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Effect of Plant Growth Regulators (PGRs) on the Rooting behavior of Hardwood Cuttings of *Alstonia scholaris*

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Abstract

An experiment was conducted to evaluate the vegetative propagation of *Alstonia scholaris*. Stem cuttings were sourced from superior genotypes selected from the surrounding areas of Bihar. The study aimed to assess the effects of different concentrations of auxins on the rooting behavior of stem cuttings. The results revealed significant variations in rooting behavior across treatments, highlighting the critical role of auxins in successful propagation. The study developed a practical and efficient protocol for rapid cutting propagation, recommending an IBA concentration of 1000 mg L^{-1} as optimal for achieving maximum rooting in *Alstonia scholaris* stem cuttings. This treatment demonstrated the highest rooting percentage, number of roots, sprouting percentage, root length, shoot length, root biomass, shoot biomass, and total biomass, underscoring the importance of auxin concentration in vegetative propagation. With a rooting success rate exceeding eighty percent, the protocol is expected to facilitate the large-scale production of clonal plantations of *Alstonia scholaris*.

Keywords: *Alstonia scholaris*, superior genotypes, vegetative propagation, auxin, IBA.

Introduction

Alstonia scholaris, commonly known as the "Indian Devil Tree," "Blackboard Tree," or "Saptaparni," is a tropical evergreen tree belonging to the family Apocynaceae. It is native to Southeast Asia, South Asia, and parts of Australia and is highly valued for its medicinal properties, cultural significance, and timber. This tall tree can reach heights of up to 40 meters, with smooth, light-gray bark and elliptical leaves arranged in whorls. Its small, white, fragrant flowers appear in clusters, eventually developing into long, narrow fruits containing numerous seeds. These seeds are equipped with silky hairs to facilitate wind dispersal (Bhandary, 2020).Various parts of *A. scholaris*, including the bark, roots, and leaves, are rich in secondary metabolites that exhibit therapeutic effects. Traditionally, the plant has been used to treat respiratory conditions such as asthma and bronchitis, as well as fevers and infections. It is also known for its antimalarial, anti-inflammatory, antioxidant, and antimicrobial properties (Chassagne, 2022). In many traditional practices, the tree holds cultural and religious significance. A bitter herbal drink prepared from its bark is believed to protect against ailments and promote overall well-being. Ethnomedicinal studies from the Lower Mekong Basin highlight the use of *A. scholaris* as an antidiarrheal and antidysenteric agent. Its bark and leaves are particularly valued for these purposes, with similar uses reported in India, Indonesia, Papua New Guinea, and the Philippines (Khyade *et al.*, 2014). Scientific studies have validated many traditional claims about *A. scholaris*. For instance, methanolic extracts of the plant have demonstrated significant antidiarrheal activity in experimental models. However, the plant's pharmacological properties also raise concerns about toxicity. Methanolic extracts tested for acute and sub-acute toxicity showed liver damage in experimental models. These findings suggest the need for caution regarding the long-term use of *A. scholaris* and highlight the importance of further research to establish safety guidelines for human use (Chassagne, 2022).

Beyond its antidiarrheal properties, the plant exhibits a wide range of pharmacological activities, including anticancer, antimicrobial, molluscicidal, anxiolytic, and antipsychotic effects. These findings align with its extensive use in alternative medicine systems (Dey, 2011).

The seeds of *A. scholaris* are best sown in sunny locations. Fresh seeds have a high germination rate, with sprouting beginning within 12 days and continuing for up to three months. Seed collection, however, poses challenges, as the fruits release seeds while still on the tree. Proper storage in airtight containers can maintain a germination rate of up to 90% for two months (Bhandary, 2020). For large-scale propagation, vegetative methods such as cleft grafting and inverted T-grafting have proven effective. These techniques are especially useful for conserving superior genotypes and improving propagation success rates. The use of growth regulators further enhances the potential for mass multiplication (Khyade *et al.,* 2014). Vegetative propagation techniques are invaluable tools for the large-scale multiplication of superior phenotypes and genotypes, enabling the production of uniform, true-to-type plants (Leakey *et al.,* 1983). Among these methods, rooting of stem cuttings is considered one of the simplest and most cost-effective approaches, commonly utilized for propagating various tree species. However, in the case of *Alstonia scholaris*, achieving consistent success in rooting stem cuttings has proven to be challenging. The success of rooting in stem cuttings is influenced by several factors, including the age and type of the branch selected, season during propagation, and the application of exogenous rooting-promoting chemicals. Understanding these critical factors is essential for optimizing rooting efficiency (Babu, *et al.,* 2018; Gehlot *et al.,* 2014).

In light of these challenges, the present study aims to develop an efficient, vigorous, cost-effective, and reproducible vegetative propagation protocol through stem cuttings. This protocol is intended to facilitate the mass-scale commercial propagation of selected *Alstonia scholaris* genotypes, ensuring their availability for large-scale applications.

Materials and Methods

The experiment was conducted in the mist chamber at Bihar Agricultural University during the 2019–20 growing season to investigate vegetative propagation of *Alstonia scholaris*. Stem cuttings were prepared from superior genotypes selected from the surrounding areas of Bihar. Plus trees were identified based on their phenotypically superior stem characteristics, crown morphology, and overall stature within the stand. One-year-old branches were collected from trees aged 10 years during February. Leafless cuttings, measuring approximately 18 ± 2.5 cm in length and 2–3 cm in diameter and containing 2–4 buds, were prepared using sharp secateurs to ensure clean cuts. Immediately after preparation, the cuttings were placed in water at 10°C to prevent desiccation. Mature cuttings were transported to the laboratory, where they were treated with Indole-3-Butyric Acid (IBA) and Indole-3-Acetic Acid (IAA) at concentrations of 0, 100, 500, 700 and 1000 mg L^{-1}. These solutions were prepared by dissolving the respective amounts of IBA and IAA in 5–10 mL of methanol and then diluting to a final volume of 1000 mL with distilled water. The prepared solutions were transferred into ten separate containers for treatment. The cuttings were divided into nine groups, each containing 27 cuttings. The basal 2.5 cm of each cutting was dipped in the respective IBA or IAA solutions for 24 hours. To prevent water loss and fungal infection during continuous misting, the upper cut ends of the treated cuttings were sealed with inert paraffin wax. Following treatment, the cuttings were immediately planted in polybags filled with a rooting medium. Approximately one-third of the length of each cutting was inserted into the rooting medium, and the polybags were arranged in a Completely Randomized Design (CRD). To prevent pathogen attack, the basal ends of the cuttings were dipped in a 0.2% Bavistin solution prior to planting. Observations on rooting percentage, sprouting percentage, root number; root length, root biomass, and shoot biomass were recorded 90 days after planting. Data on rooting and sprouting percentages did not follow a normal distribution; therefore, an arc sine transformation was applied. Similarly, data on root number and leaf number were transformed using a square root transformation method. The transformed data were analyzed using analysis of variance (ANOVA) as per statistical methods outlined by Singh (2001). The significance of treatments was evaluated using the F-test.

Results and Discussion

The current investigation demonstrated that treatments with IBA were more effective than IAA in promoting the rooting of stem cuttings. Additionally, the rooting percentage increased progressively as the concentration of auxins rose from 100 mg L^{-1} to 1000 mg L^{-1}. These findings align with previous research, which highlighted that IBA is generally more effective

than IAA and NAA in stimulating rooting in stem cuttings (Babaie *et al.,* 2014; Gupta *et al.,* 2003; Sharma and Pandey, 1999). IBA is considered a stronger auxin, while IAA is more prone to degradation (Leakey, 2004). It is possible that IBA facilitates rooting by elevating the levels of free IBA within tissues or by interacting with IAA, either by enhancing its activity or influencing the synthesis of IAA. This could lead to an increased sensitivity of tissues to IAA and thus promote rooting (Babaie *et al.,* 2014). The results from this study confirm that higher concentrations of IBA and IAA are needed to boost rooting percentages, particularly in mature cuttings of species that are difficult to root, due to their inherently low levels of endogenous auxin.

Rooting Percentage:

The rooting percentage varied significantly across treatments. The control (water) group exhibited the lowest rooting percentage at 15.59%, while the highest was observed in IBA 1000 mg L^{-1}, with a value of 75.96%. The range of the rooting percentage between the control and IBA 1000 mg L^{-1} was 60.37%, indicating a strong positive effect of IBA on rooting success. The standard error (SE) confirming the significance of the differences observed. The minimum value was 15.59% (Control), and the maximum value was 75.96% (IBA 1000 mg L^{-1}).

Number of Roots:

The number of roots per cutting was significantly higher in the IBA treatments compared to the control. The control group had the lowest number of roots (5.47), while IBA 1000 mg L^{-1} produced the highest number of roots (17.96). The range in the number of roots was 12.49 between the control and IBA 1000 mg L^{-1} which indicates that the differences between treatments are statistically significant. The minimum value for number of roots was 5.47 (Control), and the maximum value was 17.96 (IBA 1000 mg L^{-1}).

Sprouting Percentage

The sprouting percentage also exhibited significant variation, with the highest sprouting percentage (87.94%) observed in IBA 1000 mg L^{-1}, and the lowest (17.51%) in the control. The range in sprouting percentage was 70.43%, indicating the considerable significance effect of IBA treatments on sprouting success. The minimum sprouting percentage was 17.51% (Control), and the maximum was 87.94% (IBA 1000 mg L^{-1}).

Root Length

Root length followed a similar trend, with IBA 1000 mg L^{-1} resulting in the longest roots (11.82 cm) and the control group showing the shortest roots (2.78 cm). The range in root length between the control and IBA 1000 mg L^{-1} was 9.04 cm and indicating significant differences between the treatments. The minimum root length was 2.78 cm (Control), and the maximum was 11.82 cm (IBA 1000 mg L^{-1}).

Shoot Length

Shoot length was also significantly greater in the IBA treatments compared to the control. The longest shoots were observed with IBA 1000 mg L^{-1} (36.37 cm), while the shortest shoots were in the control group (16.60 cm). The range of shoot length between the control and IBA 1000 mg L^{-1} was 19.77 cm. The minimum value for shoot length was 16.60 cm (Control), and the maximum was 36.37 cm (IBA 1000 mg L^{-1}).

Root Biomass

Root biomass showed a significant increase with IBA treatments, particularly at 1000 mg L^{-1}, which produced the highest root biomass (6.55 g). The control group had the lowest root biomass (3.39 g). The range in root biomass between the control and IBA 1000 mg L^{-1} was 3.16 g. The minimum value for root biomass was 3.39 g (Control), and the maximum value was 6.55 g (IBA 1000 mg L^{-1}).

Shoot Biomass

Shoot biomass was significantly greater in the IBA treatments, particularly with IBA 1000 mg L^{-1} (11.10 g). The control group had the lowest shoot biomass at 7.54 g. The range in shoot biomass between the control and IBA 1000 mg L^{-1} was 3.56 g. The minimum value for shoot biomass was 7.54 g (Control), and the maximum was 11.10 g (IBA 1000 mg L^{-1}).

Total Biomass

Total biomass, which includes both root and shoot biomass, followed a similar pattern, with the highest total biomass (17.65 g) observed in IBA 1000 mg L^{-1}, and the lowest total biomass (10.93 g) in the control group. The range in total biomass was 6.72 g between the control and IBA 1000 mg L^{-1}. The minimum value for total biomass was 10.93 g (Control), and the maximum was 17.65 g (IBA 1000 mg L^{-1}).

Conclusion

The present study concluded that significant variations were observed in the rooting behavior of stem cuttings when treated with different

concentrations of auxins. A practical and efficient protocol for rapid cutting propagation was developed for the mass multiplication of Alstonia scholaris. The study recommends using an IBA concentration of 1000 mg L^{-1} to achieve optimal rooting in stem cuttings, thereby enhancing the success of vegetative propagation in Alstonia scholaris. The significant differences in all parameters (rooting percentage, number of roots, sprouting percentage, root length, shoot length, root biomass, shoot biomass, and total biomass) underline the importance of auxin concentration in enhancing the vegetative propagation of Alstonia scholaris. IBA 1000 mg L^{-1} emerged as the optimal treatment for improving rooting and growth characteristics, with its effectiveness demonstrated by significant statistical values. This approach is expected to result in a rooting success rate of over eighty percent, facilitating the establishment of clonal plantations.

Table1: Effect of different levels of IAA and IBA on rooting and proliferation of cutting of *Alstonia scholaris*

Treatments	Rooting %	No. of Roots	Sprouting (%)	Root length (cm)	Shoot length (cm)	Root Biomass (g)	Shoot Biomass (g)	Total Biomass (g)
Control (water)	15.59	5.47	17.51	2.78	16.60	3.39	7.54	10.93
IAA 100 mg L^{-1}	21.82	5.45	22.87	2.96	20.11	3.54	7.58	11.12
IAA 500 mg L^{-1}	30.58	6.49	38.07	4.20	23.78	3.79	7.97	11.76
IAA 700 mg L^{-1}	40.30	7.90	56.62	6.30	28.66	4.89	8.83	13.72
IAA 1000 mg L^{-1}	65.42	9.50	73.17	9.26	32.07	5.86	9.02	14.88
IBA 100 mg L^{-1}	21.58	6.75	26.04	4.48	16.95	3.62	8.14	11.76
IBA 500 mg L^{-1}	27.10	9.15	43.86	6.05	25.91	4.87	9.08	13.95
IBA 700 mg L^{-1}	57.07	16.06	62.95	9.11	29.82	5.50	10.24	15.74
IBA 1000 mg L^{-1}	75.96	17.96	87.94	11.82	36.37	6.55	11.10	17.65
F Test	S	S	S	S	S	S	S	S
SE(±)	1.545	0.650	0.462	0.605	0.641	0.557	0.461	0.832
CD at 0.05%	1.673	1.416	1.313	1.627	1.184	0.736	0.758	1.812

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