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Effect of naphthalene acetic acid (NAA) on dragon fruit (*Hylocereus undatus*) cuttings under different growing conditions

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Abstract
The experiment, conducted during 2023-2024 at the Department of Horticulture, North-Eastern University, Tura Campus, West Garo Hills, Meghalaya, evaluated the effects of Naphthalene Acetic Acid (NAA) concentrations and growing conditions on dragon fruit (*Hylocereus undatus*) cuttings. A Factorial Randomized Block Design (FRBD) with three replications tested two factors: NAA concentrations (0, 100, 150, 200 ppm) and growing conditions (greenhouse, polyhouse, open conditions). The combination of polyhouse conditions with 200 ppm NAA recorded the highest values for number of roots per plant (20.18), root length (17.95 cm), plant height (23.51 cm), fresh weight of shoots (43.94 g), dry weight of shoots (11.29 g), fresh weight of roots (4.81 g), dry weight of roots (1.09 g), survival rate (92.59%), number of sprouts per plant (2.66), length of sprouts (21.98 cm), and girth of sprouts (26.66 mm). Untreated cuttings in greenhouse conditions exhibited the lowest performance across all parameters. Additional parameters, including root-to-shoot ratio, chlorophyll content, and days to first sprout, further supported the efficacy of the polyhouse-NAA combination. These findings highlight optimal conditions for dragon fruit propagation in subtropical regions.

Keywords: Dragon fruit, *Hylocereus undatus*, naphthalene acetic acid

Introduction
Dragon fruit (*Hylocereus undatus*), a tropical cactus species, is valued for its nutritional benefits, including high antioxidant content, vitamins, and fiber, making it a promising crop for subtropical regions like Meghalaya. Propagation through stem cuttings is widely practiced due to its simplicity and cost-effectiveness, but success depends on rooting efficiency, which is influenced by hormonal treatments and environmental conditions. Naphthalene Acetic Acid (NAA), a synthetic auxin, promotes adventitious root formation by stimulating cell division and elongation (Hartmann *et al*., 2011) [1]. Growing environments, such as greenhouses, polyhouses, and open fields, regulate temperature, humidity, and light, significantly affecting plant establishment.
West Garo Hills, Meghalaya, with its subtropical climate, offers potential for dragon fruit cultivation, but local propagation techniques require optimization to enhance nursery production. Previous studies have demonstrated the benefits of auxins and controlled environments for rooting in horticultural crops (Kumar *et al*., 2018; Le & Nguyen, 2020) [2, 3]. However, the interactive effects of NAA and growing conditions on dragon fruit in Meghalaya remain underexplored. This study aimed to assess the combined effects of NAA concentrations and growing conditions on rooting, growth, and physiological parameters of dragon fruit cuttings, with the goal of developing efficient propagation protocols for local farmers.

Materials and Methods
Study Location and Duration
The experiment was conducted from June 2023 to March 2024 at the Department of

Horticulture, North-Eastern University, Tura Campus, West Garo Hills, Meghalaya (25.517°N, 90.220°E, elevation ~600 m). The region has a subtropical climate with annual rainfall of 2,500-3,000 mm, temperatures of 15-30°C, and relative humidity of 60-90%.

Experimental Design

A Factorial Randomized Block Design (FRBD) with three replications was used, testing two factors:

1. **NAA Concentrations:** Four levels (0 ppm [control], 100 ppm, 150 ppm, 200 ppm).
2. **Growing Conditions:** Three environments (greenhouse, polyhouse, open conditions).

This yielded 12 treatment combinations, with each treatment replicated thrice, resulting in 36 experimental units (4 × 3 × 3).

Plant Material and Treatment Application

Uniform dragon fruit (*Hylocereus undatus*) stem cuttings (15-20 cm long, 2-3 nodes) were collected from healthy, mature plants. Cuttings were air-dried for 24 hours to heal wounds. NAA solutions were prepared by dissolving NAA powder in ethanol and diluting with distilled water to achieve concentrations of 0, 100, 150, and 200 ppm. Basal ends of cuttings were dipped in the respective NAA solutions for 10 seconds, air-dried briefly, and planted.

Growing Conditions and Planting

Cuttings were planted in 15 cm plastic pots containing a potting mixture of soil, sand, and vermicompost (1:1:1 ratio, pH 6.5-7.0). The growing conditions were:

- **Greenhouse:** Controlled environment with 25-30°C temperature, 70-80% humidity, and supplemental lighting (12-hour photoperiod).
- **Polyhouse:** Semi-controlled environment with 22-28°C temperature, 65-75% humidity, and diffused natural light.
- **Open Conditions:** Natural outdoor environment with 20-28°C temperature, 60-75% humidity, and exposure to sunlight and rainfall.

Pots were irrigated every 2-3 days, and no fertilizers were applied during the 90-day experiment.

Data Collection

Data were recorded 90 days after planting for the following parameters:

1. Number of roots per plant (manual count).
2. Root length (cm, longest root measured with a ruler).
3. Plant height (cm, from soil level to highest point).
4. Fresh weight of shoots (g, weighed immediately).
5. Dry weight of shoots (g, oven-dried at 70°C for 48 hours).
6. Fresh weight of roots (g, weighed after washing).
7. Dry weight of roots (g, oven-dried at 70°C for 48 hours).
8. Survival rate (%, percentage of surviving cuttings).
9. Number of sprouts per plant (manual count).
10. Length of sprouts (cm, longest sprout measured).
11. Girth of sprouts (mm, measured at base with digital caliper).
12. Root-to-shoot ratio (calculated as dry weight of roots/dry weight of shoots).
13. Chlorophyll content (mg/g fresh weight, measured using a SPAD-502 chlorophyll meter).
14. Days to first sprout (days from planting to first visible sprout).

Statistical Analysis

Data were analyzed using Analysis of Variance (ANOVA) for a factorial design in SPSS (version 25). Treatment means were compared using the Least Significant Difference (LSD) test at $p < 0.05$. Interaction effects between NAA concentrations and growing conditions were also evaluated.

Results and Discussion

Main Effects and Interactions

ANOVA indicated significant effects ($p < 0.05$) of NAA concentrations, growing conditions, and their interactions on all parameters. The combination of polyhouse conditions with 200 ppm NAA (T4P) yielded the highest values (Table 1): number of roots per plant (20.18 ± 0.45), root length (17.95 ± 0.32 cm), plant height (23.51 ± 0.41 cm), fresh weight of shoots (43.94 ± 0.78 g), dry weight of shoots (11.29 ± 0.22 g), fresh weight of roots (4.81 ± 0.09 g), dry weight of roots (1.09 ± 0.03 g), survival rate (92.59 ± 1.12%), number of sprouts (2.66 ± 0.08), length of sprouts (21.98 ± 0.39 cm), girth of sprouts (26.66 ± 0.47 mm), root-to-shoot ratio (0.097 ± 0.003), chlorophyll content (2.45 ± 0.05 mg/g), and earliest days to first sprout (12.33 ± 0.58 days). Untreated cuttings in greenhouse conditions (T1G) recorded the lowest values: number of roots (4.74 ± 0.21), root length (3.08 ± 0.15 cm), plant height (11.58 ± 0.29 cm), fresh weight of shoots (2.03 ± 0.06 g), dry weight of shoots (0.50 ± 0.02 g), fresh weight of roots (0.20 ± 0.01 g), dry weight of roots (0.05 ± 0.01 g), survival rate (48.14 ± 1.35%), number of sprouts (0.33 ± 0.04), length of sprouts (9.58 ± 0.22 cm), girth of sprouts (10.87 ± 0.31 mm), root-to-shoot ratio (0.100 ± 0.004), chlorophyll content (1.12 ± 0.03 mg/g), and latest days to first sprout (28.67 ± 0.88 days).

Table 1: Effect of NAA and Growing Conditions on Dragon Fruit Cuttings (Mean ± SE)

Treatment	Roots/Plant	Root Length (cm)	Plant Height (cm)	Shoot Fresh Wt (g)	Shoot Dry Wt (g)	Root Fresh Wt (g)	Root Dry Wt (g)	Survival Rate (%)	Sprouts/ Plant	Sprout Length (cm)	Sprout Girth (mm)	Root-to-Shoot Ratio	Chlorophyll (mg/g)	Days to Sprout
T1G (0 ppm, Greenhouse)	4.74 ± 0.21	3.08 ± 0.15	11.58 ± 0.29	2.03 ± 0.06	0.50 ± 0.02	0.20 ± 0.01	0.05 ± 0.01	48.14 ± 1.35	0.33 ± 0.04	9.58 ± 0.22	10.87 ± 0.31	0.100 ± 0.004	1.12 ± 0.03	28.67 ± 0.88
T4P (200 ppm, Polyhouse)	20.18 ± 0.45	17.95 ± 0.32	23.51 ± 0.41	43.94 ± 0.78	11.29 ± 0.22	4.81 ± 0.09	1.09 ± 0.03	92.59 ± 1.12	2.66 ± 0.08	21.98 ± 0.39	26.66 ± 0.47	0.097 ± 0.003	2.45 ± 0.05	12.33 ± 0.58
LSD ($p < 0.05$)	0.62	0.44	0.59	1.08	0.31	0.13	0.04	2.11	0.12	0.55	0.68	0.006	0.07	1.24

***Note*:** T1G and T4P represent extreme treatments; LSD values indicate significant differences.

NAA Effects
The 200 ppm NAA treatment significantly enhanced rooting and growth parameters compared to lower concentrations and the control. NAA stimulates root primordia formation by promoting cell division in the cambial region (Hartmann *et al*., 2011) [1]. The optimal 200 ppm concentration likely maximized auxin activity without causing toxicity, as higher doses can inhibit growth (Le & Nguyen, 2020) [3]. Increased root number and length facilitated nutrient uptake, supporting higher shoot biomass, sprout development, and chlorophyll content, which indicates improved photosynthetic capacity. The earlier sprouting (12.33 days in T4P) suggests NAA accelerated meristematic activity.

Growing Conditions Effects
Polyhouse conditions outperformed others due to their stable microclimate (22-28°C, 65-75% humidity, diffused light), which minimized transpiration stress and optimized photosynthesis. The greenhouse's high humidity (70-80%) and potentially lower light intensity may have caused waterlogging or reduced photosynthesis, particularly in untreated cuttings, leading to low chlorophyll content (1.12 mg/g in T1G). Open conditions exposed cuttings to environmental fluctuations, reducing survival and growth. Kumar *et al*. (2018) [2] reported similar benefits of polyhouses for rooting in fruit crops.

Additional Parameters
- **Root-to-shoot ratio**: Despite T4P's high root and shoot biomass, its root-to-shoot ratio (0.097) was slightly lower than T1G (0.100), indicating balanced growth in polyhouse conditions, as high root biomass supported vigorous shoots.
- **Chlorophyll content**: Higher in T4P (2.45 mg/g) due to enhanced nutrient uptake and optimal light in polyhouses, supporting robust growth (Singh & Choudhary, 2021) [5].
- **Days to first sprout**: Earlier sprouting in T4P (12.33 days) reflects NAA's role in hastening cell division and polyhouse's favorable conditions.

Interaction Effects
The significant interaction ($p < 0.05$) between NAA and growing conditions underscores the synergy of 200 ppm NAA and polyhouse conditions. The polyhouse amplified NAA's effects by providing an ideal environment for root and shoot development, as seen in high survival (92.59%) and sprout vigor. Untreated greenhouse cuttings suffered from insufficient hormonal stimulation and suboptimal conditions, possibly due to fungal issues in high humidity.

Implications
The polyhouse-NAA combination offers a practical, scalable solution for dragon fruit propagation in Meghalaya. Polyhouses, being cost-effective, can empower smallholder farmers to produce high-quality planting material, enhancing commercial cultivation.

Conclusion
The study confirms that 200 ppm NAA under polyhouse conditions optimizes rooting, growth, and physiological parameters of dragon fruit cuttings, achieving superior results for number of roots, plant height, biomass, survival, sprout development, chlorophyll content, and sprouting time. These findings provide a robust protocol for dragon fruit propagation in subtropical regions, supporting sustainable horticulture. Future research could explore higher NAA doses, other auxins, or field performance post-propagation.

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