Enhancing Seed Germination and Seedling Growth of Tropical Almond (*Pithecellobium dulce*) through Seed Soaking Treatments

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Abstract

A greenhouse experiment was conducted at the Department of Horticulture and Landscape Engineering, College of Agriculture, University of Diyala, during March 2024/2025, to evaluate the effects of different plant growth stimulants on the germination and early growth of Indian almond (*Pithecellobium dulce* L.) seeds were obtained from a local nursery. The study employed a Completely Randomized Design (CRD) with five treatments—control (distilled water), gibberellic acid (GA_3 , 50 mg L^{-1}), benzyl adenine (BA, 50 mg L^{-1}), 2,4-dichlorophenoxyacetic acid (2,4-D, 30 mg L^{-1}), and Etonk (4 mg L^{-1})—with three replicates of 50 seeds each. Seeds were soaked for six hours and sown individually in paper cups filled with Dutch peat moss under polyethylene cover to maintain moisture. The results revealed that GA_3 significantly enhanced germination percentage (16.3%) and reduced germination time to 12.66 days, compared to the control (5.3% and 24.33 days). Treatment with 2,4-D produced the tallest seedlings (11.88 cm) and the highest number of leaves (6.82 leaves per seedling), while BA application significantly increased leaf chlorophyll content (33.59 SPAD units). These findings indicate that different plant growth stimulants selectively enhance germination and seedling growth traits, with GA_3 being most effective in promoting germination, 2,4-D in vegetative growth, and BA in chlorophyll accumulation.

Keywords: Seedling development ,Vegetative growth , Hormonal treatments ,Gibberellins and Auxins .

Introduction

Indian Almond, *Pithecellobium dulce* L. (Fabaceae), an evergreen tropical tree native to the Americas, is globally recognized for its nutritional and medicinal significance (1). Its phytochemical profile—comprising flavonoids, saponins, and alkaloids—exhibits potent antioxidant, antimicrobial, and antidiabetic activities, positioning the species as a key resource for novel drug development (2).

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Gibberellins are natural plant hormones synthesized within plant tissues across the plant kingdom, including both higher plants (angiosperms and gymnosperms) and lower plants such as bacteria, algae, fungi, and ferns (3). And (4) demonstrated that treating avocado seeds with gibberellic acid at a concentration of 400 mg L^{-1} resulted in the highest germination rate of 95.2% and a maximum seedling height of 75.67 cm after 45 days of sowing. Furthermore (5) reported that soaking *Persea americana* cv. Fuerte seeds in gibberellin solutions at concentrations of 1000 and 2000 mg L^{-1} produced the highest germination rates, ranging from 83.3% to 100%, during evaluation periods at 30, 60, and 120 days post-sowing. These treatments outperformed the control (distilled water), indicating that gibberellin application significantly accelerates seed germination and reduces the time required for radicle emergence.

Cytokinins are plant hormones that play a crucial role in stimulating cell division and can influence morphogenesis, leading to increased cell size, flowering, and senescence. Cytokinins can be produced naturally within plants or synthesized artificially (6) and (7) demonstrated that soaking pine seeds in benzyl adenine at a concentration of 0.25 mg L^{-1} resulted in significant differences in germination rates compared to the control treatment and also improved seedling morphological traits.

Auxins are the first group of plant hormones to be discovered, characterized by their ability to regulate growth through the stimulation of cell elongation. The term “auxin” is derived from a Greek word meaning “growth.” Numerous studies have shown that pure auxinic compounds exhibit physiological behavior similar to indole acetic acid, including the phenoxy group. Among these compounds, 2,4-D is one of the oldest and most important auxins discovered since 1945, commonly used at low concentrations as a plant growth regulator (8).

Reported (9) that soaking lemon seeds in auxins at a concentration of 50 mg g^{-1} resulted in high germination rates and improved post-germination seedling growth traits.

Etonk is an aromatic compound that enhances plant activity and growth without causing deformities or toxicity symptoms, and it also elevates the endogenous auxin level in plants (10). Demonstrated (11) that foliar application of Etonk on cucumber plants at concentrations of 0.5, 0.75, and 1 mL L^{-1} significantly increased vegetative growth traits, including plant height and stem diameter, compared to the untreated control. The primary objective of this study was to investigate the effects of various plant growth stimulants—gibberellic acid, benzyl adenine, 2,4-D, and Etonk—on the germination, seedling growth, and physiological traits of Indian almond seeds, to identify the most effective treatment for enhancing germination rate, seedling height, leaf number, and chlorophyll content under controlled greenhouse conditions

Materials and Methods

The experiment was conducted in a polyethylene-covered greenhouse (Saran) at the Department of Horticulture and Landscape Engineering, College of Agriculture, University of Diyala, during March of the 2024/2025 cropping season, to investigate the effects of plant growth stimulants on the germination of Indian almond seeds. Seeds were washed with water at 38°C and gently rubbed with fine sand to remove impurities, then divided into five groups according to the experimental treatments and soaked in glass bottles in a dark place using distilled water for six hours per treatment, including the control (distilled water only), gibberellic acid (GA_3) at 50 mg L^{-1}, benzyl adenine (BA) at 50 mg L^{-1}, 2,4-dichlorophenoxyacetic acid (2,4-D) at 30 mg L^{-1}, and Etonk at 4 mg L^{-1}. Paper cups perforated at the bottom and filled with Dutch peat moss were used, moistened with water, with one seed planted per cup and covered with polyethylene to maintain moisture. The experiment was conducted as a **Completely** Randomized Design (CRD) with five independent treatments and three replicates, each replicate containing 50 seeds, and data were collected and statistically analyzed using SAS software, with mean differences compared using Duncan’s multiple range test at a 0.05 significance level (12).

Studied Traits

The studied traits were recorded 20 days after germination

1- **Germination Percentage (%)**: The percentage of seeds that successfully germinated during the experimental period relative to the total number of seeds sown
2- **Germination Rate / Speed**: The rate at which radicles emerge from seeds during the experimental period, typically calculated as the number of germinated seeds divided by the time (days) required to reach this number
3- **Seedling Height (cm)**: The vertical distance from the base of the stem at the soil level to the tip of the seedling after a specified period of germination, used as an indicator of plant growth
4- **Number of Leaves:** The total number of visible leaves per seedling after a specified period of germination, used as an indicator of vegetative growth in young plants
5- **Leaf chlorophyll content** was measured using a SPAD meter, with readings expressed in SPAD unit

Results and Discussion

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1- **Germination Percentage:** The results presented in Table 1 indicate that plant growth stimulants significantly affected seed germination. Gibberellic acid (GA_3) treatment exhibited the highest germination percentage (16.3%), followed by 2,4-D (12%), while the control treatment showed the lowest germination rate at 5.3%. No significant differences were observed among the other treatments.

2- **Germination Rate (Speed):** GA_3 treatment also significantly enhanced germination speed, with an average of 12.66 days, compared to 24.33 days for the control treatment, indicating a clear accelerating effect of gibberellic acid on radicle emergence.

3- **Seedling Height:** 2,4-D treatment significantly increased seedling height, reaching 18.33 cm, surpassing GA_3 and Etonk treatments, which recorded 12.66 cm and 16.00 cm, respectively, while the control treatment produced the shortest seedlings at 24.33 cm.

4- **Number of Leaves:** Seedlings treated with 2,4-D produced the highest number of leaves per plant (6.82 leaves), whereas the control treatment resulted in the lowest leaf number (3.66 leaves). No significant differences were observed among the other treatments.

5- **Leaf Chlorophyll Content:** Benzyl adenine (BA) significantly increased leaf chlorophyll content, reaching 33.59% SPAD units, outperforming the control treatment, which recorded 21.1% SPAD units. No significant differences were detected among the remaining treatments

Table 1 Effect of different plant growth stimulants on the germination of Indian almond seeds.

Treatments	Germination Percentage	Germination Rate	Seedling Height	Number of Leaves	Leaf Chlorophyll Content
Control	5.3c	24.33a	24.33a	3.66 b	21.1 b
GA3	16.3a	12.66 c	12.66 c	6.16 a	33.38 a
BA	8 bc	15.33 bc	15.33 bc	6.26 a	33.59 a
2,4-D	12 a	.33 b18	.33 b18	6.82 a	28.71 a
Atonic	9.3b	16 bc	16.00 bc	6.19 a	29.79 a

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The results of this study indicate the presence of significant differences among treatments in germination percentage and rate, number of leaves, chlorophyll content, and seedling height. This may be attributed to the effect of the applied substances in stimulating physiological processes within the seed. The superiority of the gibberellin treatment in terms of germination percentage and speed can be explained by its role in promoting seed germination and breaking dormancy (13). These findings are consistent with those reported by (5), where soaking avocado seeds (cv. Fuerte) in gibberellin solutions at concentrations of 1000 and 2000 mg L^{-1} resulted in the highest germination percentages (83.3–100%) during evaluation periods of 30, 60, and 120 days after sowing. This treatment outperformed the control (distilled water), as gibberellin enhanced germination and reduced the time required for radicle emergence.

Soaking coconut seeds in benzyladenine led to an increase in leaf chlorophyll content in seedlings after germination. This effect is attributed to the role of benzyladenine in stimulating cell division, which may also influence cell differentiation and result in larger cell size (14). Similarly, (7) reported that soaking pine seeds in benzyladenine at a concentration of 0.25 mg L^{-1} improved physiological traits. Additionally, soaking Moringa seeds in benzyladenine at 100 mg L^{-1} enhanced vegetative growth characteristics, particularly radicle and shoot length, compared to untreated seeds (15).

Soaking seeds with 2,4-D treatment led to an increase in the number of leaves and seedling height, which is attributed to its role in growth regulation through stimulating cell elongation (7). Similarly, (16) reported that soaking lemon seeds in auxins at a concentration of 50 mg L^{-1} improved seedling growth traits after germination

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