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Morphological Growth and Biomass Allocation Responses of *Acacia auriculiformis* Seedlings to Varying Watering Intervals

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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

Water availability strongly influences early seedling establishment and nursery performance. This study examines how varying watering intervals affect the morphological growth and biomass allocation of *Acacia auriculiformis* seedlings under controlled nursery conditions. Over 13 weeks, seedlings were watered at five different frequencies: daily and every 4, 8, 12, and 16 days. The research focused strictly on physical growth patterns rather than physiological responses to drought intensity. Results showed that extended watering intervals consistently stunted above-ground development; average shoot length dropped from 60.25 cm in the daily control group to just 18.69 cm under the 16-day interval. However, a four-day watering interval stimulated below-ground growth. These seedlings achieved a maximum root length of 29.43 cm and a total plant biomass of 9.42 gm, outperforming the daily-watered control. This adaptive growth strategy was further supported by a significantly lower shoot-to-root ratio during the 4-day and 8-day intervals. The data indicated that the seedlings actively redirected biomass to their root systems to cope with moderate water stress before the severe growth declines observed at the 12-day and 16-day intervals.

Keywords: Acacia auriculiformis; water deficit; drought; nursery management; seedling growth; root elongation; biomass partitioning.

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1. Introduction

Drought remains a pervasive and highly destructive environmental stressor affecting terrestrial ecosystems, agriculture, and global forestry initiatives (Ault, 2020; Seleiman *et al.,* 2021; Intergovernmental Panel on Climate Change, 2023). Driven by anthropogenic climate change, global drought events are increasing in frequency, duration, and severity, fundamentally altering soil moisture dynamics and seasonal precipitation patterns (Intergovernmental Panel on Climate Change, 2023; Ferraz *et al.,* 2026). Water deficits have a profound impact, leading to the development of physiological, biochemical, and morphological changes that reduce carbon assimilation, nutrient uptake, and plant vegetative growth (Gupta *et al.,* 2020; Ahluwalia *et al.,* 2021). Mitigating these impacts is critical not only for global food security but also for the success of large-scale afforestation, carbon sequestration, and land rehabilitation projects designed to combat climate change (Fahad *et al.,* 2017; Ferraz *et al.,* 2026). Recent evidence from woody seedlings indicates that drought can alter root morphology and biomass allocation alongside reductions in above-ground growth, with responses varying among species and stress conditions (Di Iorio *et al.*, 2024; Gning *et al.*, 2025).

In the context of tropical and subtropical forestry, the seedling stage represents the most vulnerable phase of stand establishment (Grossnickle, 2012; Zhang *et al.,* 2025). Water scarcity is a major constraint in seedling production in nurseries and outplanting in the field, especially in areas with irregular monsoon rains and high summer temperatures (Worthy *et al.,* 2022). Understanding the response of adaptive traits such as shoot-root allocation, internodal length, and leaf expansion to imposed progressive water deficit will help foresters identify more resilient genotypes and determine appropriate nursery irrigation schedules (Sodimu *et al.,* 2021; Medeiros *et al.,* 2026). Nursery-based experiments further show that short-term drought can affect transpiration, root morphology, and plant biomass in tree seedlings, supporting an integrated morphological assessment when evaluating watering regimes (de Quesada *et al.*, 2024). In tropical tree species, induced water stress has also produced clear species-specific differences during germination and early establishment, underscoring the sensitivity of early developmental stages to water limitation (Pal & Singh, 2026).

Acacia auriculiformis (A. Cunn. ex Benth.), or earpod wattle, is a fast-growing, evergreen, nitrogen-fixing leguminous tree native to Australia, Papua New Guinea, and Indonesia (Doran & Turnbull, 1993; Brockwell *et al.,* 2005; Orwa *et al.,* 2009). It has been widely planted throughout the tropics for its remarkable ability to grow in nutrient-poor, degraded soils and for its multi-purpose value in fuelwood, pulp, timber, and environmental protection (Rahman *et al.,* 2017; Prasanna *et al.,* 2026). While generally considered well-adapted to harsh conditions, its morphological plasticity and biomass partitioning behaviours under acute and progressive drought stress during the critical nursery stage require precise quantification to forecast its long-term viability under shifting climate scenarios (Bradbury, 1990). Controlled drought experiments have quantified shoot and root growth together with fresh and dry biomass to characterise plant responses across water-deficit levels (Chiroli *et al.*, 2025).

The climate of the region where the experiment was conducted was highly variable, with summer temperatures as high as 49.5°C and most precipitation falling during the monsoon season (Central Ground Water Board, 2022; Weather Blaze, 2026). Given the ongoing need for successful afforestation in this semi-arid, climatically unpredictable zone, it is critical to evaluate the early growth responses of *Acacia auriculiformis* before large-scale deployment. This study provides valuable empirical data by documenting morphological changes, such as root elongation and biomass trade-offs, as watering intervals increase. It explains why this species is suitable for use in water-stressed landscapes and identifies the precise moisture limits at which its adaptive features fail (Bradbury, 1990; Rahman *et al.,* 2017). However, there remains a need to quantify, within a single nursery experiment, how progressively longer watering intervals jointly influence shoot growth, root elongation, leaf development, and biomass partitioning in *Acacia auriculiformis* under semi-arid conditions. Such an integrated assessment can clarify the point at which morphological adjustment under moderate water limitation gives way to broader growth restriction under prolonged watering intervals.

1.1 Objective

This study was conducted to evaluate the effects of different watering intervals on the morphological and biomass characteristics of *Acacia auriculiformis* seedlings.

2. Materials and Methods

2.1 Experimental Site

The study was conducted in 2025 at the forestry nursery of the College of Forestry, Sam Higginbottom University of Agriculture and Technology Sciences (SHUATS) at Prayagraj, Uttar Pradesh, India (Fig. 1). The site is located at 25° 24′ 45″ N latitude and 81° 50′ 57″ E longitude, at an elevation of 91 m (298 ft) above mean sea level. The local climate is characterised by extreme heat, with historically recorded summer maxima of 49.5°C and winter minima of 1.0°C (Weather Blaze, 2026). According to the Central Ground Water Board (2022), the district receives an average annual historical rainfall of approximately 955 mm, aligning with the state average.

2.2 Experimental Design and Treatment Combinations

The study utilised a completely randomised design (CRD), which is the most appropriate statistical approach given the highly uniform microclimatic conditions within the nursery environment. A total of 600 seedlings were randomly assigned across five distinct watering frequency treatments (Table 1). Each treatment comprised four replications containing 30 seedlings each. Seedlings were cultivated in 10 × 20 cm polybags filled with a standardised 1:1:1 volumetric mixture of local soil, sand, and Farm Yard Manure (F.Y.M). To accurately isolate the variable of transpiration and minimise direct soil water evaporation, the bases of the stems and the soil surface within the polybags of all treatment groups (T_1 to T_5) were tightly covered with white plastic (Phillips & Riha, 1993). Watering frequency was controlled, with exactly 250 mL of water applied per seedling at each scheduled interval (Sodimu *et al.,* 2021). Morphological and biomass characters were recorded after 13 weeks.

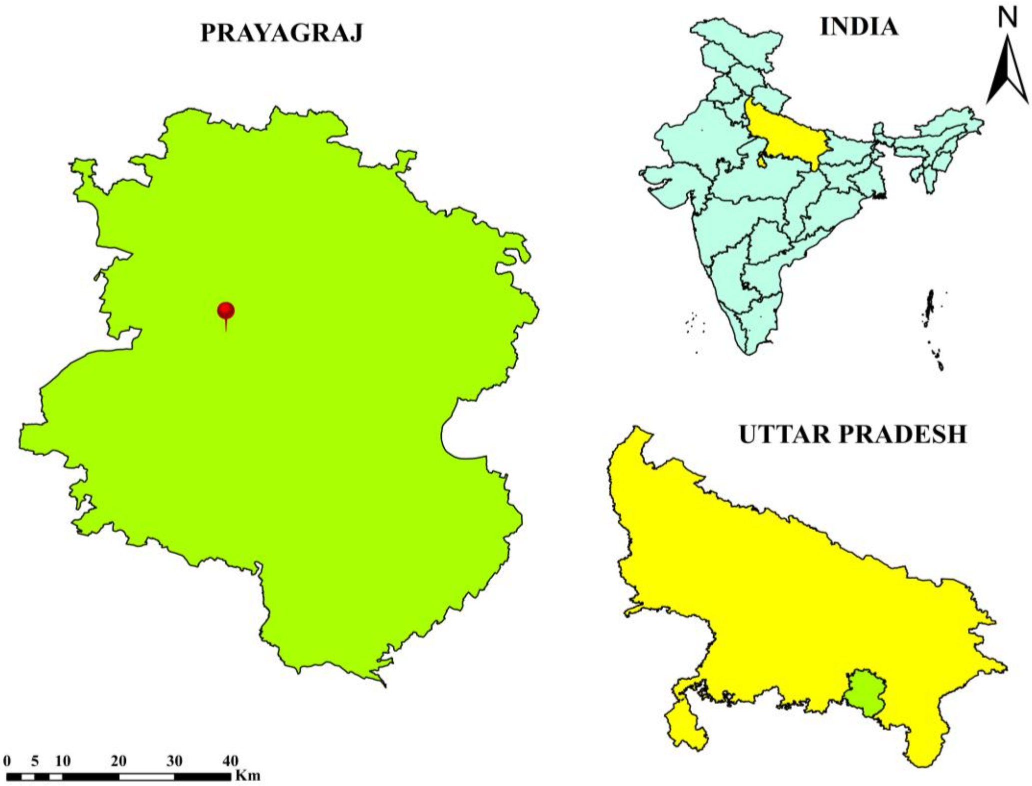

Fig. 1. Location of the experimental site in Prayagraj, Uttar Pradesh, India

Table 1. Treatments used for the experiment

Treatments	Watering Interval
T_1	Once daily
T_2	Once every 4 days
T_3	Once after 8 days
T_4	Once after 12 days
T_5	Once after 16 days

During the 13-week experimental period in 2025, the seedlings were maintained under a green agricultural shade net (providing approximately 50% shade) to protect them from direct solar radiation and buffer extreme temperatures. Meteorological data retrieved from the Department of Agro-meteorology, Naini Agricultural Institute, SHUATS, indicated that the experimental site experienced an average temperature of approximately 37.89°C and an average relative humidity of 20.46%. Ambient precipitation was negligible (approximately 0.25 mm over the period of the trial), ensuring that ambient rainfall did not interfere with the prescribed watering intervals.

2.3 Data Collection

2.3.1 Morphological Parameters

Measurements included seedling height (cm), root length (cm), collar diameter (mm), internodal length (mm), number of branches, number of leaves, leaf area (cm^2), and total length (cm).

2.3.2 Biomass Parameters

Seedlings were carefully uprooted, washed, and separated into root and shoot components. Recorded metrics included fresh shoot weight (gm), dry shoot weight (gm), fresh root weight (gm), dry root weight (gm), shoot-root ratio, and total biomass (gm).

2.4 Statistical Analysis and Software Used

All statistical computations were conducted in RStudio. An analysis of variance (ANOVA) F-test was used to evaluate the overall significance of the treatments. Because the variance across treatments was highly significant ($P < 0.001$), critical differences (C.D.) were calculated at a 95% confidence level ($P = 0.05$) to compare individual treatment means. Additionally, the spatial map of the study site (Fig. 1) was generated using QGIS (version 4.0.3).

3. Results and Discussion

3.1 Morphological Responses to Watering Intervals

The progressive drought treatments (T_1 to T_5) produced highly significant variations across all measured morphological parameters of *Acacia auriculiformis* ($P < 0.001$). While the escalating water stress generally reduced overall plant growth, the seedlings exhibited distinct adaptive responses during the early stages of the treatments (Table 2).

3.1.1 Shoot and Root Characteristics

Shoot length was significantly reduced across the treatments, with the highest value in T_1 (60.25 cm) and the lowest in T_5 (18.69 cm). In contrast, root length was greatest in T_2 (29.43 cm), followed by T_1 (26.39 cm). This initial root elongation under relatively low stress levels indicated a possible drought-avoidance mechanism, as the total plant length closely followed the pattern, reaching a maximum of 88.03 cm in T_2 and then gradually decreasing to 34.86 cm in T_5.

3.1.2 Stem Characteristics

Stem robustness was inversely related to treatment severity. Collar diameter showed a clear decreasing trend, from a maximum of 3.34 mm in T_1 to a minimum of 1.1 mm in T_5. Along the same gradient, internode length also progressively decreased from 16.27 mm to 7.72 mm, showing that the vertical growth of stems was severely hampered by greater stress. The treatments strongly inhibited the development of foliage and lateral branches.

3.1.3 Leaves and Branching Characteristics

Leaf production and expansion were greatly reduced, with a significant reduction in the number of leaves from 43.5 in T_1 to only 16.3 in T_5. The average leaf area, in turn, decreased by about 67%, from 8.85 cm^2 to 2.93 cm^2. The branching response was similar to the root-length response: branching changed little between T_1 (7.4 branches) and T_2 (7.55 branches) and then gradually decreased to 4.55 branches in T_5.

3.1.4 Total Plant Length

Total length (shoot and root) differed significantly among the treatments. The maximum total length occurred in T_2 at 88.03 cm, compared to 86.63 cm in T_1. This initial peak was caused mainly by the compensatory root growth occurring under the T_2 condition. As treatment severity increased from T_3 to T_5, total plant length decreased significantly and gradually to a minimum of 34.86 cm in T_5.

Table 2. Mean Characteristics of Morphology in *Acacia auriculiformis* Under Nursery Conditions

Treatments	Shoot Length (cm)	Root Length (cm)	Collar Diameter (mm)	Internodal Length (mm)	Number of Branches	Number of Leaves	Leaf Area (cm^2)	Total Length (cm)
T_1	60.25	26.39	3.34	16.27	7.4	43.5	8.85	86.63
T_2	58.6	29.43	3.11	15.42	7.55	39.65	8.1	88.03
T_3	38.38	24.76	1.82	11.94	6.3	28.05	5.51	63.14
T_4	25.92	21.24	1.43	9.79	5.2	21.25	3.94	47.16
T_5	18.69	16.17	1.1	7.72	4.55	16.3	2.93	34.86
F-test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
S. Ed. (±)	1.58	0.56	0.15	0.25	0.26	1.13	0.22	2.06
C. D. (P=0.05)	3.13	1.12	0.3	0.5	0.51	2.25	0.45	4.1

3.2 Biomass Accumulation and Partitioning

Biomass data showed that the nursery drought treatments (T_1 to T_5) had a significant effect on the biomass accumulation and partitioning of *Acacia auriculiformis*. All fresh and dry weight parameters were significantly different, as indicated by the F-test (Table 3).

3.2.1 Fresh and Dry Shoot Biomass

Treatment severity was significantly and negatively correlated with above-ground biomass. Fresh shoot weight decreased significantly from the highest value of 17.54 gm in T_1 to the lowest value of 5.17 gm in T_5. Dry shoot weight showed a similar trend, decreasing from 6.93 gm in T_1 to 2.12 gm in T_5.

3.2.2 Fresh and Dry Root Biomass

Root biomass, in contrast to the decreasing shoot mass, showed an initial stimulation under early stress conditions. The fresh and dry root weights were highest in T_2 (5.99 gm and 2.84 gm, respectively) compared to T_1 (5.37 gm and 2.13 gm, respectively). With increasing treatment severity (T_3 to T_5), root biomass was significantly reduced to a minimum of 2.65 gm (fresh) and 0.93 gm (dry) in T_5.

3.2.3 Total Biomass

Total biomass followed a pattern similar to root growth and reflected the plant's overall dry matter production. Total biomass was highest in T_2 (9.42 gm), slightly exceeding the 9.06 gm recorded in T_1. Total biomass then decreased steadily and significantly in all subsequent treatments, resulting in a minimum biomass of 3.04 gm in T_5.

3.2.4 Shoot-Root Ratio

The treatments resulted in a marked change in biomass allocation, suggesting a stress-induced shift in morphological growth. The shoot-root ratio was highest in T_1 (3.33). This ratio declined significantly to 2.36 under early stress (T_2) and reached a minimum of 2.25 in T_3, indicating a focus on root mass over shoot mass during early and moderate stress. Under the most severe treatment, the ratio rose slightly to 2.44 in T_5.

Table 3. Mean Characteristics of Biomass in *Acacia auriculiformis* Under Nursery Conditions

Treatments	Fresh Shoot Weight (gm)	Fresh Root Weight (gm)	Dry Shoot Weight (gm)	Dry Root Weight (gm)	Shoot-Root Ratio	Total Biomass (gm)
T_1	17.54	5.37	6.93	2.13	3.33	9.06
T_2	15.97	5.99	6.58	2.84	2.36	9.42
T_3	10.76	4.96	4.26	1.91	2.25	6.17
T_4	7.21	3.47	2.94	1.33	2.28	4.27
T_5	5.17	2.65	2.12	0.93	2.44	3.04
F-test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
S. Ed. (±)	0.33	0.11	0.15	0.08	0.2	0.15
C. D. (P=0.05)	0.65	0.21	0.31	0.16	0.39	0.3

The results demonstrated that progressive water-deficit conditions generally resulted in greater below-ground biomass allocation under moderate water stress and a marked reduction in above-ground vegetative growth of *Acacia auriculiformis*. Shoot length, stem collar diameter, and leaf area showed a gradual and severe reduction as the watering interval increased, from daily (T_1) to 16 days (T_5). Structural stunting, which is common in woody seedlings during water stress, results from a physiological process that reduces transpiration and water loss.

The most significant adaptive response was under early-to-moderate drought stress (4-day watering interval), during which seedlings exhibited compensatory root elongation and greater total plant biomass than the daily-watered control. The maximum root length was 29.43 cm, and the highest total biomass was 9.42 gm. This effect is in line with optimal partitioning theory, which suggests that plants invest in organs that are most successful in obtaining the limiting environmental factor (Yan *et al.,* 2023). A comprehensive meta-analysis of drought effects confirms that shifting biomass allocation toward the root system is a fundamental, widespread drought-avoidance strategy across plant species, enabling them to rapidly exploit deeper soil moisture (Eziz *et al.,* 2017). Furthermore, specific morphological plasticity in tree roots, including rapid elongation under mild deficits, is recognised as a critical trait for surviving progressive drought (Brunner *et al.,* 2015). Water-use adaptations and physiological plasticity have been found in other species of *Acacia*, enabling them to survive across diverse topographies and arid environments through targeted functional trait adaptations (Uni *et al.,* 2023). Additionally, dynamic shifts in biomass are known to be critical for maintaining drought tolerance in other leguminous species such as *Acacia seyal* (Abdelmalik *et al.,* 2020).

Although the initial resilience of *Acacia auriculiformis* appeared relatively high, severe stress soon exhausted the adaptive capacity of *Acacia auriculiformis*. As stress progressed, the shoot-root ratio continued to decrease before reversing; at the same time, total biomass production declined markedly under the 12-day and 16-day treatments. The greatest reduction in total length (34.86 cm) and total biomass (3.04 gm) under the most extreme water scarcity suggests that the species is unable to acclimatise to longer periods of severe water scarcity. Once the adaptive threshold is exceeded, additional moisture stress results in dramatic decreases in morphology, dry matter production, and eventually in the overall success of seedling establishment and/or long-term viability.

These results are directly applicable to nursery management in semi-arid regions such as Prayagraj. Mild stress (*e.g.,* 4-day watering intervals) acts as an effective nursery hardening technique. Research on seedling quality has demonstrated that exposing seedlings to controlled, moderate moisture deficits precondition their morphological attributes, specifically by promoting robust root systems that significantly improve field survival and drought recovery upon outplanting (Grossnickle & MacDonald, 2018). However, extending watering intervals beyond these limits severely stunts morphological development and biomass production.

4. Limitation

This study presents clear empirical data, but it is important to acknowledge certain limitations. First, the experiment was limited to a 13-week duration in a controlled nursery setting, using a specific polybag size (10 × 20 cm). As a result, the root elongation observed in the T_2 treatment may have been influenced by artificial pot-binding effects, which do not accurately represent true field conditions. Second, the study employed a single standardised soil matrix and a fixed shade level (50%), meaning that the interactive effects of different soil types, nutrient deficiencies, full sunlight exposure, and drought stress were not assessed. Future research should investigate the outplanting survival rates of these conditioned seedlings in natural field environments.

5. Conclusion

The overall growth and biomass development of *Acacia auriculiformis* seedlings were significantly affected by extended watering intervals. Less frequent watering, particularly intervals of 12 and 16 days, led to a gradual decrease in shoot growth parameters, including shoot length, stem diameter, internodal elongation, and leaf expansion. Both above-ground fresh and dry shoot mass showed a strong negative correlation with the length of the watering intervals. Despite the overall reduction in vegetative growth, a notable shift in biomass allocation occurred with a four-day watering interval. Under these conditions, the seedlings displayed compensatory root growth, resulting in maximum root length, root biomass, and total biomass that exceeded those of seedlings watered daily. This shift was also evident in a significant decrease in the shoot-root ratio, indicating a structural change toward greater investment in below-ground growth. In summary, *Acacia auriculiformis* reallocates resources to root development during mild watering intervals. However, under prolonged water deficits of 12 to 16 days, both morphological development and biomass production decline significantly.

Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. Grammarly and ChatGPT were used solely to assist with grammatical refinement and reference formatting during manuscript revision. 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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