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Research Paper

Enhancing Clonal Multiplication of *Prosopis juliflora* and *Prosopis cineraria* for Dryland Restoration and Genetic Resource Conservation

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Abstract: *Prosopis juliflora* and *Prosopis cineraria* are ecologically and economically important tree species that are found throughout arid and semi-arid regions of the world. However, large-scale propagation of unique genotypes is hampered due to physical seed dormancy, inconsistent germination, inbreeding depression, and genetic variability among seed-derived seedlings. The effects of two auxins, naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA), administered at concentrations of 3000, 4000, and 5000 ppm, on adventitious root formation and shoot growth of stem cuttings of *P. juliflora* and *P. cineraria* were assessed in this study. Effects of two rooting substrates i.e. sandy loam soil and a mixture of sand, leaf compost, and soil (1:1:1) were also evaluated. Results showed a significant difference between species and treatments in terms of root length, shoot length, and root number. IBA produced the best root and shoot development, continuously outperforming NAA, especially at 5000 ppm. As compared to sandy loam alone, the combined rooting substrate further improved rooting performance. *P. cineraria* showed a more fibrous root system and reached maximum root production under the same treatment combination, but *P. juliflora* showed the greatest root length (18 cm) and root production in cuttings treated with 5000 ppm IBA grown in the mixed medium. Auxin application significantly enhanced root quality and vegetative growth, even while rooting percentages differed only slightly between treatments. These results show that the conservation, restoration, and deployment of superior Prosopis genotypes in dryland environments can be accomplished practically and economically by auxin-mediated clonal multiplication.
Keyword: Auxins; Clonal forestry; Dryland restoration; *Prosopis cineraria*; *Prosopis juliflora*

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1. Introduction

Dryland ecosystems, supporting over two billion people globally that make up around 41% of global land area, are among the most environmentally sensitive areas with a great commercial significance (Reynolds et al., 2007). Climate change drivers such as rising temperatures, erratic rainfall patterns, prolonged droughts, and extreme land degradation are just a few of the climate variables that are negatively impacting dryland ecosystems. In the twenty-first century, these anomalies are expected to have negative impacts on dryland ecosystem productivity by accelerating desertification processes, thus resulting in a significant decline in food security of many developing countries, including Pakistan (IPCC, 2023). Consequently, global initiatives such as the Bonn Challenge and planting of climate-resilient tree species that have the potential of restoring ecosystem function have elevated the restoration efforts of degraded drylands to a top priority globally while alleviating the socio-economic status of human populations (UNEP, 2024).

Despite accounting for a modest percentage of global greenhouse gas emissions, Pakistan is one of the most climate-vulnerable countries. Over the last few decades, the country has experienced rising temperatures, irregular precipitation patterns, frequent droughts and floods, and rapid land degradation (Mobeen et al., 2025). These environmental changes are most visible in Punjab, Sindh, Baluchistan, and Khyber Pakhtunkhwa, where desertification and soil degradation are still on the rise. According to the Pakistan government climate surveys, a large part of the country constitutes dry and semi – arid climatic zones, thus making it one of the most vulnerable nations in the world to climate change-related land deterioration.

Besides an extreme threat to local biodiversity, environmental changes are negatively impacting rural life, agricultural productivity, and national economic stability. Many rural populations are dependent on the agriculture sector, thus playing a significant role in Pakistan's economy, accounting for 22-24% of GDP (Pakistan Economic Survey, 2023). Climate change drivers such as rising temperatures, declining soil fertility, water scarcity, and erratic rainfall caused serious losses to agricultural production and economic losses. Climate predictive models have raised serious concerns about the agriculture sector's productivity and regarding food security, with the prediction of significant declines in the production of the major crops such as wheat, rice, maize, and cotton (Jan Alam., 2025; Ali et al., 2017).

Pakistan is currently dealing with a serious energy crisis, particularly in rural areas where local populations depend on fuelwood for domestic energy consumption. As a result of the overharvesting of natural forests for fuelwood has caused biodiversity loss and forest degradation have resulted in a vicious cycle in which poverty and resource shortages are confounded by environmental degradation (Sulaiman, 2017). These challenges emphasize the immediate need for multipurpose tree species that can improve soil quality, strengthen ecosystem resilience, restore degraded landscapes, and produce sustainable biomass energy.

Prosopis species are known for their resilience to withstand extreme climatic conditions, thus offering valuable initiatives for the restoration of the drylands of Pakistan. Several members of the genus have unique ecological and physiological adaptive mechanisms that help them to thrive under extreme weather conditions such as prolonged water scarcity, high temperatures, and poor soil quality (Pasiecznik et al., 2001). Similarly, they have a tendency to fix atmospheric nitrogen, which helps enhance the soil fertility of degraded lands and encourages vegetation cover, particularly in damaged lands, and their deep root systems help them to extract groundwater where many other plant species fail (Felker, 2009). The wide adaptability of Prosopis species is preferred globally for arid and semi – arid regions for agroforestry development, dune stabilization, afforestation, and watershed restoration.

Prosopis juliflora and *Prosopis cineraria* are the most planted plant species for natural habitats in South Asia. Among the two species, *Prosopis juliflora* is known for its high biomass, extreme drought tolerance, and fast growth under extreme weather conditions. These characteristics have accelerated their widespread adaptation in reforestation programs for fuelwood production and land restoration in tropical drylands (Shackleton et al., 2014). Its tendency to grow on severely damaged soil makes it a significant resource for biomass production and environmental mitigation, despite the danger of its being invasive in some regions.

Conversely, *Prosopis cineraria* is considered a keystone plant in arid environments and has been an essential component of traditional agroforestry systems for many generations. The species, which is sometimes referred to as Khejri or Jand, is strongly associated with the natural and cultural environments of the deserts of Thar and Cholistan in Pakistan. Local farming communities are familiar with its potential to enhance soil fertility, increase crop yield when used in mixed cropping systems, and supply nutrient-rich feed during very dry spells, causing severe fodder shortages (Kumar et al., 2010). *P. cineraria* maintain steady growth and yield under extreme climate conditions in which many other tree species failed to survive because of physiological stress (Pareek et al., 2015). *P. cineraria,* therefore, has great potential as a climate-resilient plant in Pakistan for future restoration projects.

P. juliflora and *P. cineraria* have important social implications besides numerous economic benefits. Their wood is used for fuel and charcoal production due to its very high calorific value, leaves are a good source of fodder for cattle, and their potential to survive in marginal lands makes them a preferred choice for biomass production without directly competing with food crops (Pasiecznik et al., 2001). Despite these benefits, Prosopis species are still not widely used because of challenges such as tree shape, low biomass yield, and nursery production and propagation. Seed germination is low due to dormancy induced by a hard seed coat and inbreeding depression, resulting in weak and low biomass production (Baskin and Baskin, 2014). Therefore, the efficacy of restoration initiatives that depend on consistent and dependable planting stock may be hampered by variability.

Vegetative propagation *via* stem cuttings offers several advantages as it allows rapid multiplication of superior genotypes while maintaining desirable features like drought tolerance, growth rate, biomass production, and environmental adaptability (Hartman et al., 2018,, Sarun, 2026). It is therefore imperative that developing a successful clonal multiplication procedure for Prosopis species is not merely a horticultural goal, but offers a strategic requirement for restoration efforts on increasingly degraded and climate-stressed habitats.

Rapidly evolving challenges for Pakistan, such as deforestation, soil degradation, desertification, declining agricultural productivity, power outages that are negatively impacting the industrial sector, and low GDP, make it critical to develop effective propagation strategies for climate-resilient energy-producing species like *P. juliflora* and *P. cineraria*. Improved propagation processes in the country's dry and semi-arid regions may enable large-scale production of excellent planting material for afforestation, agroforestry, carbon sequestration, and ecological restoration projects.

This study examined how α-naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA) affect adventitious root induction and shoot growth in *Prosopis juliflora* and *Prosopis cineraria* stem cuttings. The specific goals were to: (i) determine how auxin type and concentration affect rooting performance; (ii) evaluate the role of rooting substrate in improving vegetative propagation success.

2. Materials and Methods

2.1 Study Site

The experiment was carried out in the Department of Forestry and Range Management, University of Agriculture, Faisalabad, Pakistan. The experiment was laid out under controlled nursery conditions during the active growing season. The greenhouse offers suitable conditions, such as optimal temperature and humidity, suitable for rooting and shoot development of stem cuttings. Faisalabad is situated in the Punjab province, where the climate is characterized as semi-arid, which experiences hot summers and mild winters.

The average annual precipitation ranges between 300 and 400 mm, with the majority occurring during the monsoon cycle. Annual temperatures experience between 5°C in the winter to more than 40°C in the summer.

2.2. Collection of Planting Material

Ten years old healthy, morphologically superior exhibiting vigorous growth plants of *Prosopis juliflora* and *Prosopis cineraria* were selected for this study. The cuttings were harvested in the morning to prevent moisture loss and were wrapped in moist paper towels before hormonal treatment. Each cutting exhibited the same length and girth, containing 3 – 4 leaf nodes. The leaves were removed, and the basal end of each cutting was given an oblique cut to increase hormonal absorption.

2.3. Hormonal Treatment and Growth Media

Two synthetic auxins that are frequently employed in vegetative propagation, naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA), were examined for their effects on root and shoot growth. The cuttings were treated with distilled water as a control, and each hormone was present at three distinct concentrations: 3000 ppm, 4000 ppm, and 5000 ppm. Hormone solutions were prepared by dissolving the hormonal powder in 99% ethanol to fully dissolve the powder. These solutions were subsequently diluted with distilled water to obtain the final desired hormonal concentrations. The basal ends of the cuttings (approximately 2-3 cm) were submerged in hormonal solutions and distilled water for a total duration of 15 minutes (Hartmann et al., 2018; Kan et al., 2025).

2.4. Rooting Media

Two rooting substrates with different chemical and physical characteristics were assessed.

2.5. Sandy Loam Soil

The first rooting medium tested was sandy loam soil that was readily available in the area. The soil was sieved thoroughly to remove any debris.

2.6. Mixed Rooting Medium

A composite rooting substrate was also tested by thoroughly mixing sand, agricultural soil, and leaf compost in a 1:1:1 volume ratio. The soil media was selected because sand promotes aeration and drainage, while organic matter from leaf compost enhances moisture retention, nutritional availability, and microbial activity—all of which may support adventitious root formation (Owen et al., 2020; Rouphael and Colla, 2020).

2.7. Experimental Design and Growth Conditions

All experiments were laid out in a Completely Randomized Design (CRD), and each experimental unit was randomly assigned. For each species (*P. juliflora* and *P. cineraria*), 360 cuttings were used, distributed across 12 treatments with three replicates (10 cuttings × 12 treatments × 3 replicates). In total, the experiment involved 720 treated cuttings across both species. Additionally, 30 cuttings from each species were used as a control group. To maintain high humidity, a transparent polythene sheet was installed over the entire nursery area following planting. Throughout the duration of the experiment, the mean temperature under the green net was maintained between 27°C and 31°C, with a relative humidity (RH) of approximately 75%. The cuttings were exposed to 9–11 hours of natural daylight daily.

2.8. Data collection

Data regarding shoot length, root number, and root length were recorded 60 days post-planting. To ensure the integrity of the root systems, cuttings were carefully removed from the moist growth media to prevent any breakage or loss of primary roots. After cutting removal, the roots were carefully cleaned to allow for an accurate count of primary roots.

2.8.1. Root Length (cm)

Root length was measured from the base of the cutting to the tip of the longest root using a measuring scale and expressed in centimeters.

2.8.2. Shoot Length (cm)

Shoot length was recorded as the distance from the base of the newly emerged shoot to its terminal growing point and expressed in centimeters.

2.8.3. Number of Roots

The total number of adventitious roots produced by the rooted cutting was counted manually.

2.8.4. Rooting Percentage

Rooting percentage was calculated using the following equation:

Rooting Percentage (%) = (Number of rooted cuttings / Total number of planted cuttings) /100

Rooting percentage was used as an indicator of propagation success and treatment effectiveness.

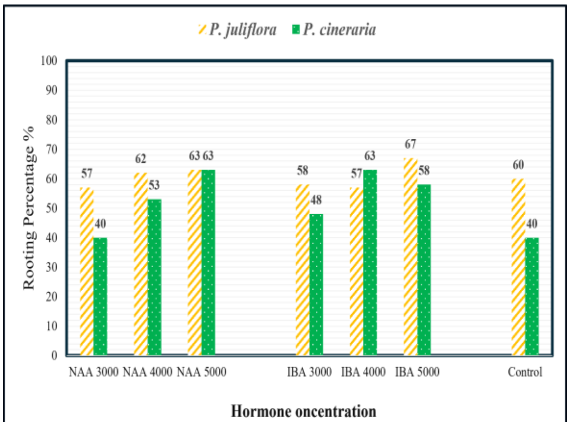

Figure 1. Rooting Percentage of *P. juliflora* and *P. cineraria* cuttings treated with different hormone concentrations.

2.9. Statistical analysis

The collected data were statistically examined using the R programming language (R Core Team, 2025), using the "*agricole"* package for agricultural research (de Mendiburu and de Mendiburu, 2019). Prior to statistical analyses, data were tested for normality using the Shapiro–Wilk test (Shapiro and Wilk, 1965). Homogeneity of variance among groups was assessed using Levene's test (Levene, 1960). Data meeting these assumptions were analyzed using parametric statistical tests at a significance level of α = 0.05. Multiple treatment comparisons using Duncan's Multiple Range Test (DMRT) were used to assess the efficiency of the hormonal treatments, as compared to the control treatment, on root length, shoot length, and number of roots. This test revealed substantial variations between treatment strategies, revealing the most effective hormone doses and growth medium for vegetative propagation of Prosopis species.

3. Results and Discussion

3.1. Effect of Auxin Type on Rooting and Shoot Development

In *Prosopis juliflora* and *Prosopis cineraria*, auxin treatment had a major impact on root and shoot growth (Tables 1 and 2). When compared to NAA-treated and control cuttings, IBA auxin-treated cuttings often showed better roots and shoot growth. In *P. juliflora*, cuttings treated with IBA had the longest mean root length (11.1 cm) and shoot length (23.0 cm), while the control treatment resulted in noticeably weak development of 7.2 cm and 14.0 cm, respectively (Table 1). Additionally, IBA-treated cuttings had considerably more roots per cutting (3.4) than NAA-treated cuttings (2.8) and control cuttings (2.9). In *P. cineraria*, the IBA treatment produced the highest mean root length (9.0 cm), shoot length (19.0 cm), and root number (3.7 roots per cutting), all of which were considerably higher than the values seen in the control treatments (Table 2). In the absence of exogenous auxin administration, control cuttings showed the lowest root length (5.7 cm) and shoot length (9.4 cm), demonstrating the efficacy of root induction and vigor.

3.2. Effect of Auxin Concentration on Stem Cuttings of *Prosopis juliflora*

Significant variations in the development of roots and shoots were observed in *P. juliflora* according to hormone concentrations (Table 3). Root length generally increased with IBA concentration, reaching a peak of 13.5 cm at 5000 ppm IBA. This result demonstrated a rise of around 87.5% as compared to untreated controls.

Additionally, auxin treatment improved shoot growth. The longest shoot was measured at 5000 ppm IBA (23.7 cm), closely followed by 4000 ppm IBA (23.6 cm). Every IBA treatment considerably boosted shoot growth as compared to the control treatment.

The root number revealed a similar trend. The highest number of roots per cutting (5.1) was observed under 5000 ppm IBA, a 75.9% increase over untreated controls. Although NAA treatments improved rooting performance when compared to controls, their advantages were usually less apparent than those of IBA. These results demonstrate the concentration dependence of *P. juliflora* rooting responses and the optimal conditions for adventitious root development at 5000 ppm IBA.

3.3. Effect of Auxin Concentration on Stem Cuttings of *Prosopis cineraria*

Additionally, *P. cineraria*'s rooting response differed considerably between hormone treatments (Table 4). Under the 5000 ppm IBA treatment, the greatest root length (11.3 cm), shoot length (20.7 cm), and root number (4.8 roots per cutting) were noted. 5000 ppm IBA increased root length by about 98.2%, shoot length by 120.2%, and root number by 100% when compared to untreated controls. Although their effects were not as strong as those seen at 5000 ppm, lower quantities of IBA still improved rooting performance.

In comparison to controls, NAA treatments resulted in modest benefits in root and shoot development. But at similar concentrations, IBA consistently performed better than NAA, showing that it was more successful in encouraging adventitious roots in *P. cineraria*.

Increasing auxin concentration improved rooting success, according to the general response pattern, with the biggest gains shown at the highest tested IBA concentration.

3.4. Interaction Between Rooting Medium and Hormone Concentration in *Prosopis juliflora*

The response of *P. juliflora* stem cuttings to auxin treatment was considerably affected by rooting substrate (Table 5). For most hormone treatments, the mixed rooting media consistently produced better root and shoot development than sandy loam soil. Cuttings treated with 5000 ppm IBA growing on the mixed substrate had the longest roots (15.4 cm). The similar treatment in sandy loam soil, on the other hand, resulted in a root length of 11.6 cm. In a similar vein, shoot length peaked at 28.5 cm when 5000 ppm IBA and mixed substrate were combined, as opposed to 23.9 cm in sandy loam soil. Root numbers also increased in response to the interaction between auxin concentration and substrate type. The greatest number of roots (5.2 roots per cutting) was observed under the 5000 ppm IBA treatment in the mixed substrate.

These results show that, as compared to sandy loam soil alone, changes in substrate quality increased the efficacy of auxin treatments and produced better rooting performance.

3.5. Interaction Between Rooting Medium and Hormone Concentration in *Prosopis cineraria*

P. cineraria showed a similar pattern (Table 6). In every hormone treatment, the mixed rooting media consistently encouraged more root and shoot growth than sandy loam soil. In the mixed substrate, the 5000 ppm IBA treatment produced the longest roots (12.6 cm). Under the same treatment combination, shoot length reached a maximum value of 25.6 cm, much higher than the values seen in untreated controls. Additionally, cuttings produced an average of 5.4 roots per cutting when 5000 ppm IBA and mixed substrate interacted. When compared to untreated controls, this number showed an increase of more than two times. The combination of

5000 ppm IBA and mixed substrate consistently produced the highest values for all investigated parameters. Overall, the interplay between auxin concentration and rooting substrate greatly increased propagation success.

Table 1: Effect of Naphthalene acetic acid (NAA) and Indole-3-butyric acid on the shooting and rooting potential of *P. juliflora* stem cuttings.

Growth hormones	Mean root length (cm)	Mean shoot length (cm)	Mean number of roots
NAA	9.3a	20.3b	2.8a
IBA	11.1a	23.0a	3.4b
Control	7.2b	14.0c	2.9a

Means with the same letter in the same column are not significantly different ($P \geq 0.05$).

Table 2. Effect of Naphthalene acetic acid (NAA) and Indole-3-butyric acid on the shooting and rooting potential of *P. cineraria* stem cuttings.

Growth hormones	Mean root length (cm)	Mean shoot length (cm)	Mean number of roots
NAA	8.1a	15.6b	2.5a
IBA	9.0a	19.0a	3.7b
Control	5.7b	9.4c	2.4a

Means with the same letter in the same column are not significantly different ($P \geq 0.05$).

Table 3. Effects of concentrations of growth hormones on root length, shoot length and number of roots on stem cuttings of *P. juliflora*.

Hormone concentration	Root length (cm)	Shoot length (cm)	Number of roots
NAA-3000	7.6	20.9	1.6
NAA-4000	9.8	21.1	2.9
NAA-5000	6.9	18.9	3.8
IBA-3000	9.5	21.8	2.0
IBA-4000	10.5	23.6	3.1
IBA-5000	13.5	23.7	5.1
Control	7.2	14.0	2.9

3.6. Rooting Percentage Response

In both species, the proportion of rooting changed depending on the hormone therapy (Figure 1). Rooting percentages in *P. juliflora* varied from 57% to 67%, with the 5000 ppm IBA treatment showing the greatest value. Rooting percentages in *P. cineraria* varied from 40% to 63%, with the 5000 ppm IBA treatment showing the highest rooting rate. Auxin-treated cuttings generally showed better rooting capability compared to untreated controls, even while differences in rooting percentage among treatments were lower than those shown for root length, shoot length, and root number. The effectiveness of IBA in encouraging adventitious root production in both Prosopis species is further supported by the consistently greater rooting percentages attained under IBA treatments.

4. Discussion

For the protection, genetic enhancement, and widespread use of woody species in restoration and agroforestry initiatives, vegetative propagation is becoming an increasingly significant technique. The creation of dependable clonal propagation procedures can greatly improve restoration performance in arid and semi-arid habitats, where drought, low soil fertility, and environmental unpredictability frequently limit seedling establishment. In stem cuttings of *Prosopis juliflora* and *Prosopis cineraria*, the current study showed that auxin treatment and rooting substrate composition had a significant effect on adventitious root formation and shoot growth. In comparison to sandy loam soil, the mixed rooting substrate consistently improved root and shoot development, while IBA was generally more effective than NAA, especially at higher concentrations. These results provide a useful foundation for the large-scale multiplication of genetically superior Prosopis genotypes and support the significance of both hormonal regulation and substrate optimization in influencing propagation success.

4.1. Effect of Auxin Treatments on Adventitious Root Formation

The establishment of adventitious roots is a highly coordinated process that includes cellular dedifferentiation, the origination of root primordia, and the eventual appearance of roots. Because auxins regulate cell division, vascular differentiation, and root meristem development, they are widely acknowledged as the primary regulators of root growth and development (Lakehal and Bellini, 2019). Recent findings in plant developmental biology have shown that auxin signaling regulates rooting competence in stem cuttings through interactions with nitric oxide signaling pathways, reactive oxygen species, carbohydrate metabolism, and transcriptional regulatory networks (Druege et al., 2019; Fattorini et al., 2024).

In comparison to NAA and untreated controls, the current study found that IBA considerably increased root length, shoot length, and root number in both *P. juliflora* and *P. cineraria*. IBA caused the mean root length of *P. juliflora* to increase from 7.2 cm in control cuttings to 11.1 cm, and the shoot length to increase from 14.0 cm to 23.0 cm. Similar reactions were seen in *P. cineraria*, where IBA-treated cuttings developed noticeably more roots, longer roots, and more shoot growth than untreated cuttings. These results are in line with earlier studies showing that IBA generally promotes adventitious roots in woody perennials more successfully than other auxins (Hartmann et al., 2018; Druege et al., 2019).

IBA performs better than naturally occurring auxins because it is more physiologically stable and less susceptible to enzymatic breakdown. Because IBA lasts longer in plant tissues, cambial activity and the growth of root primordia can be continuously stimulated (Pacurar et al., 2014). Additionally, it has been demonstrated that IBA increases endogenous auxin accumulation at rooting points, which in turn stimulates the expression of genes linked to root initiation and elongation (Lakehal and Bellini, 2019). *Acacia mangium* (Yusnita et al., 2021), *Dalbergia sissoo* (Kumar et al., 2022), Eucalyptus species (Mankessi et al., 2023), and several other woody species utilized in forestry and restoration initiatives have all shown comparable benefits from IBA. Root length, root number, and root architecture are frequently more significant predictors of post-transplant performance, even if rooting percentage is frequently used as a measure of propagation success. Field establishment and long-term survival are improved by larger and more extensive root systems because they improve water uptake, nutrient acquisition, and resistance to transplant shock (Druege et al., 2019). As a result, rooting percentage alone may not be as useful as the improvements in root shape seen after IBA treatment.

4.2. Species-Specific Differences in Rooting and Growth Responses

The roots and shoot development patterns of the two Prosopis species differed noticeably, despite the fact that both species responded favorably to auxin treatments. While *P. cineraria* maintained relatively slower shoot growth but produced a more fibrous root structure, *Prosopis juliflora* showed better shoot elongation and overall vegetative vigor.These variations most likely represent opposing ecological strategies that have developed in various environmental settings.

It is well known that *Prosopis juliflora* is a fast-growing, extremely opportunistic species that can quickly colonize damaged areas. Its success in a variety of arid and semi-arid habitats is attributed to its strong growth habit, wide physiological adaptability, and effective resource acquisition (Shiferaw and Demissew, 2023). Therefore, the increased shoot growth seen in this study is compatible with *P. juliflora*'s ecological strategy, which emphasizes competitive establishment and quick biomass accumulation.

P. cineraria, on the other hand, is well known for its remarkable drought resistance and longevity and is regarded as a keystone plant of desert ecosystems. According to earlier research, desert-adapted wood species often maximize water absorption under moisture-limited conditions by allocating a greater percentage of absorbed carbon to root development rather than shoot expansion (Grossiord et al., 2020; Kumar et al., 2021). Therefore, *P. cineraria*'s more fibrous root system may be an adaptive mechanism that increases access to limited water resources and promotes soil exploration.

It is important to recognize the ecological relevance of these rooted differences. Because root characteristics directly affect drought tolerance, nitrogen uptake, and stress tolerance, they have become important factors in determining plant performance under climate change (Nasrulla et al., 2022; Freschet et al., 2021). Extensive and highly branching root systems are frequently more resilient to extended drought periods, thus increasing environmental variability. As a result, *P. cineraria*'s strong root development, brought about by auxin treatment, may significantly increase its suitability for restoration initiatives aimed at areas with limited water resources.

4.3. Influence of Auxin Concentration on Rooting Performance

The findings showed a significant response to auxin treatment that was concentration-dependent. Increasing hormone concentration typically increased rooting performance in both species, and the 5000 ppm IBA treatment produced the greatest values for root length, shoot length, and root number. According to this research, adventitious root induction in Prosopis species necessitates relatively high auxin concentrations to maximize physiological response.

According to recent research, the development of localized auxin maxima inside competent stem tissues is essential for proper root initiation. Cell division and the production of root primordia are triggered by the activation of developmental pathways involved in root induction once a threshold concentration is achieved (Lakehal and Bellini, 2019). Thus, raising auxin concentration raises the likelihood that enough cells will join the root developmental pathway, improving rooting performance in the end.

The 5000 ppm IBA treatment performed better in this trial, which is in line with studies for other woody species that are challenging to root. While Kumar et al. (2022) noted comparable reactions in Dalbergia sissoo, Thakur et al. (2018) showed better rooting in Acacia catechu with increasing IBA concentration. Research on forest trees has also shown that in order to get beyond physiological obstacles related to rooting in mature or semi-hardwood cuttings, rather high IBA concentrations are frequently needed (Hartmann et al., 2018).

It's interesting to note that while IBA continued to enhance rooting performance, *P. juliflora*'s root length decreased at the greatest NAA concentration. Numerous woody species have shown similar

Table 4. Effects of concentrations of growth hormones on stem cuttings of P. cineraria.

Hormone concentration	Root length (cm)	Shoot length (cm)	Number of roots
NAA-3000	6.6	12.9	1.4
NAA-4000	7.8	15.1	2.6
NAA-5000	9.9	18.9	3.6
IBA-3000	7.6	16.6	2.9
IBA-4000	8.3	19.8	3.5
IBA-5000	11.3	20.7	4.8
Control	5.7	9.4	2.4

Table 5. Interactive effects of growth media and different hormone concentrations on stem cuttings of *P. juliflora*.

Growth media						
Hormonal Concentration	Root length (cm)		Shoot length (cm)		Number of roots	
	Sandy loam	Soil Mixture	Sandy loam	Soil Mixture	Sandy loam	Soil Mixture
NAA-3000	7	8.2	20.5	26.3	1.5	1.7
NAA-4000	9.1	10.5	22	25.2	3.1	2.7
NAA-5000	6	7.8	20.2	22.6	4	3.6
IBA-3000	8.9	10.1	23.7	24.9	2	2
IBA-4000	9	12	25.6	26.6	2.7	3.5
IBA-5000	11.6	15.4	23.9	28.5	5.1	5.2
Control	7.2	7.8	14.0	16.9	2.1	2.9

Table 6. Interactive effects of growth media and different hormone concentrations on stem cuttings of *P. cineraria*.

Growth media						
Hormone Concentration	Root length (cm)		Shoot length (cm)		Number of roots	
	Sandy loam	Soil Mixture	Sandy Loam	Soil Mixture	Sandy loam	Soil Mixture
NAA3000	6.1	7.1	13.8	15	1.6	1.2
NAA4000	7.0	8.6	15.7	17.5	2.7	2.5
NAA5000	9.3	10.5	18.4	22.4	3.2	3.9
IBA3000	5.9	9.3	17.0	19.2	2.6	3.1
IBA4000	7.6	9.0	19.4	23.2	3.2	3.8
IBA5000	10.0	12.6	18.8	25.6	4.2	5.4
Control	5.7	6.9	9.4	13.6	1.9	2.4

inhibitory reactions to increased NAA concentrations, which are frequently associated with hormonal toxicity, disruption of endogenous hormone balance, or inhibition of cell elongation processes (Druege et al., 2019). These results provide more evidence for IBA's advantage as a rooting hormone for Prosopis vegetative proliferation.

4.4. Role of Rooting Substrate in Propagation Success

Rooting substrate had a major effect on root and shoot growth in addition to auxin treatments. Sandy loam soil was consistently outperformed by the mixed medium composed of sand, soil, and leaf compost, especially when paired with higher concentrations of IBA. These results highlight how crucial substrate characteristics are in controlling the microenvironment around growing roots. A careful balance between oxygen transport and moisture availability is necessary for root initiation and elongation. While very porous substrates might not hold enough moisture for healthy root development, excessively compacted soils might impair aeration and root respiration (Hartmann et al., 2018). By combining the drainage qualities of sand with the water-holding capacity and nutrient availability of compost-enriched soil, the mixed substrate employed in this study probably offered a more favorable balance between these requirements.

By boosting microbial activity, improving nutrient cycling, and strengthening substrate structure, organic additives like leaf compost can further enhance rooting (Rouphael and Colla, 2020). Through enhanced nutrient uptake and the synthesis of growth-promoting substances, beneficial rhizosphere bacteria are associated with organic substrates that may also indirectly support root development. Numerous woody species propagated in nurseries have shown comparable advantages from compost-amended substrates (Owen et al., 2020; Kumar et al., 2022).

These observation on the interplay between exogenous auxin and substrate composition suggests that hormonal stimulation is not enough to optimize rooting performance. Rather, simultaneous optimization of physiological and environmental variables is necessary for effective proliferation. From an operational standpoint, using an inexpensive mixed substrate along with IBA treatment provides a financially viable method for large-scale nursery output.

4.5. Implications for Dryland Restoration and Climate Change Adaptation

The current findings are significant not just for nursery propagation but also for conservation biology, restoration ecology, breeding, and climate adaptation. With rising temperatures, extended droughts, and land degradation endangering ecological stability and production, dryland ecosystems are among the areas most at risk from climate change (IPCC, 2023). Plant species that can sustain ecosystem functions and local livelihoods while enduring increasingly harsh environmental circumstances are necessary for the mitigation of these ecosystems.

Deep root systems, nitrogen fixation, drought tolerance, and the capacity to establish damaged soil are all traits that make *P. juliflora* and *P. cineraria* extremely desirable for restoration initiatives. The use of climate-resilient species that can improve ecosystem recovery under future environmental conditions is emphasized in recent restoration frameworks (Mansourian et al., 2024). Therefore, scaling restoration efforts requires the establishment of effective propagation techniques for such species.

In this situation, vegetative propagation has several benefits. First, it makes it possible for superior genotypes with desired characteristics like high biomass production, quick growth, resistance to disease, and drought tolerance to proliferate. Second, by maintaining distinct genotypes that could otherwise be lost due to habitat degradation or population reduction, clonal propagation might aid in the ex-situ conservation of priceless genetic resources. Third, by lowering field performance variability, homogeneous planting material generated by clonal propagation might enhance restoration results.

For *P. cineraria*, which has significant ecological and cultural value throughout South Asian drylands, the conservation implications are especially pertinent. Concerns about this species' long-term survival in some areas of its natural range have been raised by population decreases linked to environmental stress, overexploitation, and changes in land use (Kumar et al., 2021; Arshad et al., 2024). Therefore, the propagation procedure created in this study offers a useful tool for conservation initiatives aiming to protect and replenish important populations.

Additionally, the importance of woody legumes in mitigating climate change and sequestering carbon is receiving increased attention. In addition to mitigating ecosystem functions, species that can quickly establish themselves and remain over an extended period in damaged areas can help store carbon. The propagation methods found in this work may allow more extensive climate-smart restoration efforts throughout arid and semi-arid regions by enabling the large-scale multiplication of elite Prosopis genotypes.

5. Limitations and Future Research Directions

Even though the current study successfully identified efficient propagation conditions, a few limitations should be considered. The long-term field performance of propagated plants is not clear because the experiment was carried out under nursery conditions. Survival after transplantation into challenging field conditions is not always ensured by rooting success alone under controlled conditions. Therefore, field establishment, growth performance, drought tolerance, and propagating material survival under restoration conditions should be assessed in future research.

Furthermore, the molecular and physiological processes that underline rooting responses were not examined. The regulatory mechanisms controlling adventitious roots in Prosopis species may be better understood by future studies that incorporate physiological measurements, hormonal analyses, and gene expression studies. Such data would enhance understanding of species-specific rooting behavior and enable additional optimization of propagation techniques. Lastly, evaluating a wider variety of genotypes, cutting kinds, and seasonal effects would aid in the creation of reliable propagation systems appropriate for running nursery programs.

Overall, the present study demonstrates that the combination of 5000 ppm IBA and a compost-enriched rooting substrate provides an effective strategy for enhancing vegetative propagation of *P. juliflora* and *P. cineraria*. The resulting improvements in root growth and shoot development highlight the potential of auxin-mediated clonal propagation as a practical tool for conservation, restoration, improved genotypes, and climate-resilient forestry initiatives in arid and semi-arid environments.

6. Conclusion

Prosopis juliflora and *Prosopis cineraria* are economically and ecologically significant dryland tree species for which the current study successfully developed an effective macropropagation procedure using exogenous auxins. The findings showed that adventitious root formation and shoot development were much improved by exogenous auxin treatment, with indole-3-butyric acid (IBA) consistently outperforming naphthalene acetic acid (NAA) across all tested parameters. Auxin concentration gradually increased root length, shoot length, and root number; the 5000 ppm IBA treatment typically proved effective with enhanced root growth and development.

The success of propagation also depended heavily on rooting substrates. Compared to sandy loam soil alone, the mixed medium of sand, soil, and leaf compost offered a more conducive rooting environment, which enhanced root growth and shoot development. Auxin administration and substrate composition interacted to further improve propagation effectiveness, suggesting that both physiological and environmental elements must be optimized for successful roots.

Significant improvements in root system quality were noted, despite relatively small increases in rooting percentage. Because root shape and root system architecture are frequently more accurate indicators of transplant survival and field establishment than rooting frequency alone, this finding is especially significant. It is predicted that the development of larger and more robust root systems will enhance drought tolerance, nitrogen uptake, and water acquisition after transplantation. Practically speaking, using a compost-enriched rooting substrate in conjunction with 5000 ppm IBA is an economical and workable method for clonal reproduction of superior Prosopis genotypes. The created propagation method has significant uses in programs for climate-resilient forestry, agroforestry development, genetic resource conservation, and dryland restoration.

The encouraging results obtained under nursery conditions need to be verified by the long-term field performance and post-transplant survival of the propagated plants. Future research should focus on assessing field establishment, drought tolerance and growth in restoration settings, and examining the physiological, hormonal and molecular mechanisms underlying rooting responses. Further investigations of a greater number of genotypes, cutting types and seasonal conditions would allow the development of more robust and scalable propagation protocols for large-scale conservation and restoration programs.

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