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Influence of Rooting Hormones on Root Induction in *Diospyros lotus* L. Branch Cuttings

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Abstract

An experiment was conducted during 2024–2025 at the Experimental Farm of the Division of Silviculture and Agroforestry, SKUAST-Jammu, Chatha, to evaluate the effects of indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) on the rooting and early growth of *Diospyros lotus* L. branch cuttings. The study used a completely randomised design with a factorial arrangement comprising seven rooting-hormone treatments and two planting seasons, giving 14 treatment combinations. Each combination had three replications of 10 cuttings. Cuttings were soaked for 24 h in IBA and NAA at 25, 50 and 100 ppm, while untreated cuttings served as the control. Planting was undertaken during spring (March–April) and the rainy season (July–August). Sprouting percentage, rooting percentage, fresh root biomass, average shoot length, and average root length were recorded. Rooting-hormone treatments significantly improved all measured parameters compared with the control. IBA at 100 ppm produced the highest sprouting percentage (52.58%), rooting percentage (44.45%), fresh root biomass (1.21 g), average shoot length (9.25 cm), and average root

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length (4.24 cm). Spring planting produced higher mean values than rainy-season planting for all assessed traits. The hormone × season interaction was non-significant, indicating that the relative treatment responses were broadly consistent across the two seasons. Under the conditions of this study, soaking branch cuttings in IBA at 100 ppm for 24 h was the most effective treatment for improving nursery establishment of *Diospyros lotus* L.

Keywords: Diospyros lotus L.; adventitious rooting; indole-3-butyric acid (IBA); naphthalene acetic acid (NAA); vegetative propagation; branch cuttings; nursery propagation.

1. Introduction

Diospyros lotus L., commonly known as date plum or Caucasian persimmon, belongs to the family Ebenaceae and is an economically and ecologically important deciduous fruit tree species distributed across temperate and subtropical regions of Asia and Europe. The species is native to parts of Central Asia, China, the Caucasus, and the Himalayan region, where it grows under diverse agro-climatic conditions and is widely valued as a rootstock for persimmon cultivation owing to its adaptability, drought tolerance, and strong root system (Liu *et al*., 2023). The genus *Diospyros* comprises numerous species with significant nutritional, medicinal, and economic value. Many species are known for their edible fruits, medicinal properties, and valuable timber. Different plant parts contain bioactive compounds such as polyphenols, tannins, terpenoids, and naphthoquinones that contribute to antioxidant, antimicrobial, anti-inflammatory, and antidiabetic activities (Rauf *et al*., 2017; Mallavadhani *et al*., 1998). Fruits and leaves of *Diospyros lotus* L. have traditionally been used in folk medicine and possess considerable antioxidant potential, further increasing the significance of this species for horticultural and pharmaceutical applications (Hwang *et al*., 2008; Zhang *et al*., 2018). Despite its importance, large-scale cultivation and conservation of *Diospyros lotus* L. are often limited by inefficient propagation methods. Conventionally, the species is propagated through seeds; however, seed propagation is associated with genetic variability, irregular germination, prolonged juvenile periods, and lack of true-to-type progeny. Such limitations reduce uniformity and delay orchard establishment, thereby necessitating the development of efficient vegetative propagation techniques (Hartmann *et al*., 2011). Vegetative propagation through branch cuttings is considered one of the most practical and economical methods for multiplying woody perennial species. Propagation by cuttings ensures genetic uniformity and preserves desirable characteristics of the mother plant, including vigour, growth habit, and stress tolerance. However, successful rooting in woody species depends on several interacting factors such as the physiological condition of the cutting, the season of collection, the rooting medium, environmental conditions, and the application of growth regulators (Hartmann *et al*., 2011). Among the various factors affecting rooting, auxin-based rooting hormones, particularly indole-3-butyric acid (IBA), play a central role in stimulating adventitious root initiation and development. Numerous studies have demonstrated that IBA enhances rooting percentage, root number, and root quality in woody plant cuttings. In *Diospyros* species, the application of IBA has been reported to improve rooting success and plant establishment, indicating its importance in vegetative propagation protocols (Tetsumura, 2000; Hwang *et al*., 2008). Likewise, recent propagation studies in *Diospyros lotus* L. have shown positive responses to auxin-mediated rooting, emphasising the potential for developing standardised multiplication techniques (Liu *et al*., 2023). Considering the increasing demand for quality planting material and the limitations of seed propagation, standardisation of branch cutting propagation in *Diospyros lotus* L. is essential. Investigations involving rooting hormones and suitable propagation media may contribute significantly to improving rooting efficiency, nursery production, and large-scale cultivation of this valuable species. Recent studies indicate that auxin effects on cuttings are shaped by the physiological condition of the propagule and the rooting environment, with responses varying across woody taxa and treatment protocols (Zine El Abidine *et al*., 2019; Zhao *et al*., 2022; Liu *et al*., 2025). Experimental evidence also shows that both conventional IBA/NAA treatments and sustained auxin delivery can enhance adventitious rooting, although the magnitude of response remains species- and protocol-dependent (Sivakumar *et al*., 2024; Roth *et al*., 2024).

Research Gap: However, the comparative effects of low-concentration IBA and NAA soaking treatments on *Diospyros lotus* L. branch cuttings across spring and rainy-season nursery conditions remain insufficiently documented. The combined assessment of sprouting, rooting, shoot and root growth, and fresh root biomass under these seasonal conditions is particularly limited.

Objective of the Study: The objective of the present study was to evaluate the effects of IBA and NAA at 25, 50 and 100 ppm, applied as 24-hour soaking treatments, on sprouting percentage, rooting percentage, fresh root biomass, average shoot length and average root length of *Diospyros lotus* L. branch cuttings during spring and rainy seasons.

2. Material and Methods

Experimental Details: The present investigation, entitled "Influence of rooting hormones on root induction in *Diospyros lotus* L. branch cuttings", was conducted at the Agroforestry Farm, Chatha, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu (SKUAST-J). The experiment was conducted using a completely randomised design (CRD) with a factorial arrangement consisting of two factors, namely rooting hormones and growing seasons, with 14 treatment combinations and three replications.

Factor I comprised seven rooting hormone treatments, including soaking in indole-3-butyric acid (IBA) at 25, 50 and 100 ppm, soaking in naphthalene acetic acid (NAA) at 25, 50 and 100 ppm, and an untreated control. Factor II consisted of two growing seasons, namely the spring season (March–April) and the rainy season (July–August). Thus, a total of fourteen treatment combinations were evaluated to study their influence on rooting and growth behaviour in branch cuttings of *Diospyros lotus* L.

Material Used: The experimental material consisted of branch cuttings of *Diospyros lotus* L. collected from 10 healthy, disease-free, phenotypically superior mother plants (approximately 8–12 years old) growing in Poonch Division of Jammu and Kashmir (with the necessary institutional permission and in compliance with applicable local guidelines and regulations). Uniform cuttings measuring 15–20 cm in length and 0.8–1.5 cm in diameter, each containing 3–4 nodes, were prepared using sterilised secateurs. All leaves were removed before hormone treatment to minimise transpiration losses. Each treatment consisted of 10 cuttings per replication with three replications, resulting in 30 cuttings per treatment combination. Following hormone treatment, the cuttings were planted in unamended field nursery soil, which served as the rooting medium. The basal ends of the cuttings were cut slantwise to increase the absorption surface area and facilitate uptake of growth regulator solutions. The materials used during the investigation included secateurs, pruning knife, measuring cylinder, weighing balance, plastic containers, rooting trays/polybags, distilled water, and growth regulators, namely indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA). Appropriate standard nursery management practices were employed for successful establishment of the cuttings.

Preparation of IBA and NAA Solutions: The growth regulator formulations of indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) were prepared at concentrations of 25 ppm, 50 ppm and 100 ppm using analytical-grade chemicals and distilled water. Since both IBA and NAA are sparingly soluble in water, the required quantity of each growth regulator was first dissolved in a small quantity of ethyl alcohol (95%) to ensure complete dissolution and then diluted with distilled water to make up the final volume. For the preparation of the stock solutions, the required amount of hormone was weighed accurately using an electronic balance. The concentration of plant growth regulator solutions was calculated on a ppm basis, where 1 ppm corresponds to 1 mg of solute per litre of solution.

To prepare a 25 ppm solution, 25 mg of IBA or NAA was weighed and dissolved in a small quantity of ethyl alcohol (approximately 2–5 mL). After complete dissolution, distilled water was added gradually and the final volume was made up to 1 litre in a volumetric flask or measuring cylinder.

To prepare a 50 ppm solution, 50 mg of IBA or NAA was accurately weighed and dissolved in a small quantity of ethyl alcohol. The solution was then diluted with distilled water and the final volume was adjusted to 1 litre to obtain the desired concentration.

To prepare a 100 ppm solution, 100 mg of IBA or NAA was weighed and initially dissolved in a small quantity of ethyl alcohol. Distilled water was subsequently added and the total volume was made up to 1 litre to obtain a 100 ppm solution. Separate glass containers were used for each hormone and concentration to prevent contamination and ensure accuracy of treatment preparation. Fresh solutions were prepared prior to treatment application to maintain the effectiveness of the growth regulators. The basal portions of branch cuttings were soaked in the respective hormone solutions for 24 h according to the treatment schedule before planting.

Formula Used:

The quantity of growth regulator required was calculated using the formula:

$$\text{ppm} = \frac{\text{Weight of solute (mg)}}{\text{Volume of solution (L)}}$$

or

$$\text{Weight required (mg)} = \text{Desired concentration (ppm)} \times \text{Volume (L)}$$

This procedure ensured accurate and uniform preparation of IBA and NAA formulations for treatment of *Diospyros lotus* L. branch cuttings.

Treatment of branch cuttings and planting: The treatments included soaking in IBA at 25, 50 and 100 ppm, soaking in NAA at 25, 50 and 100 ppm, and an untreated control. After the soaking period, the treated cuttings were planted in beds under nursery conditions during two planting seasons, namely the spring season (March–April) and the rainy season (July–August). Cuttings were inserted 6–8 cm deep, and the surrounding soil was gently firmed to hold them in place while avoiding compaction to ensure adequate aeration of the rooting zone. They were watered twice daily (morning and evening), and weeds were removed as required.

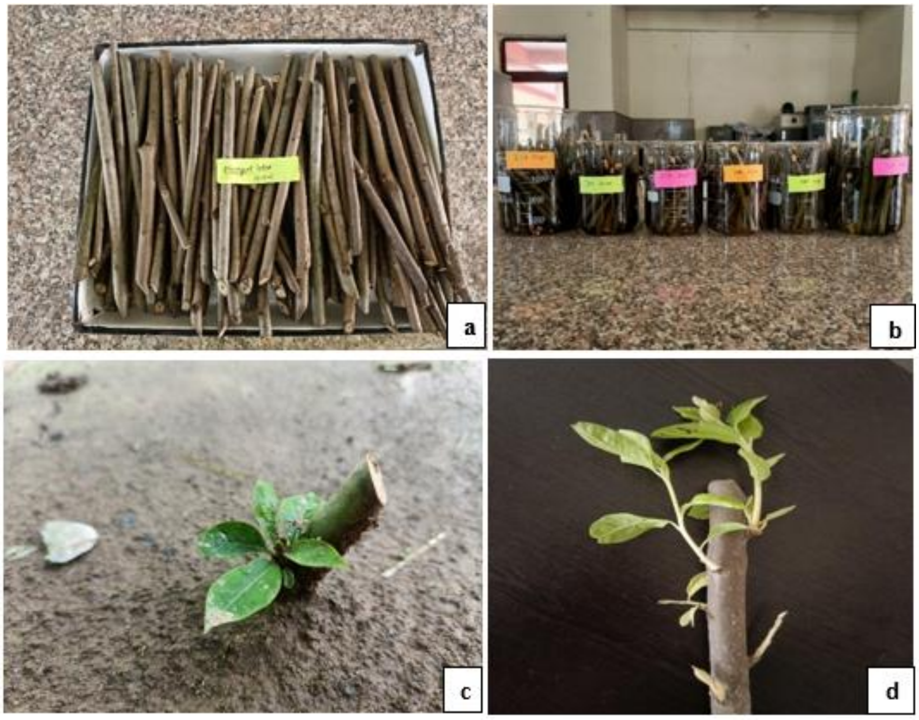

Fig. 1. (Clockwise from a to d): a. Preparation of branch cuttings; b. Dipping of branch cuttings in rooting hormonal solution; c & d. Sprouting of branch cuttings

The observations recorded during the study included sprouting percentage, rooting percentage, fresh root biomass, average shoot length, and average root length. The data from the current investigation were analysed statistically using OP STAT and Microsoft Excel. A critical difference (CD) at the 5% level of significance was employed to assess differences among the treatment means (Gomez & Gomez, 1984). Before conducting the analysis of variance (ANOVA), the assumptions of normality of residuals and homogeneity of variances were assessed and found to be satisfied. Therefore, the sprouting and rooting percentage data were analysed using the original (untransformed) values.

3. Results and Discussion

The corrected numbering is as follows: the main effects are presented in Table 1, the hormone x season interaction is presented in Table 2, and the graphical summaries are presented sequentially as Figs. 2-6.

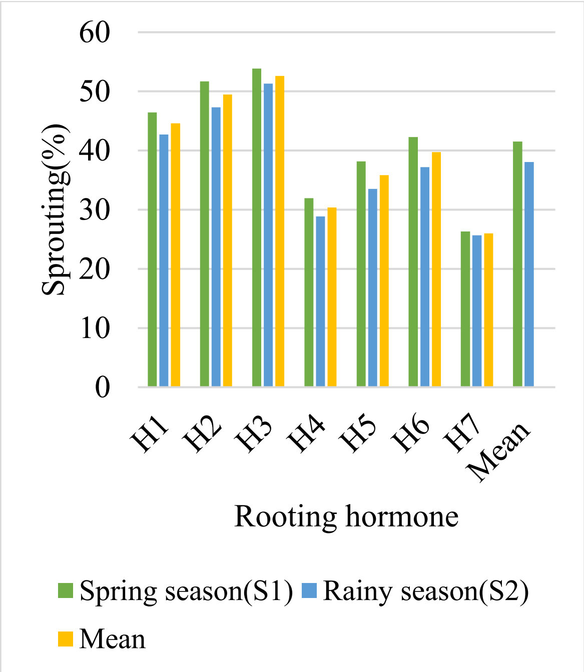

Fig. 2. Effect of different hormonal treatments and seasons on sprouting percentage (%) of *Diospyros lotus L.* branch cuttings

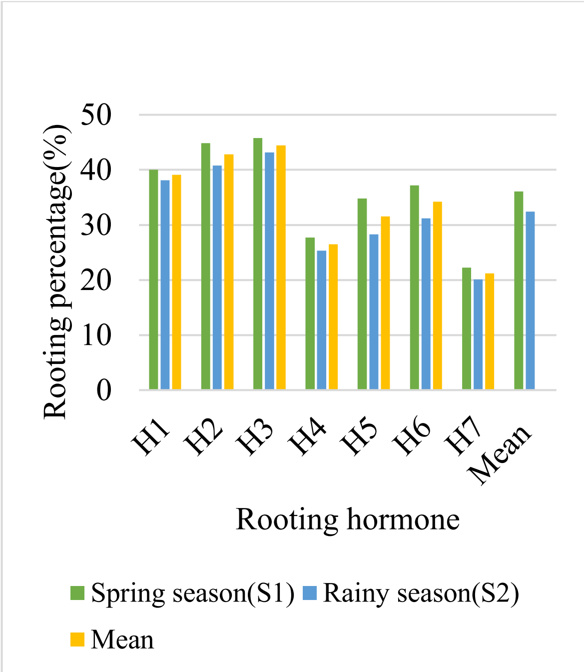

Fig. 3. Effect of different hormonal treatments and seasons on rooting percentage (%) of *Diospyros lotus L.* branch cuttings

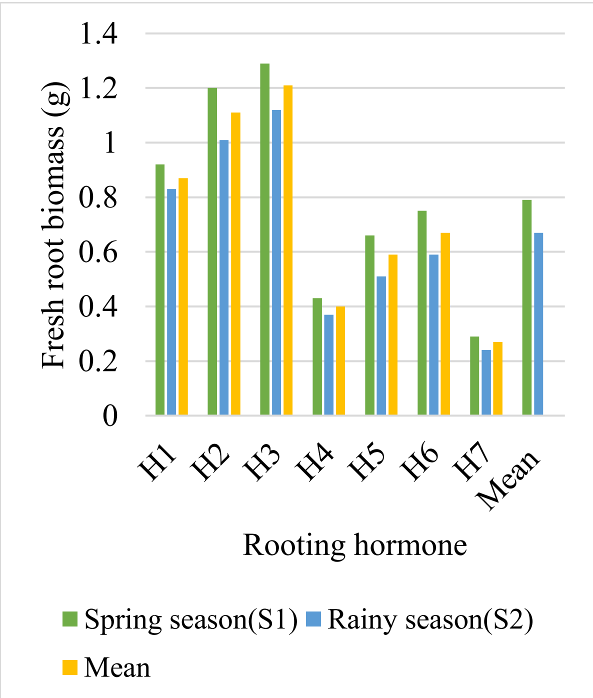

Fig. 4. Effect of different hormonal treatments and seasons on fresh root biomass (g) of *Diospyros lotus L.* branch cuttings

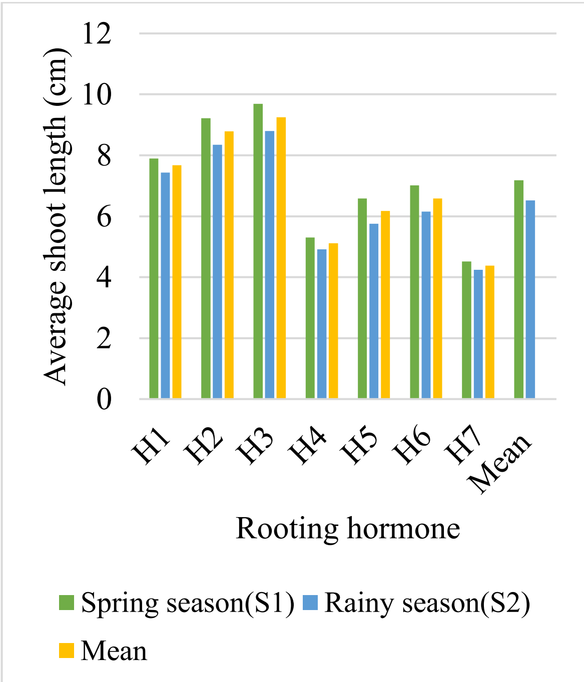

Fig. 5. Effect of different hormonal treatments and seasons on average shoot length (cm) of *Diospyros lotus L.* branch cuttings

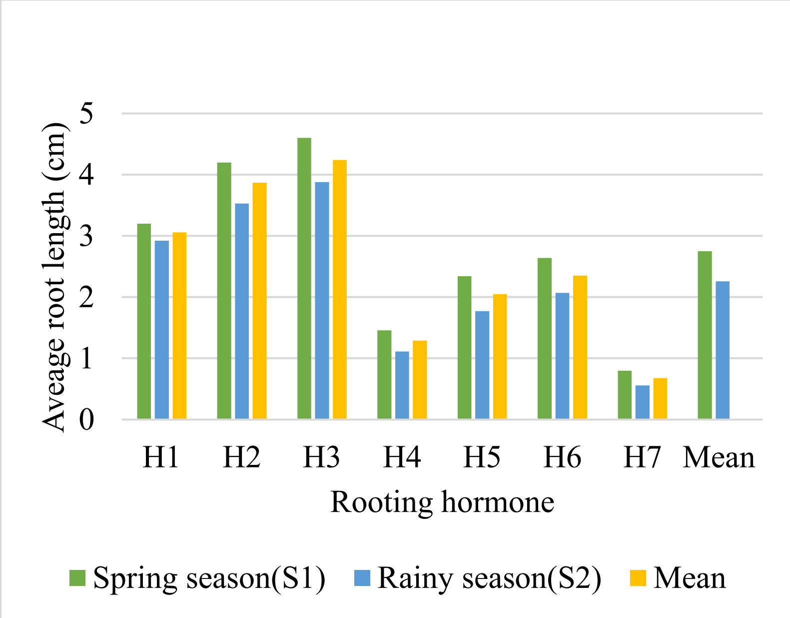

Fig. 6. Effect of different hormonal treatments and seasons on average root length (cm) of *Diospyros lotus L.* branch cuttings

Table 1. Effect of rooting hormones and growing seasons on sprouting, rooting and growth parameters of *Diospyros lotus* L. branch cuttings

Treatment	Sprouting (%)	Rooting (%)	Fresh root biomass (g)	Average shoot length (cm)	Average root length (cm)
A. Rooting hormone (H)					
H_1 (IBA@25ppm) for 24 hrs	44.58	39.07	0.87	7.67	3.06
H_2 (IBA@50ppm) for 24 hrs	49.49	42.81	1.11	8.79	3.87
H_3 (IBA@100ppm) for 24 hrs	52.58	44.45	1.21	9.25	4.24
H_4(NAA)@25ppm) for 24 hrs	30.39	26.51	0.40	5.11	1.29
H_5(NAA)@50ppm) for 24 hrs	35.84	31.55	0.59	6.17	2.05
H_6(NAA)@100ppm) for 24hrs	39.73	34.22	0.67	6.58	2.35
H_7(Control)	25.99	21.18	0.27	4.38	0.68
SE(m) (±)	0.526	0.4	0.01	0.079	0.034
CD (P=0.05)	1.532	1.16	0.031	0.231	0.10
B. Growing season (S)					
S_1 – Spring	41.53	36.09	0.79	7.18	2.75
S_2 – Rainy	38.07	32.43	0.67	6.52	2.26
SE(m) (±)	0.984	0.74	0.02	0.148	0.064
CD (P=0.05)	2.866	2.16	0.057	0.432	0.187

Table 2. Interaction effect of rooting hormones and growing seasons on sprouting, rooting and growth parameters of *Diospyros lotus* L. branch cuttings

Treatment	Treatment combination	Sprouting (%)	Rooting (%)	Fresh root biomass (g)	Average shoot length (cm)	Average root length (cm)
T_1	H_1S_1	46.45	40.01	0.92	7.90	3.20
T_2	H_1S_2	42.72	38.13	0.83	7.43	2.92
T_3	H_2S_1	51.69	44.84	1.20	9.22	4.20
T_4	H_2S_2	47.28	40.79	1.01	8.35	3.53
T_5	H_3S_1	53.87	45.76	1.29	9.69	4.60
T_6	H_3S_2	51.29	43.14	1.12	8.80	3.88

Treatment	Treatment combination	Sprouting (%)	Rooting (%)	Fresh root biomass (g)	Average shoot length (cm)	Average root length (cm)
T_7	H_4S_1	31.93	27.72	0.43	5.30	1.46
T_8	H_4S_2	28.85	25.3	0.37	4.92	1.11
T_9	H_5S_1	38.17	34.82	0.66	6.58	2.34
T_{10}	H_5S_2	33.51	28.28	0.51	5.75	1.77
T_{11}	H_6S_1	42.29	37.21	0.75	7.01	2.64
T_{12}	H_6S_2	37.18	31.22	0.59	6.15	2.07
T_{13}	H_7S_1	26.33	22.23	0.29	4.52	0.80
T_{14}	H_7S_2	25.65	20.12	0.24	4.24	0.56
SE(m) (±) H × S		1.392	1.05	0.028	0.21	0.091
CD (P=0.05) H × S		NS	NS	NS	NS	NS

Effect of rooting hormones on sprouting and rooting percentages of cuttings: It is evident from the overall mean data that rooting hormone treatment H_3 recorded the maximum sprouting percentage (52.58), whereas the minimum sprouting percentage (25.99) was observed under H_7. Among the seasons, the spring season (S_1) recorded a higher sprouting percentage (41.53) than the rainy season (S_2) (38.07). The rooting percentage was significantly influenced by the rooting hormone treatments. H_3 recorded the maximum rooting percentage (44.45), whereas the minimum rooting percentage (21.18) was observed under H_7. Seasonal averages revealed that spring (S_1) registered a higher rooting percentage (36.09) than the rainy season (S_2) (32.43). Cuttings treated with IBA showed a higher sprouting rate than untreated controls. This response may be due to the increased level of auxins, resulting in the earlier completion of physiological processes involved in the rooting and sprouting of cuttings. The increase in the number of sprouts may be due to the improved utilisation of stored carbohydrates, nitrogen, and other factors with the help of growth regulators (Singh, 2014). Auxin application may have promoted the hydrolysis and translocation of carbohydrates and nitrogenous substances at the bases of the cuttings, thereby accelerating cell division and elongation. It has also been found to enhance histological features such as callus and tissue formation and vascular tissue differentiation. IBA is widely used because it is less toxic to plants over a wider concentration range than NAA or IAA (Hartmann *et al.,* 2002) and is also effective in promoting rooting of a large number of plant species (Teklehaimanot *et al.,* 1996; Henrique *et al.,* 2006). IBA is less sensitive than IAA to non-biological degradation such as photo-oxidation (Epstein & Ludwig-Müller, 1993; De Klerk *et al.,* 1997). Alternatively, there may be differences in the rates of uptake and metabolism of the two auxins as demonstrated in apple and *Grevillea* microcuttings (De Klerk *et al.,* 1997; Krisantini *et al.,* 2006; Kesari *et al.,* 2009). The differences among auxins could also be related to other factors such as greater stability and a slower rate of conjugation of IBA, so that the free IBA required to induce rooting would remain available for a longer period of time than IAA or NAA (Krisantini *et al.,* 2006). Adventitious rooting is a coordinated developmental response involving wound signalling, cell reprogramming, auxin transport and hormone crosstalk; hence, the superior performance under H_3 is consistent with reports that auxin supply improves rooting most effectively when the physiological and resource status of the cutting remains favourable. The higher spring response may also reflect seasonal differences in cutting maturity, reserve mobilisation and environmental suitability, although this interpretation should be treated cautiously because individual microclimatic variables were not separately quantified in the present experiment.

Effect of rooting hormones on shoot length: Treatment H_3 achieved the maximum average shoot length of 9.25 cm. In contrast, treatment H_7 recorded the minimum average shoot length of 4.38 cm. Across seasons, spring (S_1) exhibited a higher average shoot length of 7.18 cm than the rainy season (S_2), which recorded 6.52 cm. This may be because IBA promoted the greatest aerial growth. Kaur *et al*. (2018) reported that the emergence of the longest shoots on cuttings may be attributed to the well-developed root system in such cuttings, which may have promoted shoot growth by ensuring adequate mobilisation of water and nutrients from the soil or substrate to the growing apices. Similar observations were reported by Venkatesh and Pandey (2006) in *Pterocarpus dalbergioides* (Padauk), where IBA at 100 ppm produced the longest shoot length among the auxin treatments, while untreated cuttings exhibited comparatively poor growth. The authors attributed the response to enhanced rooting, which facilitated greater nutrient uptake and translocation to developing shoots. In poplar cuttings, Haider *et al*. (2015) observed that 100 ppm IBA significantly increased the number of sprouts per cutting compared with the control, demonstrating the positive role of IBA in promoting shoot initiation in forestry species. The non-significant interaction between rooting hormone treatments and seasons confirmed the stability of treatment effects. The improvement in shoot length under H_3 can be interpreted as a secondary response associated with improved rooting and early establishment, because a functional root system supports water uptake, mineral acquisition and assimilate balance required for shoot extension. Recent evidence indicates that shoot growth after cutting establishment depends on coordinated source-sink allocation and hormone-regulated developmental reprogramming, rather than on auxin application alone (Bannoud & Bellini, 2021; Druege *et al*., 2019; Pacurar *et al.,* 2014). Therefore, the higher shoot length observed in IBA-treated cuttings should be discussed as a growth response associated with improved cutting establishment, not as an independent direct effect of IBA on shoot elongation (da Costa *et al*., 2013; Lakehal & Bellini, 2019).

Effect of rooting hormones on root characters: Treatment H_3 produced the maximum average root length of 4.24 cm, significantly exceeding the remaining treatments. Conversely, treatment H_7 exhibited the minimum average root length of 0.68 cm. Seasonal averages indicated that spring (S_1) recorded a higher root length of 2.75 cm than the rainy season (S_2), which recorded 2.26 cm. Treatment H_3 recorded the highest fresh root biomass of 1.21 g. In contrast, the lowest fresh root biomass of 0.27 g was observed under treatment H_7. With respect to seasonal influence, the spring season (S_1) exhibited a higher average fresh root biomass of 0.79 g than the rainy season (S_2), which recorded 0.67 g. The non-significant H × S interaction confirmed the consistency of treatment effects across seasonal environments. Evidence suggests that auxins may have increased rooting and root length because the root-elongation stage is highly responsive to auxin concentration, particularly at higher concentrations (Hartmann *et al.,* 2002). Auxin application has been found to enhance histological features such as callus and tissue formation and vascular tissue differentiation (Mitra & Bose, 1957). It also affects metabolite translocation and carbohydrate metabolism. The results are consistent with the findings of Reddy *et al.* (2008) in fig and Santos *et al.* (2011) in *Annona muricata.* In addition to enhancing the rate of adventitious root development, auxin application has been found to increase the number of roots initiated per rooted cutting in a variety of species (Mesen *et al.,* 1997; Palanisamy *et al.,* 1998). The maximum fresh root weight was attributed to the enhanced auxin-mediated root initiation and the mobilisation of stored carbohydrates towards the basal region of cuttings, which provides energy and carbon skeletons required for adventitious root development (Ahkami *et al*., 2013). Auxin application stimulates the mobilisation of carbohydrates in the upper shoot, increases assimilate translocation, and increases sugar availability at the site of root primordium development (Altman & Wareing, 1975; Haissig, 1986; Husen & Pal, 2007; Agulló-Antón *et al*., 2011). The increase in fresh weight of roots might be due to the fact that the increase in root number and root length resulted in greater accumulation of dry matter. The present findings are in agreement with Lymba and Wani (2019), who reported that the application of IBA significantly enhanced root biomass in *Melia dubia* cuttings. The higher average root length and fresh root biomass under H_3 are consistent with the established role of auxin in cell-cycle reactivation, root primordium formation and local carbohydrate mobilisation at the basal region of cuttings.

4. Limitation of the Study

The study was limited to a single site and one growing cycle (2024–2025), with cuttings sourced only from mother plants in Poonch district, which may restrict the generalisability of the findings across other agro-climatic conditions. Only three concentrations of IBA and NAA were tested, and only spring and rainy season plantings were evaluated, while long-term survival and field performance of rooted cuttings after transplanting were not assessed.

5. Conclusions

The study showed that rooting hormone treatment significantly improved sprouting, rooting, and early growth of *Diospyros lotus* L. branch cuttings compared with the untreated control. Among the evaluated treatments, IBA at 100 ppm produced the highest sprouting percentage, rooting percentage, fresh root biomass, average shoot length, and average root length. The spring season produced better overall establishment than the rainy season under the conditions of this experiment. The non-significant hormone × season interaction indicates that treatment effects were broadly consistent across the two seasons. IBA at 100 ppm may therefore be considered a suitable treatment for improving nursery propagation of *Diospyros lotus* L. branch cuttings.

Declaration of AI Use

This manuscript was prepared through the combined contributions of all author(s), including contributions to the study design, data, content development, results, interpretation, and related scholarly work. The author(s) acknowledge the use of Grammarly and ChatGPT to assist with grammar checking, language refinement, reference formatting. These AI-assisted tools were not used as authors and did not replace the intellectual contributions or scholarly judgment of the author(s). All AI-assisted outputs, including content, references, and interpretations, were carefully reviewed, revised, verified, and approved by the author(s). The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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