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Effect of auxin type, concentration and planting season on rooting and growth attributes of *Diospyros lotus* L. branch cuttings

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Abstract
Diospyros lotus L. (date-plum), an important persimmon rootstock species valued for its adaptability and drought tolerance, is difficult to propagate vegetatively because of poor and inconsistent rooting of stem cuttings. A field experiment was conducted at the nursery of the Division of Silviculture & Agroforestry, SKUAST-Jammu, to evaluate the effect of two auxins – indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA), each at 25, 50 and 100 ppm applied through 24-hour soaking, along with an untreated control – on the sprouting, rooting and growth of branch cuttings planted during the spring and rainy seasons. The experiment followed a two-factor factorial Completely Randomized Design with fourteen treatment combinations, each replicated thrice. IBA at 100 ppm (H_3) recorded the highest number of leaves per cutting (14.51) and dry root biomass (0.15 g), while IBA at 50 ppm (H_2) gave a statistically comparable, and the highest, number of shoots (3.51); NAA and the untreated control performed poorly across all parameters. Cuttings planted in spring consistently outperformed those planted in the rainy season for shoot number, leaf number, dry root biomass and leaf area. The hormone × season interaction was significant for shoot number and dry root biomass, with the combination of IBA at 100 ppm with spring planting (T_3) giving the best overall rooting response (0.16 g dry root biomass), closely followed by IBA at 50 ppm with spring planting (T_2, 0.15 g). The results indicate that treating branch cuttings of *D. lotus* with IBA at 100 ppm and planting them in spring is the most effective and practically feasible approach for its vegetative propagation on a nursery scale.

Keywords: *Diospyros lotus*, auxin, IBA, NAA, branch cuttings, vegetative propagation

Introduction
Many forest tree species with considerable ecological, horticultural and economic potential remain underutilized because their propagation requirements have not been adequately investigated. *Diospyros lotus* L. (date-plum) is one such species. It is a deciduous member of the genus *Diospyros*, a large and widely distributed genus of the family Ebenaceae occurring predominantly in tropical and subtropical regions (Mallavadhani *et al*., 1998; Duangjai *et al*., 2009) [4, 12]. Although *Diospyros kaki* Thunb. is the principal commercially cultivated persimmon species, other congeners, including *D. lotus*, have considerable horticultural and economic importance? *Diospyros lotus* is particularly important as a rootstock for persimmon because of its adaptability, drought tolerance and grafting affinity, making reliable production of planting material important for persimmon cultivation and related propagation programmes (Liu *et al*., 2023) [10].

Diospyros lotus is valued not only for its edible fruit but also for its medicinal and ornamental attributes. Different parts of the plant contain biologically active compounds and have been investigated for pharmacological properties, including antinociceptive, anti-inflammatory and sedative activities (Uddin *et al*., 2014) [16]. Studies have also reported antidiabetic potential associated with *D. lotus* extracts and their phytochemical constituents (Qin *et al.*, 2022). In addition, the species has attractive deciduous foliage and small edible fruits, further increasing its horticultural value. These characteristics, together with its importance as a rootstock species, indicate considerable scope for its wider cultivation and utilization.

However, the availability of adequate and uniform planting material remains an important constraint to the wider utilization of *D. lotus*. Seed propagation can be affected by dormancy, viability and germination-related constraints, and several studies have investigated stratification, scarification and gibberellic acid treatments to improve seed germination in the species (Sayyad-Amin and Shahsavar, 2019) [18]. More recently, Khatri *et al*. (2025) [8] reported that low seed viability and prolonged dormancy can result in delayed and uneven establishment of *D. lotus* seedlings under nursery conditions. Vegetative propagation therefore provides an important alternative because it can facilitate the multiplication of selected plant material while maintaining desirable characteristics of the parent plant.

Among vegetative propagation techniques, stem cutting is a simple, economical and potentially scalable method for nursery production. Nevertheless, successful propagation through cuttings depends largely on the capacity of the species to form adventitious roots. Woody species often exhibit considerable variation in rooting ability, and *D. lotus* has previously been identified as a difficult-to-root rootstock. Sato and Sano (1999) [17] investigated the propagation of *D. lotus* through leafy stem cuttings and demonstrated that rooting was strongly influenced by propagation medium, IBA concentration, number of leaf buds, shoot position and defoliation. Their study reported rooting percentages ranging from 10 to 95% depending on cutting position and other propagation conditions, while IBA treatment substantially improved rooting under suitable conditions (Sato and Sano, 1999) [17]. These findings indicate that optimization of rooting conditions is essential for efficient vegetative propagation of the species.

Plant growth regulators, particularly auxins, are widely used to improve adventitious root formation in stem cuttings. Auxins influence the physiological and developmental processes associated with root initiation, and their application can increase rooting percentage, hasten root initiation and improve the number and quality of roots produced by cuttings (Blythe *et al*., 2007) [21]. Among synthetic auxins, indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) are commonly used in vegetative propagation, although their effectiveness varies with species, concentration, method of application and physiological condition of the cutting (Blythe *et al*., 2007) [21]. In *D. lotus*, Sato and Sano (1999) [17] demonstrated a marked response to IBA treatment, with rooting increasing as IBA concentration increased under their experimental conditions. However, their study used relatively high IBA concentrations and a basal quick-dip application, whereas information on the response of *D. lotus* branch cuttings to comparatively low concentrations of IBA and NAA applied through soaking remains limited.

The rooting response of stem cuttings is also influenced by environmental and physiological conditions prevailing during propagation. Factors such as temperature, moisture availability, physiological age of the cutting, endogenous carbohydrate and hormone status, and season can influence adventitious root formation and subsequent growth (Blythe *et al*., 2007) [21]. Consequently, the effectiveness of a particular rooting hormone treatment may vary according to the season in which cuttings are established. Evaluating rooting hormones under contrasting seasons is therefore important for developing a practical propagation protocol suitable for nursery production.

Although previous research has demonstrated the potential of IBA for rooting *D. lotus* cuttings (Sato and Sano, 1999) [17], their study primarily evaluated relatively high IBA concentrations together with different propagation beds, cutting positions and leaf-bud treatments. Consequently, comparative information on the response of *D. lotus* branch cuttings to lower concentrations of different auxins, particularly IBA and NAA, under contrasting nursery seasons remains limited. Moreover, the effectiveness of rooting treatments may vary with environmental and physiological conditions prevailing during different seasons. Therefore, systematic evaluation of auxin type and concentration under different planting seasons is necessary to identify an effective and practically feasible rooting treatment for *D. lotus* branch cuttings. Accordingly, the present study was undertaken to evaluate the effects of IBA and NAA at different concentrations on the sprouting, rooting and early growth of *D. lotus* branch cuttings during spring and rainy seasons, with the objective of identifying an effective hormone treatment and suitable planting season for nursery-scale vegetative propagation of the species.

Material and Methods

The experiment was laid out in a Completely Randomized Design (CRD) with a two-factor factorial arrangement, comprising:

- **Factor 1:** Rooting hormone (7 levels)
- **Factor 2:** Growing season (2 levels)

This resulted in a total of 14 treatment combinations, each replicated three times, with ten plants per replication (30 plants per treatment). Observations were recorded on fifteen randomly selected plants per treatment (five plants selected at random from each replication).

Planting material: Branch cuttings

Time of planting: First week of March (2024 and 2025) for the spring season trial, and first week of August (2024 and 2025) for the rainy season trial.

1. Treatment Details

Treatment	Combination	Description
T_1	$H_1 \times S_1$	IBA @ 25 ppm (24 hr soaking) + Spring season
T_2	$H_2 \times S_1$	IBA @ 50 ppm (24 hr soaking) + Spring season
T_3	$H_3 \times S_1$	IBA @ 100 ppm (24 hr soaking) + Spring season
T_4	$H_4 \times S_1$	NAA @ 25 ppm (24 hr soaking) + Spring season
T_5	$H_5 \times S_1$	NAA @ 50 ppm (24 hr soaking) + Spring season
T_6	$H_6 \times S_1$	NAA @ 100 ppm (24 hr soaking) + Spring season
T_7	$H_7 \times S_1$	Control (no hormone application) + Spring season
T_8	$H_1 \times S_2$	IBA @ 25 ppm (24 hr soaking) + Rainy season
T_9	$H_2 \times S_2$	IBA @ 50 ppm (24 hr soaking) + Rainy season
T_{10}	$H_3 \times S_2$	IBA @ 100 ppm (24 hr soaking) + Rainy season
T_{11}	$H_4 \times S_2$	NAA @ 25 ppm (24 hr soaking) + Rainy season
T_{12}	$H_5 \times S_2$	NAA @ 50 ppm (24 hr soaking) + Rainy season
T_{13}	$H_6 \times S_2$	NAA @ 100 ppm (24 hr soaking) + Rainy season
T_{14}	$H_7 \times S_2$	Control (no hormone application) + Rainy season

2. Collection and Preparation of Branch Cuttings

Branch cuttings of *Diospyros lotus* L. were collected from ten phenotypically superior, vigorously growing, and disease-free trees, identified and marked following a preliminary field survey in the Poonch district of Jammu & Kashmir.

- Cuttings measuring 12–15 cm in length, approximately pencil-thickness, and bearing 3–4 nodes were selected from the marked donor plants.
- To minimize desiccation during transit, cuttings were wrapped in moist sphagnum moss and transported to

the experimental nursery of the Division of Silviculture & Agroforestry.

- Upon arrival, cuttings were heeled into soil until the scheduled planting dates (first week of March, 2024 and 2025).
- For the rainy season trial (first week of August, 2024 and 2025), sphagnum moss packing was not required, as the prevailing high atmospheric humidity and soil moisture from monsoon precipitation provided sufficient conditions to prevent desiccation and support root induction.
- Each cutting was prepared with an oblique cut at the apical end and a horizontal cut at the basal end, made using a sharp, sterilized razor blade, prior to hormone treatment and planting.

3. Preparation of Rooting Hormone Solutions

Working solutions of Indole-3-Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA) were prepared at three concentrations each: 25, 50, and 100 ppm.

Stock solution preparation

- Analytical-grade IBA and NAA crystals were weighed precisely on an analytical balance.
- A 1000 ppm stock solution of each hormone was prepared by dissolving 1000 mg (1.000 g) in a small volume of ethanol/absolute methanol with continuous stirring until fully dissolved, followed by volume make-up to 1 litre with distilled water.
- Stock solutions were stored in amber bottles at 4°C to prevent photodegradation.

Working solution preparation

- Dilutions were calculated using the formula $C_1V_1 = C_2V_2$.
- For 100 mL of final working solution: 10 mL, 5 mL, and 2.5 mL of the 1000 ppm stock were used to obtain 100, 50, and 25 ppm solutions, respectively, made up to volume with distilled water in volumetric flasks.
- Working solutions were freshly prepared as needed, stored in labelled amber bottles at 4°C, and all glassware/solvents were maintained contaminant-free to ensure accuracy and reproducibility.

Hormone application

- Immediately prior to planting, the basal ends of cuttings were dip-treated in the respective hormone solutions for 24 hours to ensure uniform hormone uptake.
- Treated cuttings were planted immediately thereafter to prevent hormone degradation from sunlight exposure.

4. Planting of Cuttings

- Cuttings were inserted to a depth of **6–8 cm** into the rooting medium.
- Surrounding soil was gently firmed to secure the cuttings while avoiding compaction, in order to preserve adequate aeration in the rooting zone.
- Cuttings were irrigated twice daily (morning and evening), except during the rainy season trials (2024 and 2025), when natural precipitation met moisture requirements.
- Weeding was carried out as needed throughout the experimental period.

5. Observations recorded

Data on rooting and growth parameters were recorded from the fifteen randomly selected plants per treatment (five plants from each replication) at the appropriate stage of observation. The following biometric parameters were studied:

5.1 Average Number of Shoots

The total number of shoots that emerged per cutting was counted for each of the five selected plants per replication, and the mean value was computed to obtain the average number of shoots per treatment.

5.2 Average Number of Leaves per Plant

The total number of fully expanded leaves borne on each sprouted cutting was recorded by manual counting. The values obtained from the five selected plants in each replication were averaged to determine the mean number of leaves per plant for each treatment.

5.3 Dry Root Biomass (g)

At the time of final observation, the root systems of the selected plants were carefully uprooted, washed gently under running water to remove adhering soil particles, and separated from the shoot portion. The cleaned roots were then oven-dried at 65–70°C until a constant weight was achieved. The dry weight of the root biomass was recorded using an electronic analytical balance and expressed in grams (g) per plant. The mean dry root biomass was calculated for each treatment.

Average Leaf Area (cm^2)

Leaf area was measured using a leaf area meter, employing fully matured, healthy leaves selected from each of the five observed plants per replication. Readings were recorded directly in square centimetres (cm^2), and the mean leaf area per treatment was computed from the values obtained across the selected plants.

Fig a to d (clockwise): Hormonal treatment of cuttings; Planting of cuttings in the nursery bed; measurement of leaf area by leaf area meter; sprouted cutting.

Statistical analysis

The data recorded for various growth and rooting parameters were subjected to statistical analysis appropriate for a two-factor factorial experiment in Completely Randomized Design (CRD). Analysis of Variance (ANOVA) was performed to test the significance of the main effects of rooting hormone treatments, growing season, and their interaction (Hormone × Season) on the observed parameters. Treatment means were compared using the Critical Difference (CD) test at a 5% level of significance ($p \leq 0.05$). Statistical analysis was carried out using OPSTAT software (developed by CCS Haryana Agricultural University, Hisar).

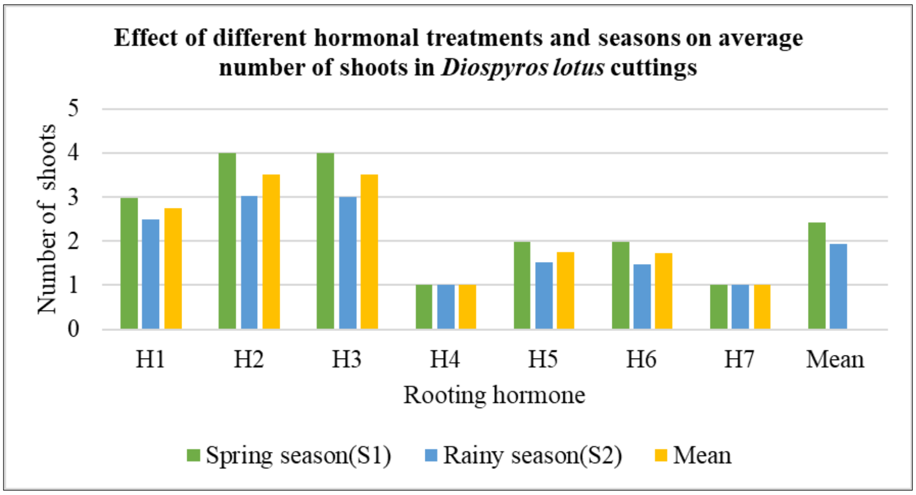
Results and Discussion

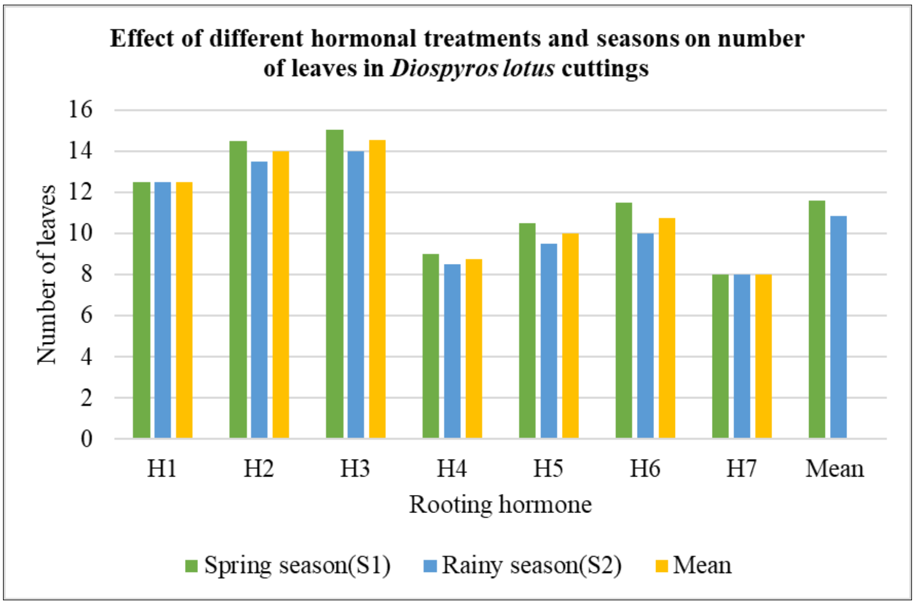
Table 1: Effect of rooting hormones and growing seasons on average number of shoots, average number of leaves per plant, dry root biomass and average leaf area(cm^2) of *Diospyros lotus* L. branch cuttings.

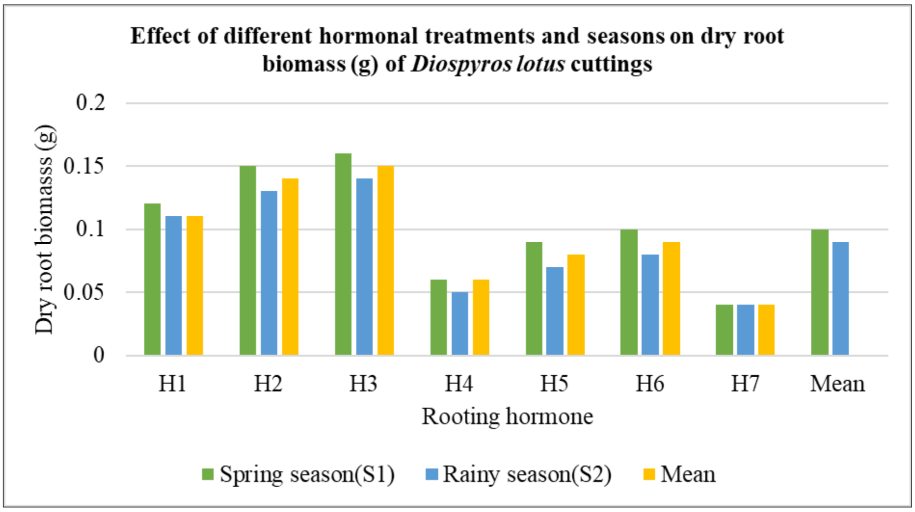
Treatment	Average number of shoots	Average number of leaves per plant	Dry root biomass	Average leaf area(cm^2)
A. Rooting hormone (H)				
H_1 (IBA@25ppm) for 24 hrs	2.74	12.50	0.11	4.68
H_2 (IBA@50ppm) for 24 hrs	3.51	14.00	0.14	4.87
H_3 (IBA@100ppm) for 24 hrs	3.50	14.51	0.15	4.94
H_4(NAA)@25ppm) for 24 hrs	1.00	8.75	0.06	4.18
H_5(NAA)@50ppm) for 24 hrs	1.75	10.00	0.08	4.39
H_6(NAA)@100ppm) for 24hrs	1.73	10.76	0.09	4.48
H_7(Control)	1.00	8.00	0.04	4.03
SE(m) (±)	0.03	0.14	0.00	0.05
CD (*P* =0.05)	0.08	0.40	0.00	NS
B. Growing season (S)				
S_1 – Spring	2.42	11.58	0.10	4.57
S_2 – Rainy	1.93	10.86	0.09	4.45
SE(m) (±)	0.05	0.25	0.00	0.10
CD (*P* =0.05)	0.15	0.74	0.01	0.29

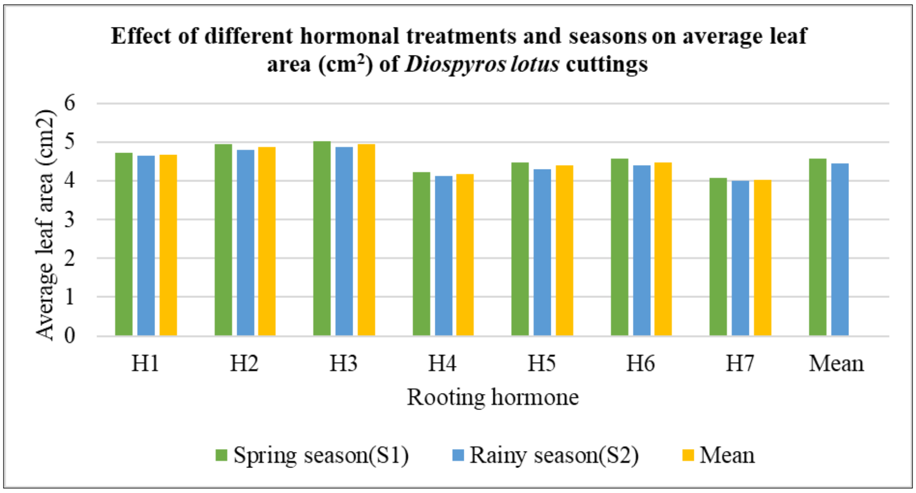
Table 2: Interaction effect of rooting hormones and growing seasons on average number of shoots, average number of leaves per plant, dry root biomass and average leaf area (cm^2) of *Diospyros lotus* L. branch cuttings.

Treatment	Treatment combination	Average number of shoots	Average number of leaves per plant	Dry root biomass (g)	Average leaf area (cm^2)
T_1	$H_1 \times S_1$	2.98	12.50	0.12	4.72
T_2	$H_2 \times S_1$	2.50	12.50	0.11	4.65
T_3	$H_3 \times S_1$	4.00	14.50	0.15	4.94
T_4	$H_4 \times S_1$	3.02	13.50	0.13	4.79
T_5	$H_5 \times S_1$	4.00	15.01	0.16	5.01
T_6	$H_6 \times S_1$	3.00	14.00	0.14	4.87
T_7	$H_7 \times S_1$	1.00	9.00	0.06	4.22
T_8	$H_1 \times S_2$	1.00	8.50	0.05	4.13
T_9	$H_2 \times S_2$	1.99	10.50	0.09	4.48
T_{10}	$H_3 \times S_2$	1.52	9.50	0.07	4.30
T_{11}	$H_4 \times S_2$	1.98	11.51	0.10	4.56
T_{12}	$H_5 \times S_2$	1.48	10.00	0.08	4.40
T_{13}	$H_6 \times S_2$	1.00	8.00	0.04	4.06
T_{14}	$H_7 \times S_2$	1.00	8.00	0.04	3.99
SE(m) (±) H × S	—	0.07	0.36	0.00	0.14
CD (*P* =0.05) H × S	—	0.21	NS	0.01	NS









Treatment H_3 produced the highest dry root biomass of 0.15 g. Conversely, the lowest dry root biomass of 0.04 g was recorded under treatment H_7. Across seasons, spring (S_1) registered a higher average dry root biomass of 0.10 g compared with rainy season (S_2), which recorded 0.09 g. Increase in dry weight of roots might be due to the fact that the increase in the root number and length of roots resulted in higher accumulation of dry matter The interaction effect (H × S) was reported significant with maximum dry root biomass reported in treatment combination T_3: H_3xS_1 (0.16), at par with T_2: H_2xS_1 (0.15) and lowest value reported in T_{14}: H_7xS_2 (0.04). The superior growth and rooting performance of cuttings during spring may be attributed to enhanced cambial activity, higher endogenous auxin concentration, greater availability of stored carbohydrates accumulated during dormancy. Similar observations have been reported by Nanda *et al.* (1968) [13] in several forest tree species, Shamet and Sharma (2004) [19] in red cedar and

Kumar *et al*. (2018) [9] in *Acer acuminatum*. The present findings agree with Lymba and Wani (2019) [11], who reported that the application of IBA significantly enhanced root biomass in *Melia dubia* cuttings. Among the hormone treatments, 100 mg L^{-1} IBA recorded the highest fresh and dry root weights, indicating that IBA promotes adventitious root formation and subsequent root biomass accumulation and it certainly has a certain role in the rooting initiation (Štefanič *et al*., 2007) [21]. Auxins control growth and development in plants, including lateral root initiation and root gravity response (Lymba and Wani, 2019) [11]. The authors attributed this improvement to the stimulatory effect of IBA on root initiation and elongation, resulting in a more extensive root system capable of greater water and nutrient absorption, thereby enhancing overall plant growth.

Treatment H_2 produced the highest number of shoots per cutting (3.51), which was statistically at par with treatment H_3 (3.50) and significantly superior to the remaining treatments. In contrast, treatment H_4 and H_7 recorded the lowest value (1.00). With regard to seasonal influence, spring season (S_1) exhibited a higher average number of shoots per cutting (2.42) than rainy season (S_2) (1.93). The interaction between rooting hormone treatments and seasons was significant, with highest value (4.00) found in treatment combination; T_2: H_2xS_1 and T_3: H_3xS_1 and lowest value (1.00) in T_4: H_4xS_1, T_{11}: H_4xS_2, T_7: H_7xS_1 and T_{14}: H_7xS_2. This might be due to the fact that IBA led to best aerial growth. Kaur *et al.* (2018) [7] reported that the emergence of longest shoots on cuttings may be attributed to the well-developed root system in such cuttings which might have tended to promote shoot growth by ensuring adequate mobilization of water and nutrients from the soil or substrate to the growing apices. Similarly, observations were reported by Venkatesh and Pandey (2006) [23] 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) [5] 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. Similar seasonal responses were reported by Rana and Sood (2012) [15] in *Ficus roxburghii*, where spring-grown cuttings treated with IBA 100 ppm consistently outperformed rainy-season cuttings for shoot growth and overall propagation success. The present findings agree with Dhillon *et al*. (2011) [3], who reported that branch cuttings of *Jatropha curcas* planted during the spring season exhibited superior sprouting and rooting responses following treatment with auxins (IAA, IBA and NAA) and thiamine. The enhanced sprouting observed during spring resulted in more vigorous shoot development, as evidenced by improved shoot growth and establishment.

Treatment H_3 produced the highest number of leaves per cutting (14.51). In contrast, treatment H_7 recorded the lowest number of leaves per cutting (8). Across seasons, spring (S_1) exhibited a higher mean leaf count (11.58) than rainy season (S_2) (10.86). Mean data revealed non – significant effects of rooting hormone on average leaf area of branch cuttings. Across seasons, spring (S_1) maintained a greater average leaf area of 4.57 cm² than rainy season (S_2), which recorded 4.45 cm². Increase in leaf number might be due to the vigorous rooting induced by the growth regulators enabling the cuttings to absorb more nutrients and thereby producing more leaves as reported by Stancato *et al.* (2003) [20]. The highest number of leaves were associated with number of sprouts as well as length of sprouts of cuttings, which in turn, was dependent on hydrolysis of reserve food materials, proper shoot and root balance (Pushpa *et al.*, 2023; Kaur and Kaur, 2017). Kaur *et al.* (2018) [6, 7, 14] observed in cuttings of fig cv. brown turkey that increase in total leaf area is related with the growth. It might be due to the fact that the plants with the vigorous growth gave more leaf area and vice versa. Plants with more roots increased nutrient uptake and increased growth with more leaf area. The superiority of IBA-treated cuttings observed in the present study with respect to leaf number is supported by the findings of Albarzinji and Swara (2024) [1] in *Dalbergia sissoo*. They reported that IBA application significantly increased both the number of leaves and leaf area compared with untreated cuttings. The enhanced foliage development was attributed to improved rooting, which facilitated greater uptake of water and nutrients and consequently promoted vigorous shoot growth and leaf expansion. The present findings are in conformity with those of Lymba and Wani (2019) [11], who reported that IBA at 100 ppm significantly improved leaf area and overall vegetative growth in *Melia dubia* cuttings compared with the control and other treatments. The enhanced vegetative growth associated with IBA application supports the higher number of leaves per cutting observed in the present investigation, which may be attributed to improved rooting and efficient uptake of water and nutrients, leading to increased leaf production and expansion. Similar findings were reported by Thakur (2020) [22], who observed that cuttings established during the spring season exhibited significantly higher sprouting, rooting and root number than those planted during the rainy season. The superior rooting and early establishment of cuttings during spring enhanced the uptake of water and mineral nutrients, resulting in vigorous vegetative growth, which supports the higher leaf number and larger leaf area recorded in the present study.

Conclusion

The present investigation demonstrated that auxin treatment and planting season significantly influence the sprouting, rooting and vegetative growth of *Diospyros lotus* L. branch cuttings. Among the auxins tested, IBA proved distinctly superior to NAA, with IBA at 100 ppm (H_3) producing the maximum number of leaves and the highest dry root biomass, and IBA at 50 ppm (H_2) producing a statistically comparable number of shoots, while the untreated control and the lower-performing NAA treatments recorded the poorest rooting and growth. Cuttings planted during the spring season consistently showed better sprouting, rooting and vegetative growth than those planted during the rainy season, reflecting more favourable cambial activity, endogenous auxin levels and carbohydrate reserves at that time of year. The significant hormone × season interaction further confirmed that the combination of IBA at 100 ppm with spring planting (T_3), closely followed by IBA at 50 ppm with spring planting (T_2), gave the best overall rooting response. Based on these findings, soaking branch cuttings of *D. lotus* in IBA at 100 ppm for 24 hours and planting them in spring can be recommended as a simple, effective and economically feasible practice for large-scale nursery multiplication of this valuable rootstock species. Further studies involving additional auxin concentrations, rooting media and a wider range of donor genotypes may help refine this protocol and improve rooting percentage and seedling quality under commercial nursery conditions.

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