Effect of IBA (Indole Butyric Acid) levels on the growth and rooting of different cutting types of Clerodendrum splendens

Abbas Jamal1*, Gohar Ayub1, Ali Rahman1, Anwar Rashid1, Jawad Ali2 and Muhammad Shahab3

1. Department of Horticulture, The University of Agriculture Peshawar, Pakistan
2. University of Swabi, Khyber Pakhtunkhwa, Pakistan
3. Department of Agronomia, Universidade Estadual de Londrina, Rodovia Celco Garcia Cid (PR 445), km 380, P.O. Box 6001, ZIP 86051-990, Londrina, PR, Brazil
*Corresponding author’s email: abbasjamal143@gmail.com

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Abstract
An experiment to evaluate different IBA levels and cutting types for achieving growth and rooting of Clerodendrum splendens was studied at Ornamental Horticulture Nursery, Department of Horticulture, The University of Agriculture Peshawar, Pakistan during the year 2012. In the experiment two types of cuttings were used i.e. stem cuttings and root cuttings and were treated with 0, 10, 20 and 30% IBA. Cuttings were then planted in polythene bags crammed with the mixture of clay, FYM and sand with the ratio of 1:1:1. IBA with the level of 20% showed best results regarding number of leaves per plant (14.0), number of roots per plant (9.8), root diameter (2.4mm) and survival percentage (60.0%). A significant increase in sprout length (29.1cm) and root length (24.8cm) were observed in cuttings treated with 10% IBA level over the control. Among cuttings, stem cuttings gave good results in all parameters as compared to the root cuttings. Interaction revealed that stem cuttings proved superior when treated with 20% IBA followed by 10% IBA, while root cuttings did not need IBA for rooting and resultant growth as these cuttings rooted and grew well without IBA. Hence it is recommended that stem cuttings need IBA at the rate of 20%, while root cuttings should be used without treating with IBA.

Keywords: IBA; Cuttings; Clerodendrum splendens

Introduction
Clerodendrum splendens is an ornamental flowering plant in the genus Clerodendrum of the family Lamiaceae, native to tropical Western Africa [1]. It is quick growing, vigorous and evergreen climber, commonly called as Flaming Glorybower. Usually growing up to a height of 4 meter. Leaves are large, lush green and arranged oppositely on stem. Flowers are bright red in color, borne in a large terminal or axillary corymbose clusters in winter months. It is grown in evenly moist organically rich, well-drained soil in full sun to partial shade and is propagated through layering, stem and root cuttings [2].

The discovery of auxins as plant growth regulating chemicals, its physiological
effects and practical applications form an attracting story of horticultural research [3]. From these studies, the finding that auxins could stimulate adventitious roots in cuttings was a major breakthrough commercially for plant propagation [4]. Thimann and Koepfli [5] reported that the synthetic Indole-3-Acetic Acid (IAA) has the capability of forming roots. They demonstrated its practical use in stimulating root formation on cuttings. In the same year, the growth regulators Indole-3-Butyric Acid (IBA) and 1-Naphthalene Acetic Acid (NAA) were shown to be more effective than IAA for rooting [6]. All the growth regulators are not equally suitable for rooting performances. Among the growth regulators Indole Butyric Acid is the most commonly and widely used to achieve high percentage of rooting success for the ornamental species [7]. Since IBA has a higher activity, a broader range of effective concentration without toxicity and it is effective in more plant species. Therefore IBA is used more frequently than NAA [8].

When propagation through cuttings becomes very difficult, treatments with growth regulators i.e. IBA are applied in optimum concentration to promote rooting in stem cutting. Activity of growth regulators depends upon the amount of synthetic hormone applied and a particular concentration of growth regulator may be more effective for initiation of root in stem cuttings. Hence, the present investigation was carried out in order to improve the rooting ability of cuttings of this difficult to root climber by using IBA (Indole Butyric Acid). Objectives of the study were to find out best level of IBA for growth and rooting of Clerodendrum splendens, to explore which cutting show better response to IBA and to find out best interaction between IBA and cuttings.

Materials and methods

An experiment entitled “Effect of IBA levels on the growth and rooting of different cutting types of Clerodendrum splendens” was conducted at Ornamental Nursery Farm, Department of Horticulture, The University of Agriculture, Peshawar during the year 2012. The experiment was laid out as RCBD (randomized complete block design) with split-plot arrangement. Stem cuttings and root cuttings of Clerodendrum splendens were assigned to main plot and levels of IBA were randomly assigned to sub plots. The experiment was replicated three times. The experiment included the following factors and their levels:

Cuttings: C1= Stem Cuttings, C2= Root Cuttings

IBA Levels: T0 = 0% (Control), T1 = 10%, T2 = 20%, T3 = 30%

Hardwood stem cuttings containing two buds were taken from healthy and vigorous growing Clerodendrum splendens plant. Slanting cuts were given to the stem cuttings just below the bud at the proximal end. In case of root cuttings, young, vigorous pencil thick roots close to the crown were selected. Any fibrous lateral roots were discarded. Each root was divided into 5cm long cuttings. Required IBA levels, 0%, 10%, 20% and 30% were prepared by mixing IBA with Talc Powder. To prepare 10% IBA, 50mg of IBA was mixed with 450mg of Talc Powder. 20% and 30% IBA were prepared accordingly. 0% IBA contains only Talc Powder. Root and stem cuttings were dipped in the mixture of IBA and Talc Powder. Polythene bags were filled with the planting media which was prepared from the combination of FYM, clay and sand at the ratio of 1:1:1. There were 10 cuttings of each root and stem in each treatment with a total number of 80 cuttings in each replication that makes a total of 240 cuttings for the whole experiment. Cuttings were then planted in polythene bags. The whole
experiment was covered in a plastic tunnel to keep the humidity level high in order to prevent the water loss from the cuttings. Sprout length (cm) and root length (cm) of randomly selected plants for all treatments in each replication were measured with the help of measuring tape and then average was calculated. Average number of roots per cutting and leaves per cutting were recorded for all treatments in each replication. Root diameter (mm) was measured by taking the thickness of the roots that emerged from the cutting with the help of vernier caliper and then mean was calculated and the percentage of survival of cuttings was calculated with the help of the following formula:

\[
\text{Plant Survival } \% = \frac{\text{Total No. of survived cuttings} \times 100}{\text{Total No. of sprouted cuttings}}
\]

Statistical Analysis
Least significant difference (LSD) test was used for the mean comparison. The recorded data collected on each parameter was subjected to analysis of variance (ANOVA) in order to observe the difference between treatments as well as their interaction. For calculating both LSD and ANOVA, computer statistical software MSTATC was applied [9].

Results and Discussions
Sprout length (cm)

Mean data regarding sprout length are described in Table 1. Analysis of variance showed that IBA had significant effect on sprout length and the interaction between cutting types and IBA was also significant. From the mean values of mean table of sprout length, it revealed that highest sprout length (29.1) was recorded in cuttings treated with 10% IBA followed by 20% (27.8) and 30% IBA (23.2) respectively. Whereas minimum sprout length (23.1) was noticed in cutting treated with no IBA. Stem cuttings showed better result (35.0) as compare to the root cuttings (16.7). In interaction, maximum sprout length (41.3) was noticed in stem cutting at 10% IBA, on the other hand root cuttings showed minimum sprout length (15.0) at 30% IBA level. By increasing IBA levels in root cuttings, sprout length decreases this is because root cells are significantly more sensitive to auxin concentrations as compare to the cells of the shoot [10]. The increase in sprout length in stem cuttings is related with rooting. Cuttings treated with IBA gave more rooting which helped in more nutrient uptake and ultimately increased the sprout length. These findings are in accordance with the results of Hussain and Khan [11] who achieved tallest plants and longer roots in two Rosa species by treatment with IBA.

Table 1. Mean table for sprout length of cutting types as affected by IBA levels

| IBA levels (%) | Cutting types | Means |
|----------------|---------------|-------|
| 0              | Stem          | 27.4  |
| 10             | Stem          | 41.3  |
| 20             | Stem          | 39.8  |
| 30             | Stem          | 31.4  |
| Means          | Stem          | 35.0a |
| 0              | Root          | 18.9  |
| 10             | Root          | 17.0  |
| 20             | Root          | 15.8  |
| 30             | Root          | 15.0  |
| Means          | Root          | 16.7b |

LSD value for Cuttings (C) at 5% level of probability = 2.842975
LSD value for IBA (I) at 5% level of probability = 1.776487
LSD value for C x I at 5% level of probability = 2.512332
**Root Length (cm)**

The mean data regarding root length are presented in mean Table 2. The ANOVA table showed that the length of root is significantly affected by IBA at different levels, while interaction between cuttings type and IBA had also shown a significant effect on the length of root of *Clerodendrum splendens* cuttings. Mean values shows that maximum root length (24.8) was noticed in cuttings at concentration of 10% IBA while minimum root length (19.4) was recorded in control. In different cutting types stem cuttings showed better result (30.3) as compare to the root cuttings (13.3). Specific trend was observed so far in interaction. Longest roots (34.6) were observed at 10% IBA in stem cutting, while root cuttings showed poor result (10.8) at 30% IBA. The above results might be due to the reason that the sprouted cuttings were having high foliage and leaves are the main site of food production which is translocated to the roots for development. On the other hand, IBA helped the roots to grow deeper in the soil for locating more nutrients and thus resulted in increased root length. These results are in partial agreement with Hussain and Khan [11] who reported that IBA produced significantly longer roots and maximum root percentage in *Rosa* species than control.

| IBA levels | Cutting types | Means |
|------------|---------------|-------|
|            | Stem          | Root  |
| 0          | 25.0          | 15.5  | 20.2c |
| 10         | 34.6          | 15.1  | 24.8a |
| 20         | 33.7          | 11.7  | 22.7b |
| 30         | 28.0          | 10.8  | 19.4c |
|            | 30.3a         | 13.3b |

LSD value for Cuttings (C) at 5% level of probability = 1.228963
LSD value for IBA (I) at 5% level of Probability = 2.00454
LSD value for C x I at 5% level of probability = 2.834847

**Number of leaves per plant**

The data pertaining to the number of leaves per plant are presented in Table 3. On the basis of Analysis of variance, number of leaves per plant was significantly affected by various concentrations of Indole Butyric Acid, while types of cuttings and their interaction had also a significant effect.

It is evident from the results that maximum number of leaves per plant (14.0) was observed on cuttings which were treated with 20% IBA, followed by 10% IBA (12.3) and 0% IBA (12.0). Minimum number of leaves per plant (10.6) was recorded in cuttings treated with 30% IBA. The mean value for the cutting types showed that maximum number of leaves (15.1) were noted in stem cutting as compare to the root cuttings (9.4). This is because the root cuttings took more than 40 days to sprout as compare to the stem cuttings which sprouted in about two weeks. In interaction more no. of leaves (18.6) were produced in stem cutting treated with 20% IBA, while root cuttings showed less number of leaves (6.9) at 30% IBA level.

The increase in number of leaves on 20% IBA concentration may be due to more number of roots which resulted in increased plant height and subsequently branches per plant. These results are partial in agreement with Siddiqui and Hussain [12] who reported that after treatment of *Ficus hawaii*...
cuttings with IBA produced more no. of leaves. Bhattacharjee and Balakrishna [13] also conducted an experiment on several ornamental climbers including *Clerodendrum splendens* and noticed that by dipping the stem cuttings in IBA solutions can increase the rooting and the number of leaves in the cuttings.

**Table 3. Mean table for number of leaves per plant of cutting types as affected by IBA levels**

| IBA levels (%) | Cutting Types | Means |
|----------------|---------------|-------|
| 0              | Stem          | 13.3  |
| 10             | 14.1          | 10.5  |
| 20             | 18.6          | 9.4   |
| 30             | 14.4          | 6.9   |
| Means          | 15.1          | 9.4b  |

LSD value for Cuttings (C) at 5% level of probability = 1.309794  
LSD value for IBA (I) at 5% level of probability = 1.562742  
LSD value for C x I at 5% level of probability = 2.210051

**Number of roots per plant**

The data regarding number of roots per plant is presented in table 4. According to ANOVA, IBA levels and its interaction had significant effect on number of roots per plant. More number of roots per plant (9.8) occurred with IBA at the concentration of 20% followed by 10% IBA (8.8) and 30% IBA (7.6) whereas at 0% IBA plants produced least number of roots per plant (7.1). In case of cutting types more number of roots per plant (10.6) were recorded in stem cuttings. Minimum number of roots per plant (6.6) in root cuttings were also noticed. As already mentioned that there was a significant interaction between cutting types and IBA levels, Stem cuttings at 20% IBA level produced maximum number of roots per plant (12.6) while minimum number of roots (5.8) were observed in root cuttings with at the rate of 30%. The poor results regarding root cuttings was due to the sensitivity of root cells toward IBA levels as compare to shoot cells. So roots cannot tolerate high concentrations of auxin [10]. These results are in conformity with the results of Bhattacharjee and Balakrishna [13] who noticed that dipping the stem cuttings of *Clerodendrum splendens* in IBA solutions (1000-6000 ppm) improved the number of roots.

**Table 4. Mean table for number of roots per plant of cutting types as affected by IBA levels**

| IBA levels (%) | Cutting Types | Means |
|----------------|---------------|-------|
| 0              | Stem          | 8.3   |
| 10             | 9.8           | 7.8   |
| 20             | 12.6          | 7.0   |
| 30             | 9.4           | 5.8   |
| Means          | 10.0a         | 6.6b  |

LSD value for Cuttings (C) at 5% level of probability = 1.842436  
LSD value for IBA (I) at 5% level of Probability = 0.862316  
LSD value for C x I at 5% level of probability = 1.219499
Root Diameter (mm)
The data pertaining to the root diameters are presented in Table 5. ANOVA showed that root diameter is significantly affected by IBA levels, as well as the interaction between cutting types and IBA was also significant. Maximum root diameter (2.4) was observed with IBA at 20%, followed by 10% and 30% (2.3), (2.2) whereas cuttings in control produced roots with minimum diameter (2.1). By comparing cutting types, stem cuttings produced roots with maximum diameter (2.5) as compared to the root cuttings (2.0). In interaction linear relationship was observed, stem cuttings at 20% IBA level showed best results (2.8) regarding the diameter of root, while poor results in root cuttings (1.8) was observed with 30% IBA. This may due to the inhibitory or toxic effect of IBA at higher concentration [14]. These current results are also in positive relation with Singh and Singh [15] who also observed maximum root diameter in Clerodendrum thomsoniae cuttings when treated with IBA.

### Table 5. Mean table for root diameter of cutting types as affected by IBA levels

| IBA levels (%) | Cuttings type | Means |
|----------------|---------------|-------|
|                | Stem          | Root  |       |
| 0              | 2.2           | 2.0   | 2.1c  |
| 10             | 2.4           | 2.2   | 2.3ab |
| 20             | 2.8           | 2.0   | 2.4a  |
| 30             | 2.5           | 1.8   | 2.2bc |
| Means          | 2.5a          | 2.0 b |       |

LSD value for Cuttings (C) at 5% level of probability = 0.224462
LSD value for IBA (I) at 5% level of Probability = 0.134532
LSD value for C x I at 5% level of probability = 10.190257

Survival percentage
The data pertaining to the percentage of survival are presented in Table 6, the analysis of variance for IBA levels and cutting types showed highly significant effect on survival percentage of sprouted cuttings. And their interaction was also significant. According to the mean value, lowest survival percentage (46.7%) was recorded in cuttings treated at 30% IBA level while best survival percentage (60%) was noted in cuttings treated with IBA at 20% concentration. Comparing different types of cuttings, maximum survival percentage (57.2%) was observed in stem cuttings followed by root cuttings (47.5%). The interaction revealed that the stem cuttings showed best result (73.3%) regarding survival percentage at 20% IBA, In case of root cuttings minimum survival percentage (40%) was noted at 30% IBA. Maximum percentage of survival at 20% IBA may be due to the reason that IBA enhanced lengthy roots which subsequently increased the number of leaves per plant. Nutrients uptake and photosynthates production provides sufficient food contents for the metabolic activities of the plants. These results are in accordance with Ahmad et al. [16] who reported that the Bougainvillea cuttings when treated with IBA showed maximum survival percentage. Root cutting shows good result at 0% IBA due to the fact that root cells are more sensitive and showed inhibitory effect to the synthetic auxin. These results in root cuttings are also in accordance with Campagnolo and Rafael [17] who concluded best survival percentage in the root cuttings of blackberries at 0% IBA.
Table 6. Mean table for survival percentage of cutting types as affected by IBA levels

| IBA levels | Cutting types | Means  |
|------------|---------------|--------|
|            | Stem          | Root   |
| 0          | 43.3          | 56.7   |
| 10%        | 60.0          | 46.7   |
| 20%        | 73.3          | 46.7   |
| 30%        | 53.3          | 40.0   |
| Means      | 57.5a         | 47.5b  |

LSD value for Cuttings (C) at 5% level of probability = 6.210344
LSD value for IBA (I) at 5% level of Probability = 8.516282
LSD value for C x I at 5% level of probability = 12.04384

Conclusion
The following conclusions were drawn from the findings of above experiment. IBA level of 20% showed best results regarding, number of leaves per plant, number of roots per plant, root diameter and survival percentage. Sprout length and root length were best recorded at IBA level of 10%. Stem cuttings showed good result at 20% IBA followed by 10% IBA in almost all parameters. Root cuttings showed better result without IBA.

Recommendations
The following recommendations are drawn from the study for future. Since IBA level at 20 % has shown best results regarding rooting and growth, therefore it is recommended for Stem cuttings of Clerodendrum splendens. It is also recommended that root cuttings may not be treated with IBA. However, further research to narrow the intervals of IBA levels (10, 15, 20 and 25%) should be carried out on Stem cuttings of Clerodendrum splendens.

Authors’ contributions
Conceived and designed the experiments: G Ayub. Performed the experiments: A Jamal & M Shahab. Analyzed the data: A Jamal, A Rahman, J Ali, G Ayub, A Rashid & M Shahab. Contributed reagents/ materials/ analysis tools: A Rashid & M Shahab, Wrote the paper: A Jamal & G Ayub.

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