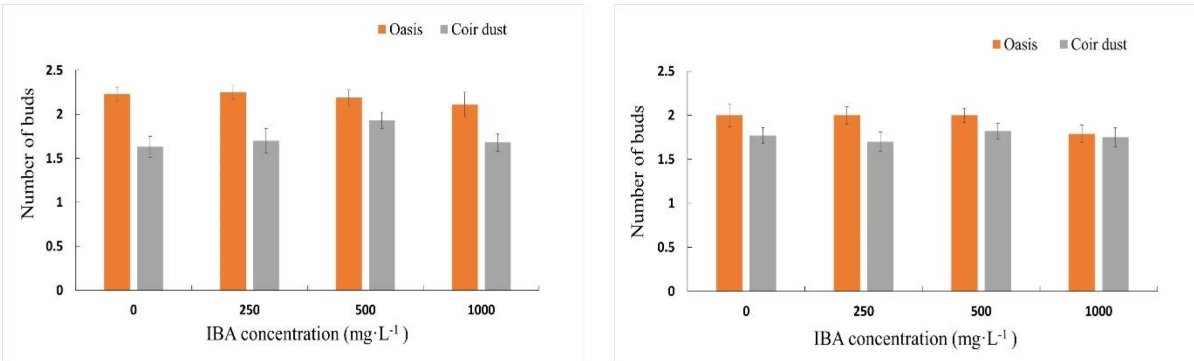


(A) (B)

Figure 5. Effect of rooting medium and IBA concentration on the number of roots of ‘Marble Queen’ cuttings in experiments one (A) and two (B). Experimental data represent means ± standard error with n = 10. ‘***’ significantly different at P≤0.001, ‘**’ significantly different at P≤0.01, and ‘*’ are significantly different at P≤0.05, according to Chi-square test.

3.1.4. Number of Buds

In experiment one, the highest number of buds per cutting (2) was observed in three concentrations of IBA except for 1,000 mg L^{-1}. The lowest mean number of buds (1.7) was observed in a coir dust medium with 250 mg·L^{-1} IBA application. In the second experiment, the highest mean number of buds per cutting (2.25) was observed in oasis medium with 250 mg·L^{-1} IBA application, and the lowest (1.63) was observed in coir dust medium with 0 mg·L^{-1} IBA application (Fig. 6).

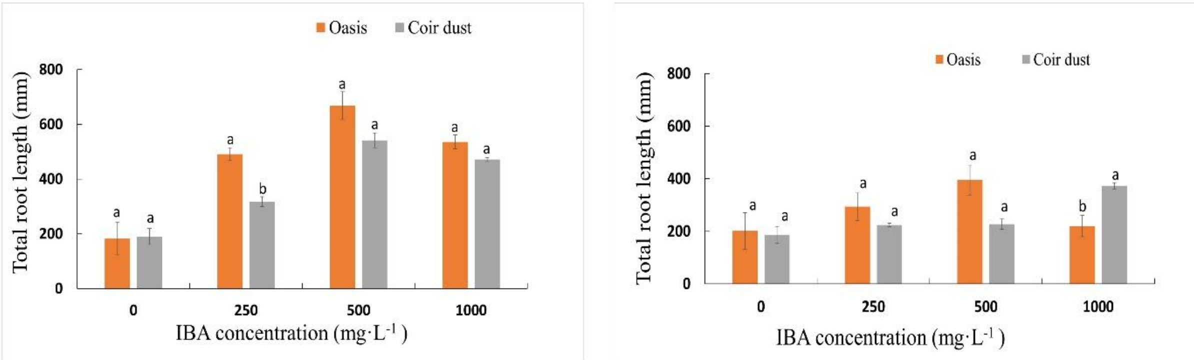


(A) (B)

Figure 6. Effect of rooting medium and IBA concentration on the number of buds per replicate of 'Marble Queen' cuttings in experiments one (A) and two (B). Experimental data represent means ± standard error with n = 10.

3.1.5. Total Root Length

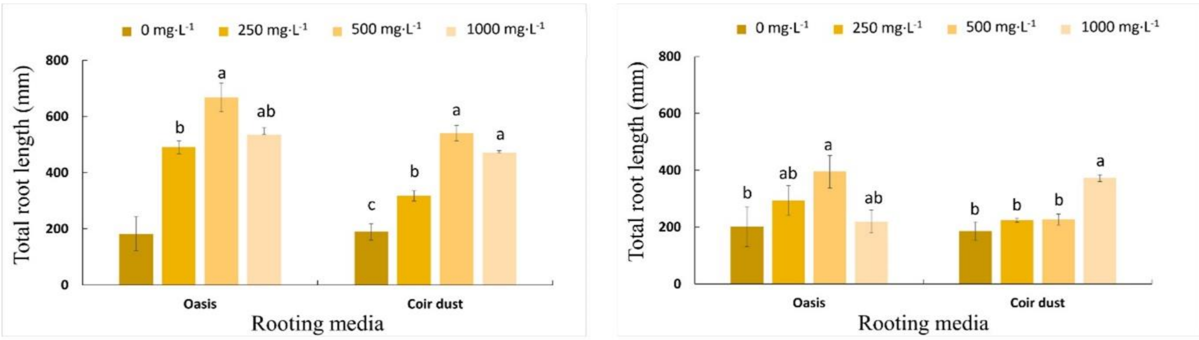
Total root length per cutting was higher in the oasis medium (P≤0.05) than that in the coir dust medium except on two occasions, *i.e.*, 0 mg·L^{-1} IBA application in experiment one and 1,000 mg·L^{-1} IBA application in experiment two where coir dust recorded longer roots. Results from experiment one reported the longest roots (668.1 mm) in the oasis medium with 500 mg·L^{-1} IBA application. In contrast, the shortest roots (182.7 mm) were recorded in the oasis medium with 0 mg·L^{-1} IBA application. There was a significant difference between the values for two rooting media in 250 mg·L^{-1} IBA treatment (Fig. 7). In experiment two, the oasis medium had a higher (P≤0.05) total root length compared to the coir dust medium at 500 mg·L^{-1} IBA. However, the coir dust medium had significantly higher values (P≤0.05) than the oasis medium at 1,000 mg·L^{-1} IBA. The longest roots (395.3 mm) were recorded in 500 mg·L^{-1} IBA with oasis medium, while the shortest roots (185.8 mm) were recorded in 0 mg·L^{-1} IBA with coir dust medium (Fig. 7).



(A) (B)

Figure 5. Effect of rooting medium and IBA concentration on total root length in 'Marble Queen' cuttings in experiments one (A) and two (B). Experimental data represent means ± standard error with n = 03. According to Duncan's new multiple range test, means followed by the same letters are not significantly different at P≤0.05.

The effect of hormones on the root length of 'Marble Queen' cuttings was significant (P≤0.05; Table 2). Results from the 1st experiment showed that total root length varied significantly between IBA concentrations within each rooting medium (Fig. 8). The length of the longest root (668.1 mm) was observed in the oasis medium with 500 mg·L^{-1} IBA application followed by coir dust medium with 500 mg·L^{-1} IBA application. The minimum (182.7 mm) root length was recorded in the oasis medium with 0 mg·L^{-1} IBA application. In the 2nd experiment, the highest value of total root length (395.3 mm) was recorded in the oasis medium with 500 mg·L^{-1} IBA application (Fig. 8).

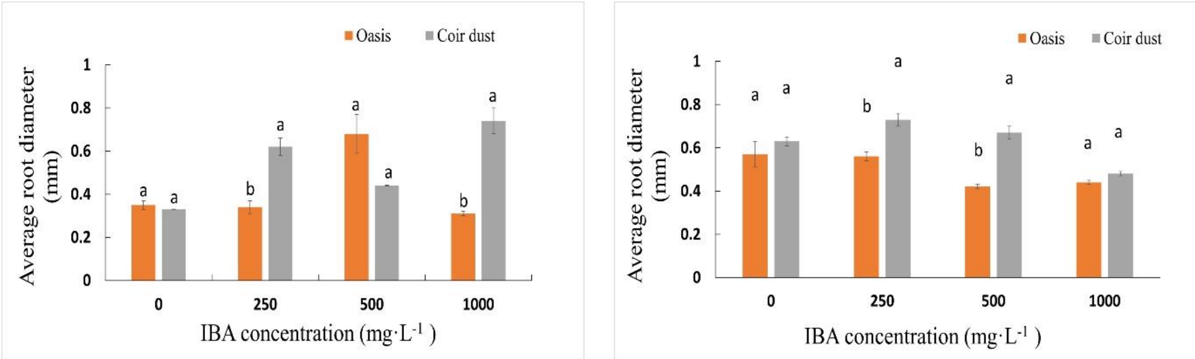


(A) (B)

Figure 6. Effect of rooting medium and IBA concentration on total root length in 'Marble Queen' cuttings in experiments one (A) and two (B). Experimental data represent means ± standard error with n = 03. Means followed by the same letters are not significantly different at P≤0.05, according to Duncan's new multiple range test.

3.1.6. Average Root Diameter

In most treatment combinations, the average root diameter in the coir dust medium was higher than in the oasis medium. In experiment one, the highest (0.74 mm) and lowest (0.31 mm) root diameter values were recorded in 1,000 mg·L^{-1} IBA application with coir dust and oasis mediums, respectively. The root diameter values significantly differed (P≤0.05) between the tested growth media at 250 mg·L^{-1} IBA application (Fig. 9). In experiment two, the coir dust medium showed higher root diameter values than that in the oasis medium at all IBA concentrations. Combining coir dust medium with 250 mg·L^{-1} IBA recorded the highest root diameter (0.73 mm), while oasis medium with 500 mg·L^{-1} IBA recorded the lowest root diameter (0.42 mm; Fig. 9).



(A) (B)

Figure 7. Effect of rooting medium and IBA concentration on average root diameter in 'Marble Queen' cuttings in experiments one (A) and two (B). Experimental data represent means ± standard error with n = 03. Means followed by the same letters are not significantly different at P≤0.05, according to Duncan's new multiple range test.

3.1.7. Root-to-Shoot Ratio on Fresh Weight Basis

In both experiments, cuttings propagated in the coir dust medium had higher ratio values to cuttings propagated in the oasis medium. In experiment one, the ratio values on a fresh weight basis varied between 0.11 and 0.24, whereas in experiment two, they were between 0.09 and 0.21 (Tables 2 & 3).

Table 2. Root–to–shoot ratio on a fresh weight basis for 'Marble Queen' cuttings grown in two different rooting media under four different IBA concentrations in experiment one

Rooting medium	IBA concentration (mg·L^{-1})			
	0	250	500	1000
Oasis	0.13 ± 0.01^{a}	0.12 ± 0.01^{b}	0.14 ± 0.02^{b}	0.11 ± 0.01^{a}
Coir dust	0.14 ± 0.01^{a}	0.22 ± 0.03^{a}	0.24 ± 0.009^{a}	0.15 ± 0.01^{a}

Experimental data represent means ± standard error with n = 03. According to Duncan's new multiple range test, means followed by the same letters are not significantly different at P≤0.05.

Table 3. Root–to–shoot ratio in fresh weight basis for 'Marble Queen' cuttings grown in two different rooting media under four different IBA concentrations in experiment two

Rooting medium	IBA concentration (mg·L^{-1})			
	0	250	500	1000
Oasis	0.09 ± 0.005^{b}	0.12 ± 0.003^{b}	0.1 ± 0.007^{b}	0.09 ± 0.01^{b}
Coir dust	0.19 ± 0.02^{a}	0.18 ± 0.01^{a}	0.17 ± 0.01^{b}	0.21 ± 0.007^{a}

Experimental data represent means ± standard error with n = 03. According to Duncan's new multiple range test, means followed by the same letters are not significantly different at P≤0.05.

3.1.8. Root–to–Shoot Ratio on Dry Weight Basis

The root–to–shoot ratio value on a dry weight basis showed a different pattern than the ratio on a fresh weight basis. Root–to–shoot ratio on a dry weight basis was not significantly affected ($P \geq 0.05$) by individual effects of rooting medium, IBA concentrations, and the interaction effect of IBA and rooting medium (Table 1). In experiment one, the root–to–shoot ratio between the two rooting media and IBA concentrations were similar ($P \geq 0.05$) (Table 4). In experiment two, the highest ratio was observed in coir dust medium for both 1,000 $mg \cdot L^{-1}$ and 250 $mg \cdot L^{-1}$ IBA treatments. Significant differences existed between the growth media in 0 $mg \cdot L^{-1}$ and 500 $mg \cdot L^{-1}$ IBA (Table 5).

Table 4. Root–to–shoot ratio on dry weight basis for 'Marble Queen' cuttings grown in two different rooting media under four different IBA concentrations in experiment one.

Rooting medium	IBA concentration ($mg \cdot L^{-1}$)			
	0	250	500	1000
Oasis	0.08 ± 0.01^{a}	0.09 ± 0.01^{a}	0.08 ± 0.02^{a}	0.07 ± 0.01^{a}
Coir dust	0.06 ± 0.02^{a}	0.11 ± 0.02^{a}	0.1 ± 0.04^{a}	0.11 ± 0.01^{a}

Experimental data represent means ± standard error with n = 03. According to Duncan's new multiple range test, means followed by the same letters are not significantly different at $P \leq 0.05$.

Table 5. Root–to-shoot ratio on dry weight basis for 'Marble Queen' cuttings grown in two different rooting media under four different IBA concentrations in experiment two.

Rooting Medium	IBA Concentration ($mg \cdot L^{-1}$)			
	0	250	500	1000
Oasis	0.06 ± 0.01^{b}	0.09 ± 0.01^{a}	0.04 ± 0.01^{b}	0.07 ± 0.02^{a}
Coir dust	0.09 ± 0.01^{a}	0.11 ± 0.01^{a}	0.1 ± 0.01^{a}	0.1 ± 0.01^{a}

Experimental data represent means ± standard error with n = 03. According to Duncan's new multiple range test, means followed by the same letters are not significantly different at $P \leq 0.05$.

3.2. Discussion

The results of this experiment revealed that most of the variables studied were affected by the rooting media and the IBA concentration. Therefore, using perfect substrate and concentration of IBA for the vegetative propagation of *E. aureum* 'Marble Queen' is essential when using single nodal stem cuttings.

3.2.1. Days to Bud Initiation

The rooting medium affected the time required for bud initiation. Here, better results were attained in the oasis medium. During vegetative propagation, leaf buds' early differentiation and growth depend on food reserves available in the cuttings (Netam et al., 2018). In general, the top growth of a cutting may reflect earlier growth of the root system, but other environmental factors can also impact the new shoot growth. Thus, the early bud initiation in oasis foams might be associated with the early root growth in this oasis medium. The above phenomena cannot be explained entirely by the differences in the water/air relationship of the various rooting media. They may have also been affected by other factors, such as porosity and mechanical impedance of the medium.

3.2.2. Days to Root Initiation

An ideal substrate for rooting stem cuttings should possess both porosity for root aeration and drainage, as well as the ability to retain an adequate amount of water. Rooting substrates significantly impact the rooting of stem cuttings in various plant species. In the present study, the rooting medium affected the days required for root initiation (Table 1). The time required to initiate rooting was shorter in the oasis medium than in the coir dust. This could be due to the excellent aeration and the ability to maintain a high air-to-water ratio even after heavy watering due to its higher drainage rate promoting root growth (Nemati et al., 2021). Similar success with IBA has been documented in Barbados cherry (*Malpighia emarginata*) (Samim et al., 2021) and Marigold (*Tagetes erecta* L; Watane et al., 2018) propagated by stem cuttings.

E. aureum 'Marble Queen' single nodal stem cuttings treated with IBA had shortened the rooting period. The observed promotional effect of applying IBA on the rooting of cuttings could be attributed to the effective utilization of stored resources in cuttings treated with plant growth regulators, which can trigger early root formation (Samim et al., 2021). This is likely due to the attraction of assimilates to the cutting base and stimulation of the meristematic differentiation by auxins. However, plant species show considerable variations in the optimal application rate and much intraspecific variation (Nasri et al., 2015). Synthetic forms, such as IBA and NAA, are more effective than endogenous auxins like IAA in promoting root formation in cuttings because they are not toxic to plants in a wide range of concentrations, stimulate rooting in a large number of species, and have more excellent photostability (Babu et al., 2018). Contrary to the above findings, Oni and Ojo (2002) reported that *Massularia acuminatea* (West African chewing chick) could be vegetatively propagated with or without auxin treatment.

3.2.3. Number of Roots

Sufficient root volume is essential for adequate water and nutrient absorption and the successful establishment of a young plant. If a rooted cutting lacks a well-developed root system, it will be of inferior quality and may cause delays in shipping, transplantation, and the growth of the final product (Kohler & Lopez, 2021). According to Pandey et al. (2019), the ideal substrate would allow high aeration and water retention capacity while being well-drained and free of pathogens. A possible explanation for the significant difference between the average number of roots between the two rooting media could be the availability of nutrients and the organic matter in coir dust compared to the oasis. Different rooting substrates significantly impact the rooting of cuttings in various plant species. The central aspect that differentiated the substrates used in the present experiment was the availability of nutrients since the oasis is an inert substrate. The availability of nutrients, although not a fundamental characteristic of rooting stem cuttings, is an essential factor for the early development of seedlings (Pigatto et al., 2018). The results confirm the findings of Liyanage et al. (2021).

The maximum number of roots per cutting might be due to the application of auxin with appropriate concentration inducing early and better root initiation. As a result, the maximum number of roots were produced in those treatments that received the appropriate concentration of auxin, which initiates early and more roots per cutting. In both experiments, the cuttings not treated with IBA recorded the least number of roots. This showed that IBA application increased the root number while enhancing the root formation along the whole stem base (Gehlot et al., 2014). The enhanced rooting ability, along with IBA, can be attributed to the high stability of this auxin inside the plant (Gehlot et al., 2014; Markovic, 2021). The present findings are consistent with those of (Mehta et al., 2018) for the pomegranate (*Punica granatum*) cuttings.

3.2.4. Number of Buds

The leaves are the primary sites of major food manufacturing in plants. As a result, the number of leaves in a plant indicates its overall vigor and various environmental conditions strongly influence its development and quality. The results demonstrated that the oasis medium resulted in more new buds per replicate. According to Kaur and Kaur (2017), the utilization of stored carbohydrates, nitrogen, and other factors can be improved with the help of growth regulators, which may contribute to early sprouting. The fact that the maximum efficiency of sprouting is lower than that observed for rooting in coir dust might be associated with the fact that the synthetic auxin mobilized nutrients for root formation, inhibiting sprouting when applied to the base of the cutting (Pigatto et al., 2018). As discussed earlier, the number of roots per replicate was significantly higher in the coir dust medium compared to the oasis medium. Other than this, environmental factors, such as bright sunshine hours, air, and medium temperatures, also impact the new shoot generation (Devi et al., 2016). The present findings did not seem consistent with other research on *E. aureum*, which demonstrated a higher shoot growth in a medium containing coco peat (Liyanage et al., 2021).

3.2.5. Total Root Length

The cellular division plays a crucial role in the elongation of roots. The attainment of maximum root length can be attributed to the early differentiation of cells and the augmented elongation of cells induced by auxin. Auxins trigger the synthesis of structural enzyme proteins during the development of adventitious roots, thereby facilitating root elongation. This process is associated with acidification mechanisms that increase root length (Osmont et al., 2007; Overvoorde et al., 2010; Watane et al., 2018). The increment in root length of 'Marble Queen' cuttings submitted to auxin application may be attributed to the increase in the sensitivity of the basal tissue of the cutting, as well as to an improvement in the capacity for hydrolysis of reserves essential for root growth and development (Osman et al., 2013).

Cold-stored cuttings produced long roots compared to the cuttings that did not receive cold treatment (Fig. 7). This demonstrated that some changes occurred in cold storage that modified the rooting process in 'Marble Queen' cuttings that enhanced the cell elongation process. These results do not agree with the findings of Khayyat et al. (2007) in *E. aureum*, which reported higher root lengths in a coco peat medium. This may be due to the limited space in the planting vessel when compared with the oasis medium, which reported higher values for the root length.

3.2.6. Average Root Diameter

Rooting media is critical in providing a supportive root growth and development environment. They act as a reservoir for essential nutrients, water, and oxygen, which are vital for adequately functioning and expanding root systems. The effect of the rooting medium on increasing the diameter of roots is likely attributed to several factors, foremost among them being the enhanced intake and availability of nutrients for the development of hairy roots in plants. This is because the oasis is an inert material lacking essential nutrients, whereas coir dust, an organic material, can supply nutrients to the growing medium.

3.2.7. Root-to-Shoot Ratio on Fresh Weight Basis

The root–to–shoot ratio measures the number of roots compared to the number of shoots. It provides insights into resource allocation strategies and reflects a plant's adaptation to environmental conditions and growth stage. A plant with a higher proportion of roots than a neighboring plant will be able to absorb more nutrients. In the early phases of the propagation periods, it is essential to have a more significant proportion of roots so that the cutting can survive by absorbing more nutrients from the rooting medium. The maximum fresh weight of roots recorded in cuttings grown in coir dust could be related to better aeration and drainage conditions and water maintenance capability, resulting in a higher number of roots. These favorable conditions promote better root growth, leading to a higher number of roots than the oasis medium. These findings follow the findings of Wahab et al. (2001) in guava (*Psidium guajava* L), Khayyat et al. (2007) in pothos (*Epipremnum aureum* Lindl. and Andre ‘Golden Pothos’), Moreno et al. (2009) in cape gooseberry (*Physalis peruviana* L.), and Rajkumar et al. (2016) in pomegranate (*Punica granatum L*).

3.2.8. Root–to–Shoot Ratio on Dry Weight Basis

The root-to-shoot ratio on a dry weight basis is an essential parameter in plant ecology and physiology, indicating the balance of resource allocation and the functional strategy of plants. A higher root-to-shoot ratio in dry weight basis indicates that a more significant proportion of the plant's dry biomass is allocated to the roots, signifying a more significant investment in below-ground structures. The higher number of roots and the availability of nutrients for the root growth in the coir dust medium when compared with the oasis medium might be the reason for the enhanced root–to–shoot ratios on a dry weight basis observed in the experiment.

4. Conclusion

The rooting medium and the IBA concentration influenced the various root and shoot growth parameters (days to bud and root initiation, number of roots and new buds, total root length, average root diameter, and root–to–shoot ratio on fresh weight and dry weight basis) in ‘Marble Queen’ cuttings. According to the findings of the present study, the oasis medium showed better responses in days to root and bud initiation and number of buds. But the coir dust medium recorded the highest number of roots per replicate, roots-to-shoot fresh weight ratio, and average root diameter, in both experiments. Auxin seems to be a useful root initiation hormone and in the present study, the highest number of roots, root length, and days to root initiation were observed in 500 $mg{\cdot}L^{-1}$ of IBA concentration. According to the results of the study, it is recommended to use the coir dust medium with 500 $mg{\cdot}L^{-1}$ IBA application for the efficient growth and development of ‘Marble Queen’ cuttings.

Further studies are needed to confirm the results under mass production and to find out the ability of these cuttings to survive after transplanting. In addition, investigations on the response of physiological parameters such as photosynthesis will help better understand the effects of rooting medium and rooting hormone (IBA).

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