



Effects Auxins and Brassinosteroids on Adventitious Rooting and Vegetative Growth of *Cannabis sativa* L. Cuttings

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ABSTRACT :

Effects of auxins and 24-epibrassinolide (BS) on adventitious root development and vegetative growth of *Cannabis sativa* L. cv. FloSee stem cuttings were studied by treating stem cuttings with NAA 100 mg/L, IBA 3,000 mg/L, BS 1.0 mg/L, and combinations of IAA 100 mg/L, IBA 3,000 mg/L, and BS 1.0 mg/L. Root and shoot development were evaluated at 7, 14, 21, and 28 days after treatment. At 7 days, the combination of IAA 100 mg/L + IBA 3,000 mg/L + BS 1.0 mg/L produced the highest number of root primordia (1.75 ± 2.36), indicating an early rooting response. At 28 days, IBA 3,000 mg/L + BS 1.0 mg/L produced the highest root number (36.75 ± 3.94 roots/cutting), root weight (1.36 ± 0.01 g), and total plantlet fresh weight (4.14 ± 0.18 g), representing increases of 390.0%, 267.6%, and 61.1%, respectively, compared with the control (distilled water). IAA 100 mg/L + IBA 3,000 mg/L produced the highest number of newly emerged leaves (8.00 ± 2.94 leaves/cutting). In this study, it could be concluded that IBA 3,000 mg/L + BS 1.0 mg/L was the suitable formula for cannabis cutting.

Keywords: *Cannabis sativa* L.; adventitious rooting; stem cuttings; indole-3-butyric acid; indole-3-acetic acid; 24-epibrassinolide; plant growth regulators; vegetative propagation

Introduction

Cannabis sativa L. is an economically significant medicinal and industrial crop, and commercial propagation relies predominantly on vegetative stem cuttings to maintain clonal uniformity and stable production traits (Caplan et al., 2018; Hwang et al., 2026; Ioannidis et al., 2022). Successful propagation depends on adventitious root (AR) formation, which is a common bottleneck in clonal propagation because rooting speed and rooting success can vary substantially among species, genotypes, and propagation conditions (Druege et al., 2019; Huang et al., 2025; Liu et al., 2025). In cannabis specifically, rooting performance is strongly influenced not only by hormone treatment but also by substrate, vessel system, leaf retention, and cutting type, indicating that inconsistent establishment is a multifactorial problem rather than a hormone-only issue (Caplan et al., 2018; Hwang et al., 2026; Ioannidis et al., 2022).

Adventitious rhizogenesis is a multi-stage developmental process that is commonly divided into induction, initiation or primordium formation, and elongation or emergence phases (Druege et al., 2019; Huang et al., 2025; Zhang et al., 2026). Anatomical studies further show that AR primordia can arise from cambium, phloem, cortex, callus, or other responsive basal tissues depending on the species and rooting route (Huang et al., 2025). The developmental stages and tissue origin of AR formation are illustrated clearly in anatomical series that track callus formation, primordium initiation, and root emergence (Zhang et al., 2026).

Auxins are the core phytohormones governing AR induction, and the principal rooting auxins used in cuttings are indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and 1-naphthaleneacetic acid (NAA) (Druege et al., 2019; Liu et al., 2025; Roussos, 2023). After excision, endogenous IAA accumulates near the wound at the stem base, where it helps trigger cellular reprogramming and subsequent root initiation (Druege et al., 2019; Roussos, 2023). More specifically, studies of cuttings show that vascular disruption after excision leads to auxin accumulation in the basal region within hours, linking wounding to early AR signaling (Druege et al., 2019; Roussos, 2023).

Exogenous application of synthetic auxins such as IBA and NAA is widely used to accelerate rhizogenesis, but their effects depend strongly on concentration, exposure duration, and tissue context (Liu et al., 2025). IBA is generally preferred over IAA because it is more effective in many AR systems and functions as a precursor that is converted into active IAA within plant tissues (Justamante et al., 2022; Song et al., 2024). However, prolonged or excessive auxin exposure can inhibit later stages of root development, including AR elongation (Justamante et al., 2022; Marquez et al., 2016). NAA can promote lateral root formation or AR-related responses at low doses, but higher concentrations suppress cell elongation and can reduce root development (Alarcón et al., 2019; Marquez et al., 2016) Alarcón2019Marquez2016. Single-auxin treatments also do not fully determine final root system architecture, which additionally depends on lateral root patterning, carbon metabolism, redox status, and environmental or propagation conditions (Alarcón et al., 2019; Liu et al., 2025; Zhang et al., 2026).

To overcome these physiological limitations, recent plant biotechnology research emphasizes hormonal crosstalk between auxins and secondary growth regulators, particularly brassinosteroids (BRs), in the control of root development (Altamura et al., 2023; Devi et al., 2022). Brassinosteroids are steroidal phytohormones that regulate cell division, cell elongation, and vascular tissue differentiation (Furuya et al., 2024; Planas-Riverola et al., 2019). At the cellular level, BR signaling intersects with the auxin transcriptional cascade through shared transcriptional targets and regulation of auxin transport

and signaling components (Nolan et al., 2023; Planas-Riverola et al., 2019). Whereas auxins initiate root primordium development and lateral root founder-cell specification, BRs modulate auxin transport, auxin homeostasis, and cell elongation programs that support downstream root development (Jia et al., 2021; Zhang et al., 2026). Co-application of exogenous auxins and brassinosteroids can produce synergistic effects on rooting and lateral root proliferation, although these responses are strongly dependent on dose, tissue context, and developmental stage (Betti et al., 2021; Pacholczak et al., 2021).

In pre-experiments many concentrations of IAA, IBA, NAA and BS were individually treated to cannabis cutting after that the concentration of each substances which gave the best results were selected to further studied in this experiment (data did not show). Therefore, this study evaluated the single and combined effects of exogenous plant growth regulators—including IAA, IBA, NAA, and BS—on the temporal rooting dynamics and vegetative growth of *Cannabis sativa* L. cv. FloSee stem cuttings. Morphophysiological parameters, including root primordia initiation, root length, root number, root biomass, and shoot development, were systematically quantified at 7, 14, 21, and 28 days post-cutting. The primary objective was to elucidate the physiological synergy between auxins and brassinosteroids, establishing an optimized, science-based propagation protocol to improve micropropagation efficiency, reduce crop cycle duration, and enhance transplant quality for commercial cannabis production.

Materials and Methods

1.1. Plant material and experimental design

Healthy, disease-free mother plants of *Cannabis sativa* L. cv. FloSee were maintained under uniform vegetative growth conditions (18-h photoperiod, 25±2°C). Uniform apical stem cuttings (15 cm in length) possessing 1–2 nodes and fully expanded leaves were harvested using sterile scalpel blades. The lower leaves were removed to reduce transpiration, leaving 2–3 upper leaves trimmed by 50% to minimize water loss. The experiment was arranged in a completely randomized design (CRD) with eight plant growth regulator (PGR) treatments and four biological replications per treatment evaluation interval.

1.2. Plant growth regulator (PGR) treatments and gel formulation preparation

To evaluate the effects of exogenous PGRs and a gel-based delivery matrix on *Cannabis sativa* L. cv. FloSee stem cuttings, eight experimental treatments were designed. Treatment 1 served as the absolute control using distilled water (1,050 mL) whereas Treatments 2 through 8 incorporated targeted PGR combinations into a standardized carrier gel matrix. The eight experimental treatments were formulated as follows:

- Treatment 1 (Control - Water): Distilled water (0 mg/L)
- Treatment 2 (NAA 100): 100 mg/L NAA incorporated in carrier gel base
- Treatment 3 (IBA 3000): 3,000 mg/L IBA incorporated in carrier gel base
- Treatment 4 (BS 1.0): 1.0 mg/L BS incorporated in carrier gel base
- Treatment 5 (IAA 100 + IBA 3000): 100 mg/L IAA + 3,000 mg/L IBA incorporated in carrier gel base
- Treatment 6 (IAA 100 + BS 1.0): 100 mg/L IAA + 1.0 mg/L BS incorporated in carrier gel base
- Treatment 7 (IBA 3000 + BS 1.0): 3,000 mg/L IBA + 1.0 mg/L BS incorporated in carrier gel base
- Treatment 8 (IAA 100 + IBA 3000 + BS 1.0): 100 mg/L IAA + 3,000 mg/L IBA + 1.0 mg/L BS incorporated in carrier gel base

The standardized carrier gel matrix utilized for Treatments 2 through 8 was prepared by homogenizing specified auxiliary components to achieve a uniform working volume of 1,050 mL per formulation. Each gel batch contained anhydrous lanolin (350 g in 800 mL warm water) as a lipophilic paste, mineral oil (14 mL in 1,400 mL water) as a protective moisture barrier, a non-ionic sticker/wetting agent (7 mL in 700 mL water) to enhance cuticular adhesion, a broad-spectrum fungicide (7 g in 700 mL water) to prevent basal rot, and synthetic dyes (12.81 mL purple and 3.5 mL red dye in 815.5 mL water) for visual tracking of application coverage. Active PGR compounds were completely dissolved in minimal solvent volumes and thoroughly blended into the gel base. The basal cut ends (1.5 cm) of uniform stem cuttings were dipped into their respective treatment formulations for a standardized quick-dip duration prior to planting into pre-moistened rooting media.

1.3. Environmental control and data collection

Cuttings were kept in a micropropagation chamber 25±2°C, 85±5% relative humidity, 16-h light / 8-h dark cycle). Morphophysiological parameters were destructively harvested and recorded at 7, 14, 21, and 28 days post-cutting:

- Root Parameters: Number of root primordia, root length (cm), total root number (roots/cutting), and root fresh biomass (g).
- Shoot Parameters: Length of new leaves (cm), number of new leaves (leaves/cutting), and total plantlet fresh weight (g).

1.4. Statistical analysis

All data were expressed as Mean ± Standard Deviation. One-way Analysis of Variance (ANOVA) was conducted, and treatment means were compared using Duncan's multiple range test (DMRT) at $p < 0.05$. Percentage changes relating to the control were determined on Day 28.

Results and Dis

1.5. Effects of plant growth regulators on root development

The effects of plant growth regulators on root development of *Cannabis sativa* L. cv. FloSee stem cuttings were evaluated at 7, 14, 21, and 28 days after treatment (Table 1). Root initiation was generally limited during the first 7 days, as no root development was observed in the control, NAA 100 mg/L, IBA 3,000 mg/L, or BS 1.0 mg/L treatments. In contrast, the combined treatments induced root primordia formation at this early stage. The combination of IAA 100 mg/L + IBA 3,000 mg/L + BS 1.0 mg/L produced the highest number of root primordia (1.75 ± 2.36), which was significantly greater than the other treatments ($p < 0.05$). The combinations of IAA + IBA, IAA + BS, and IBA + BS also initiated root primordia, although at lower levels.

Table 1 - Effects of plant growth regulators on root development (root primordia initiation, root length, root number, and root weight) of *Cannabis sativa* L. cv. FloSee stem cuttings at 7, 14, 21, and 28 days post-cutting.

Treatments	Primordia	Root length (cm)	Root number (roots/cutting)	Root weight (g)
7 Days				
Control (Water)	(-)	(-)	(-)	(-)
NAA 100 mg/L	(-)	(-)	(-)	(-)
IBA 3,000 mg/L	(-)	(-)	(-)	(-)
BS 1.0 mg/L	(-)	(-)	(-)	(-)
IAA 100 + IBA 3000 mg/L	0.25 ± 0.50^b	(-)	(-)	(-)
IAA 100 + BS 1.0 mg/L	0.25 ± 0.50^b	(-)	(-)	(-)
IBA 3000 + BS 1.0 mg/L	0.25 ± 0.50^b	(-)	(-)	(-)
IAA 100 + IBA 3000 + BS 1.0 mg/L	1.75 ± 2.36^a	0.75 ± 0.15^a	0.25 ± 0.50^a	(-)
14 Days				
Control (Water)	1.75 ± 3.50^b	(-)	0.50 ± 0.57^d	0.03 ± 0.05^c
NAA 100 mg/L	3.00 ± 3.16^{ab}	1.35 ± 1.57^{cd}	3.75 ± 3.77^{cd}	0.32 ± 0.33^{abc}
IBA 3,000 mg/L	2.25 ± 1.25^{ab}	12.47 ± 3.37^{ab}	11.50 ± 4.65^{abcd}	0.51 ± 0.13^{abc}
BS 1.0 mg/L	3.25 ± 1.50^{ab}	16.37 ± 3.36^a	5.25 ± 3.09^{cd}	0.18 ± 0.11^{ab}
IAA 100 + IBA 3000 mg/L	1.75 ± 0.50^b	6.40 ± 3.20^{bc}	23.25 ± 17.34^a	0.80 ± 0.48^a
IAA 100 + BS 1.0 mg/L	1.75 ± 0.50^b	7.07 ± 3.28^{bc}	10.25 ± 8.80^{bcd}	0.59 ± 0.52^{ab}
IBA 3000 + BS 1.0 mg/L	4.25 ± 0.50^{ab}	5.05 ± 1.00^c	22.25 ± 8.95^{ab}	0.65 ± 0.42^{ab}
IAA 100 + IBA 3000 + BS 1.0 mg/L	5.75 ± 3.86^a	10.82 ± 1.12^{abc}	14.75 ± 2.50^{abc}	0.39 ± 0.15^{abc}
21 Days				
Control (Water)	1.75 ± 2.06^b	5.50 ± 8.27^{bc}	(-)	(-)
NAA 100 mg/L	(-)	1.75 ± 1.57^{abc}	1.50 ± 1.91^d	0.01 ± 0.20^c
IBA 3,000 mg/L	5.00 ± 2.44^b	14.02 ± 6.39^a	7.50 ± 1.91^{bcd}	0.07 ± 0.04^c
BS 1.0 mg/L	2.67 ± 1.25^a	14.40 ± 6.39^a	12.50 ± 8.10^{bc}	0.31 ± 0.25^{ab}
IAA 100 + IBA 3000 mg/L	2.25 ± 1.25^b	7.47 ± 3.52^{bc}	12.50 ± 6.45^{bc}	0.14 ± 0.06^{bc}
IAA 100 + BS 1.0 mg/L	9.25 ± 9.77^b	14.90 ± 6.60^a	4.50 ± 4.04^{cd}	0.09 ± 0.01^c
IBA 3000 + BS 1.0 mg/L	1.75 ± 1.50^b	5.25 ± 1.38^{cd}	28.50 ± 8.58^a	0.48 ± 0.08^a
IAA 100 + IBA 3000 + BS 1.0 mg/L	9.00 ± 6.00^b	12.62 ± 3.37^{ab}	17.75 ± 12.78^b	0.36 ± 0.17^a
28 Days				
Control (Water)	2.00 ± 2.00^a	15.82 ± 3.30^a	7.50 ± 3.00^{cd}	0.37 ± 0.20^b
NAA 100 mg/L	1.00 ± 0.81^b	3.50 ± 2.48^c	2.50 ± 1.91^d	0.09 ± 0.10^c
IBA 3,000 mg/L	0.25 ± 0.50^b	5.85 ± 1.21^{bc}	19.25 ± 4.99^b	0.23 ± 0.07^{bc}
BS 1.0 mg/L	(-)	11.62 ± 3.90^{ab}	10.00 ± 5.03^c	0.12 ± 0.06^c
IAA 100 + IBA 3000 mg/L	(-)	7.37 ± 1.24^{bc}	12.75 ± 2.98^{bc}	0.24 ± 0.06^{bc}
IAA 100 + BS 1.0 mg/L	0.75 ± 0.95^b	9.80 ± 7.68^{abc}	2.37 ± 2.86^d	0.09 ± 0.07^c
IBA 3000 + BS 1.0 mg/L	2.25 ± 1.25^b	9.95 ± 3.98^{abc}	36.75 ± 3.94^a	1.36 ± 0.01^a
IAA 100 + IBA 3000 + BS 1.0 mg/L	1.00 ± 0.81^b	9.27 ± 3.83^{bc}	19.25 ± 7.50^b	0.24 ± 0.12^{bc}

Remarks: abcd Means in the same column followed by the same letter in the same column were not significant differences at $p < 0.05$ by Duncan's Multiple Range Test (DMRT).

(-) Indicated that no observed change or development.

At 14 days, root development increased considerably in most treatments. The three-component treatment of IAA 100 mg/L + IBA 3,000 mg/L + BS 1.0 mg/L produced the highest number of root primordia (5.75 ± 3.86), whereas BS 1.0 mg/L alone resulted in the greatest root length (16.37 ± 3.36 cm). The highest root number was observed with IAA 100 mg/L + IBA 3,000 mg/L (23.25 ± 17.34 roots/cutting), followed closely by IBA 3,000 mg/L + BS 1.0 mg/L (22.25 ± 8.95 roots/cutting). The former treatment also produced the highest root weight (0.80 ± 0.48 g). At 21 days, IBA 3,000 mg/L + BS 1.0 mg/L produced the greatest number of roots (28.50 ± 8.58 roots/cutting) and the highest root weight (0.48 ± 0.08 g). At 28 days, this treatment maintained the strongest root development, producing 36.75 ± 3.94 roots/cutting and 1.36 ± 0.01 g root weight, both significantly higher than several other treatments ($p < 0.05$). In contrast, NAA 100 mg/L resulted in relatively poor root development, with a root length of only 3.50 ± 2.48 cm and 2.50 ± 1.91 roots/cutting at day 28.

1.6. Effects of plant growth regulators on shoot growth and plantlet biomass

Shoot growth also differed among treatments and observation periods (Table 2). At 7 days, the control treatment produced the longest newly emerged leaves (3.65 ± 0.38 cm), whereas BS 1.0 mg/L resulted in the highest total plantlet fresh weight (1.96 ± 0.24 g). The number of newly emerged leaves ranged from 1.50 to 2.25 leaves/cutting, with NAA 100 mg/L and BS 1.0 mg/L producing 2.25 ± 0.50 and 2.25 ± 0.57 leaves/cutting, respectively.

At 14 days, leaf length increased substantially in all treatments compared with day 7. IBA 3,000 mg/L produced a leaf length of 6.05 ± 0.51 cm, while IAA 100 mg/L + IBA 3,000 mg/L and the three-component treatment produced 5.07 ± 1.34 and 5.27 ± 1.17 cm, respectively. The highest number of newly emerged leaves was observed in IBA 3,000 mg/L + BS 1.0 mg/L and IAA 100 mg/L + IBA 3,000 mg/L + BS 1.0 mg/L, both with 3.00 ± 0.00 leaves/cutting. However, no significant differences were detected among treatments in total plantlet fresh weight at this stage.

Table 2 - Effects of plant growth regulators on shoot growth (new leaf length, number of newly emerged leaves, and total plantlet weight) of *Cannabis sativa* L. cv. FloSee stem cuttings at 7, 14, 21, and 28 days post-cutting.

Treatments	Length of new leaves (cm)	Number of new leaves (leaves/cutting)	Total plantlet fresh weight (g)
7 Days			
Control (Water)	3.65±0.38^a	2.00±0.00 ^{ab}	1.56±0.47 ^{ab}
NAA 100 mg/L	3.30±0.24 ^{ab}	2.25±0.50^a	1.38±0.38 ^b
IBA 3,000 mg/L	2.80±0.86 ^{bc}	2.00±0.00 ^{ab}	1.66±0.41 ^{ab}
BS 1.0 mg/L	2.42±0.09 ^c	2.25±0.57^a	1.96±0.24^a
IAA 100 + IBA 3000 mg/L	2.42±0.05 ^c	2.25±0.57^a	1.41±0.34 ^b
IAA 100 + BS 1.0 mg/L	2.50±0.08 ^c	2.00±0.00 ^{ab}	1.68±0.19 ^{ab}
IBA 3000 + BS 1.0 mg/L	3.00±0.55 ^{abc}	2.00±0.00 ^{ab}	1.54±0.27 ^{ab}
IAA 100 + IBA 3000 + BS 1.0 mg/L	2.47±0.51 ^c	1.50±0.57 ^b	1.44±0.30 ^b
14 Days			
Control (Water)	3.00±0.57 ^b	1.50±0.57 ^d	1.96±0.55 ^a
NAA 100 mg/L	5.27±0.94 ^a	1.75±0.50 ^{cd}	2.34±0.80 ^a
IBA 3,000 mg/L	6.05±0.51^a	2.50±0.57 ^{ab}	2.77±0.39 ^a
BS 1.0 mg/L	5.02±0.82 ^a	2.75±0.50 ^{ab}	2.03±0.23 ^a
IAA 100 + IBA 3000 mg/L	5.07±1.34 ^a	1.75±0.50 ^{cd}	2.79±0.85 ^a
IAA 100 + BS 1.0 mg/L	4.72±1.76 ^a	2.25±0.50 ^{bc}	2.82±1.00^a
IBA 3000 + BS 1.0 mg/L	4.65±0.23 ^a	3.00±0.00^a	2.71±0.93 ^a
IAA 100 + IBA 3000 + BS 1.0 mg/L	5.27±1.17 ^a	3.00±0.00^a	2.41±0.54 ^a
21 Days			
Control (Water)	3.90±1.47 ^{cd}	3.00±0.00 ^{bc}	1.34±0.14 ^c
NAA 100 mg/L	4.07±0.69 ^{bcd}	2.75±0.50 ^c	1.42±0.20 ^c
IBA 3,000 mg/L	6.77±1.63^a	4.25±0.95 ^{ab}	1.98±0.79 ^{bc}
BS 1.0 mg/L	2.45±0.58 ^d	4.00±0.00 ^{abc}	3.08±0.87 ^a
IAA 100 + IBA 3000 mg/L	5.65±0.75 ^{ab}	3.25±1.50 ^{bc}	1.98±0.51 ^{bc}
IAA 100 + BS 1.0 mg/L	6.27±1.07 ^a	3.75±0.95 ^{abc}	2.26±0.81 ^{abc}
IBA 3000 + BS 1.0 mg/L	5.17±0.09 ^{abc}	4.75±0.50^a	3.19±0.88^a
IAA 100 + IBA 3000 + BS 1.0 mg/L	6.30±1.31 ^a	2.75±0.50 ^c	2.82±0.57 ^{ab}
28 Days			
Control (Water)	5.42±1.70 ^a	5.25±0.95 ^{bc}	2.57±0.48 ^{bc}
NAA 100 mg/L	5.30±1.67 ^a	5.25±0.95 ^{bc}	2.41±0.53 ^c
IBA 3,000 mg/L	5.22±0.26 ^a	4.75±0.50 ^c	2.53±0.32 ^{bc}
BS 1.0 mg/L	5.90±0.94 ^a	7.00±0.81 ^{ab}	2.47±0.52 ^{bc}
IAA 100 + IBA 3000 mg/L	6.10±0.21 ^a	8.00±2.94^a	3.20±0.51 ^b
IAA 100 + BS 1.0 mg/L	5.45±0.19 ^a	5.50±0.57 ^{bc}	2.56±0.34 ^{bc}
IBA 3000 + BS 1.0 mg/L	6.00±0.43 ^a	5.75±0.50 ^{bc}	4.14±0.18^a
IAA 100 + IBA 3000 + BS 1.0 mg/L	6.22±0.26^a	6.00±0.81 ^{bc}	2.72±0.63 ^{bc}

Remarks: abcd Means in the same column followed by the same letter in the same column were not significant differences at $p < 0.05$ by Duncan's Multiple Range Test (DMRT).

(-) Indicated that no observed change or development.

By 21 days, IBA 3,000 mg/L produced a leaf length of 6.77 ± 1.63 cm, while IAA 100 mg/L + BS 1.0 mg/L and the three-component treatment produced 6.27 ± 1.07 and 6.30 ± 1.31 cm, respectively. The highest number of newly emerged leaves was recorded for IBA 3,000 mg/L + BS 1.0 mg/L (4.75 ± 0.50 leaves/cutting), which was significantly higher than several treatments. This treatment also produced the highest total plantlet fresh weight (3.19 ± 0.88 g), although it was statistically comparable with BS 1.0 mg/L (3.08 ± 0.87 g).

At 28 days, leaf length did not differ significantly among treatments, ranging from 5.22 ± 0.26 to 6.22 ± 0.26 cm. However, significant differences were observed in the number of newly emerged leaves and total plantlet fresh weight. IAA 100 mg/L + IBA 3,000 mg/L produced the highest number of newly emerged leaves (8.00 ± 2.94 leaves/cutting), whereas IBA 3,000 mg/L + BS 1.0 mg/L produced the highest total plantlet fresh weight (4.14 ± 0.18 g), significantly exceeding the control (2.57 ± 0.48 g) and the other treatments ($p < 0.05$).

1.7. Key performance indicators on day 28

To facilitate comparison among treatments, root and shoot performance at day 28 was summarized using key developmental indicators (Table 3). Relative to the control, IBA 3,000 mg/L + BS 1.0 mg/L produced the greatest increase in root number, from 7.50 ± 3.00 to 36.75 ± 3.94 roots/cutting, corresponding to a 390.0% increase. Root biomass also increased from 0.37 ± 0.20 g in control to 1.36 ± 0.01 g, representing a 267.6% increase. In addition, total plantlet fresh weight increased from 2.57 ± 0.48 g to 4.14 ± 0.18 g, corresponding to a 61.1% increase. These values represented the highest root number, root biomass, and total plantlet fresh weight among the treatments evaluated at day 28.

Table 3 - Key performance indicators (KPIs) and relative enhancement over control at Day 28 post-cutting.

Treatment	Root length (cm)	Root number (roots/cutting)	Root biomass / Fresh weight (g)	Total plantlet fresh weight (g)	Key developmental advantage
Control (Water)	15.82 ± 3.30^a	7.50 ± 3.00^{cd}	0.37 ± 0.20^b	2.57 ± 0.48^{bc}	Baseline elongation
NAA 100 mg/L	3.50 ± 2.48^c	2.50 ± 1.91^d (-66.7%)	0.09 ± 0.10^c (-75.7%)	2.41 ± 0.53^c (-6.2%)	Inhibitory to root growth
IBA 3,000 mg/L	5.85 ± 1.21^{bc}	19.25 ± 4.99^b (+156.7%)	0.23 ± 0.07^{bc} (-37.8%)	2.53 ± 0.32^{bc} (-1.6%)	Moderate root initiation
BS 1.0 mg/L	11.62 ± 3.90^{ab}	10.00 ± 5.03^c (+33.3%)	0.12 ± 0.06^c (-67.6%)	2.47 ± 0.52^{bc} (-3.9%)	Moderate length expansion
IAA 100 + IBA 3,000 mg/L	7.37 ± 1.24^{bc}	12.75 ± 2.98^{bc} (+70.0%)	0.24 ± 0.06^{bc} (-35.1%)	3.20 ± 0.51^b (+24.5%)	Promotes new leaf count
IAA 100 + BS 1.0 mg/L	9.80 ± 7.68^{abc}	2.37 ± 2.86^d (-68.4%)	0.09 ± 0.07^c (-75.7%)	2.56 ± 0.34^{bc} (-0.4%)	Weak combination effect
IBA 3,000 + BS 1.0 mg/L	9.95 ± 3.98^{abc}	36.75 ± 3.94^a (+390.0%)	1.36 ± 0.01^a (+267.6%)	4.14 ± 0.18^a (+61.1%)	The most effective treatment combination for root proliferation
IAA 100 + IBA 3,000 + BS 1.0 mg/L	9.27 ± 3.83^{bc}	19.25 ± 7.50^b (+156.7%)	0.24 ± 0.12^{bc} (-35.1%)	2.72 ± 0.63^{bc} (+5.8%)	Early primordia trigger (Day 7)

Remarks: abcd Means in the same column followed by the same letter in the same column were not significant differences at $p < 0.05$ by Duncan's Multiple Range Test (DMRT).

IBA 3,000 mg/L alone increased root number by 156.7% relative to the control, although root biomass remained lower than that of the control. BS 1.0 mg/L increased root length by approximately 33.3% but reduced root biomass relative to the control. The combination of IAA 100 mg/L + IBA 3,000 mg/L increased total plantlet fresh weight by 24.5% and was associated with the highest number of newly emerged leaves on day 28. Conversely, NAA 100 mg/L reduced root length, root number, and root biomass relative to the control. The three-component treatment of IAA 100 mg/L + IBA 3,000 mg/L + BS 1.0 mg/L was notable for its early effect on root primordia formation at day 7, but its day-28 root and biomass values were lower than those obtained with IBA 3,000 mg/L + BS 1.0 mg/L.

1.8. Practical comparison of treatments

Based on the developmental responses observed throughout the 28-day period, the treatments differed according to the specific developmental parameter considered (Table 4). The combination of IAA 100 mg/L + IBA 3,000 mg/L + BS 1.0 mg/L was associated with the earliest detectable root primordia formation, with 1.75 ± 2.36 primordia at day 7 and a statistically significant difference from the other treatments ($p \leq 0.05$). For subsequent root system development, IBA 3,000 mg/L + BS 1.0 mg/L produced the highest root number and root biomass at day 28, with 36.75 roots/cutting and 1.36 g, respectively. The same treatment also produced the highest total plantlet fresh weight (4.14 g). In terms of shoot development, IAA 100 mg/L + IBA 3,000 mg/L produced the highest number of newly emerged leaves on day 28 (8.00 leaves/cutting), significantly exceeding the control and single-regulator treatments. Thus, the observed responses indicate that different regulator combinations were associated with different developmental outcomes, with early root initiation, root system proliferation, biomass accumulation, and leaf production showing distinct treatment responses.

Table 4 - Practical recommendation matrix for cannabis (*Cannabis sativa* L. cv. FloSee) micropropagation.

Objective / Target outcome	Recommended treatment	Primary benefit observed	Statistical significance
1. Earliest root initiation (Rapid primordia setup)	IAA 100 mg/L + IBA 3,000 mg/L + BS 1.0 mg/L	Earliest primordia emergence at Day 7 (1.75 ± 2.36)	Significantly higher than all other treatments at Day 7 ($p \leq 0.05$)
2. Maximum root system development (Profuse rooting)	IBA 3,000 mg/L + BS 1.0 mg/L	Highest root number (36.75 roots/cutting) and root biomass (1.36 g) at Day 28	~4.9-fold increase in root number vs. Control ($p \leq 0.05$)
3. Highest overall plantlet biomass and vigor	IBA 3,000 mg/L + BS 1.0 mg/L	Highest total plantlet fresh weight (4.14 g) at Day 28	~1.6-fold increase over Control ($p \leq 0.05$)
4. Maximum shoot / Foliar proliferation	IAA 100 mg/L + IBA 3,000 mg/L	Highest new leaf production (8.00 leaves/cutting) at Day 28	Significantly superior to Control and single-auxin treatments

4. Discussions

The present study demonstrated that exogenous application of auxins and BS substantially influenced adventitious root development and shoot growth of *Cannabis sativa* L. cv. FloSee stem cuttings, with the magnitude and timing of responses varying according to the type and combination of plant growth regulators. A major finding was that the effects of the treatments were stage dependent. The three-component treatment containing IAA, IBA, and BS was associated with the earliest detectable root primordia formation, whereas the combination of IBA and BS produced the most extensive root system and the greatest plantlet fresh weight by day 28. These findings indicate that early root initiation and subsequent root system development were not

necessarily maximized by the same treatment, suggesting that different phases of adventitious rooting may respond differently to exogenous growth regulators.

During the early rooting phase, no visible root development was observed in the control, NAA, IBA, or BS treatments at 7 days after cutting, whereas root primordia were detected in treatments containing combinations of growth regulators. The IAA + IBA + BS treatment produced the highest number of root primordia at this stage. This response may indicate that the combined application of auxins with BS promoted the early developmental processes associated with adventitious root initiation, because adventitious rooting is a multiphase process and exogenous growth regulators can affect the induction and initiation phases before visible root emergence (Guan et al., 2019; Huang et al., 2025; Wang et al., 2022). Auxins are central regulators of adventitious root formation, particularly during the initiation and differentiation of root founder cells and root primordia (Guan et al., 2019; Liu et al., 2025). IAA and IBA can contribute to the establishment of local auxin responses required for the transition of competent cells toward a root-forming pathway, because early IAA accumulation in the cutting base, auxin transport, and auxin-responsive transcription are linked to founder-cell activation and primordium initiation (Druege et al., 2019; Guan et al., 2019). IBA specifically has been shown to promote cell division in vascular tissues and root primordium formation, while IAA is closely associated with stage-specific hormonal balance during root induction (Uddin et al., 2024; Wang et al., 2022; Zhang et al., 2026). The presence of BS in the combined treatment may have further supported these developmental processes, because brassinosteroids can positively affect rooting and are often more effective when combined with auxin than when applied alone (Pacholczak et al., 2021; Uddin et al., 2024). Rooting improvement under BR-related treatments and auxin combinations is shown clearly in comparative rooting tables and stage-dependent histology (Pacholczak et al., 2021).

However, because the present experiment did not directly measure endogenous hormone concentrations, gene expression, or cellular events, the observed response should be interpreted as an association between the treatment and early root initiation rather than direct evidence of a specific hormonal mechanism; this caution is consistent with reviews emphasizing that hormone homeostasis, signaling, and phase-specific interactions during adventitious rooting remain incompletely resolved (Druege et al., 2019).

The rooting response changed considerably as the cuttings developed. At 14 days, BS alone produced the longest roots, whereas IAA + IBA produced the greatest number of roots and root biomass. By 21 days, IBA + BS produced the highest root number and root weight, and this treatment became clearly superior for root system development by day 28. This shift is biologically plausible because root initiation and later proliferation are distinct developmental phases, and different treatments can differentially affect primordium initiation, elongation, and later root-system expansion (Guan et al., 2019; Zhang et al., 2026). In several systems, exogenous auxin increases primordium number more strongly than final root length, whereas later root-system traits depend on continued development through extension and elongation phases (Guan et al., 2019). The anatomical progression from callus or cambial activation to root primordium formation and then root emergence is illustrated clearly in developmental series of rooted cuttings (Zhang et al., 2026). The progressive increase in root number under IBA + BS suggests that this treatment was particularly effective during the root proliferation and biomass accumulation phases rather than solely during the initial establishment of root primordia. This interpretation aligns with studies showing that BR-related treatments can increase fine-root formation and that BR–auxin combinations can improve rooting degree, root length, and overall rooting parameters relative to single treatments (Pacholczak et al., 2021). The distinction between root initiation and subsequent root proliferation is important because successful vegetative propagation depends not only on root emergence but also on the development of a sufficiently large and functional root system capable of improving water and nutrient uptake after transfer (Liu et al., 2025).

The response to IBA + BS was particularly notable at day 28. Root number increased from 7.50 ± 3.00 roots/cutting in the control to 36.75 ± 3.94 roots/cutting, while root weight increased from 0.37 ± 0.20 to 1.36 ± 0.01 g. Total plantlet fresh weight also increased from 2.57 ± 0.48 to 4.14 ± 0.18 g. These results indicate that the treatment was associated with both enhanced root proliferation and greater overall plantlet biomass. Similar co-improvement of root number, root mass, and whole-plant biomass has been reported under effective auxin-based rooting treatments in several species (Ghimire et al., 2022; Nale et al., 2024). Nevertheless, the present study evaluated plantlets only during the 28-day period; therefore, whether these differences translate into improved acclimatization, survival, or subsequent growth should be tested in follow-up experiments, ideally by extending observations beyond rooting to transplant performance and survival (Druege et al., 2019; Wang et al., 2022).

The effects of the auxin treatments were not uniform. IBA alone increased root number relative to the control on day 28, but root length and root biomass remained lower than those of the control. In contrast, NAA 100 mg/L was associated with markedly reduced root length, root number, and root biomass at the final observation. This pattern is consistent with the broader evidence that the rooting response of stem cuttings depends not only on the presence of an auxin, but also on auxin type and concentration (Ghimire et al., 2022; Liu et al., 2025). Across species, IBA often produces stronger overall rooting responses than other tested auxins, although the optimal response remains concentration- and genotype-dependent (Delgado-Rafael et al., 2026; Ghimire et al., 2022). By contrast, NAA can promote adventitious rooting at suitable doses but can become less effective or inhibitory outside its optimal range, including reductions in root formation and biomass at higher or non-optimal concentrations (Ghimire et al., 2022; Wang et al., 2022). The relatively weak response to NAA compared with IBA may reflect differences in physiological activity and metabolism in cutting tissues, because exogenous auxins differ in their effects on endogenous IAA balance, IAA-oxidase activity, and related biochemical processes during root induction (Wang et al., 2022). However, because only one concentration of NAA was evaluated, the present results cannot establish that NAA is generally unsuitable for rooting in this cultivar; other species show that NAA can be effective at different concentrations or in different auxin combinations (Liu et al., 2025; Wang et al., 2022).

The results also revealed a distinct effect on shoot development. At day 28, the IAA + IBA treatment produced the highest number of newly emerged leaves, whereas IBA + BS produced the highest total plantlet fresh weight. This kind of divergence among shoot traits is plausible because morphological development and rooting do not always respond in parallel, and morphophysiological traits often provide complementary rather than redundant information about cutting performance (Delgado-Rafael et al., 2026; Ghimire et al., 2022; Nale et al., 2024). Evidence from propagation studies also shows that auxin treatments can differentially affect leaf number, shoot growth, root biomass, and total plant biomass, rather than shifting all growth variables in the same direction or magnitude (Ghimire et al., 2022; Nale et al., 2024). The absence of a corresponding treatment effect on leaf length therefore supports evaluating plant growth with multiple shoot and root parameters, not a single shoot trait (Delgado-Rafael et al., 2026).

The observation that the IAA + IBA + BS treatment induced earlier root primordia formation but did not produce the largest final root system is also biologically credible. Adventitious rooting is a multistage developmental process with distinct induction, initiation, primordium formation, and

extension phases, and treatments can influence these phases differently over time (Druege et al., 2019; Wang et al., 2022). Auxin often has its clearest effect on founder-cell activation and primordium initiation, whereas later root elongation and system expansion can follow a partially different trajectory (Guan et al., 2019). The anatomical progression from early primordium formation to later root emergence and extension is shown clearly in developmental series of auxin-treated cuttings.

The observed responses may also reflect interactions between auxin-mediated root induction and brassinosteroid-regulated growth processes. Brassinosteroids regulate plant development, including cell expansion, cell division, and root-system formation, but their effects depend strongly on developmental context and hormone balance (Altamura et al., 2023; Planas-Riverola et al., 2019). In stem cuttings, BR-containing or BR-related treatments can improve rooting traits, and BRs are often more effective when combined with auxin than when applied alone (Pacholczak et al., 2021). In barberry, for example, BL + IBA increased rooting degree and root length beyond IBA alone, illustrating that a combination treatment can favor later root-system development rather than only early initiation. The comparative rooting outcomes under BR, IBA, and BR + IBA are shown directly in a treatment table (Pacholczak et al., 2021).

The present findings are better described as a strong response to the IBA + BS combination than as definitive hormonal synergism. Reviews emphasize that BR–auxin crosstalk in adventitious rooting is real but still incompletely resolved mechanistically (Altamura et al., 2023; Druege et al., 2019). A formal claim of synergism would require a factorial design and explicit testing of interaction effects, as used in studies that separate genotype, treatment, and genotype × treatment components (Delgado-Rafael et al., 2026). Several limitations should remain explicit. Results from a single cultivar and a limited set of concentrations should not be generalized without further validation (Delgado-Rafael et al., 2026; Druege et al., 2019). Morphological measurements alone cannot identify the hormonal or molecular basis of the response. Follow-up work should use factorial designs, multiple concentrations, and longer-term outcomes such as rooting percentage, root quality, acclimatization, transplant survival, and later vegetative growth (Altamura et al., 2023; Delgado-Rafael et al., 2026; Druege et al., 2019).

5. Conclusion

The present study demonstrated that exogenous application of auxins and 24-epibrassinolide influenced both adventitious root development and vegetative growth of *Cannabis sativa* L. cv. FloSee stem cuttings, with responses varying according to the developmental stage and growth parameter evaluated. The combination of IAA 100 mg/L + IBA 3,000 mg/L + BS 1.0 mg/L promoted the earliest detectable root primordia formation at 7 days, whereas IBA 3,000 mg/L + BS 1.0 mg/L produced the greatest root proliferation, root biomass, and total plantlet fresh weight at 28 days. The IAA 100 mg/L + IBA 3,000 mg/L treatment produced the highest number of newly emerged leaves at the final observation. These results indicated that different plant growth regulator combinations may provide distinct advantages during different stages of vegetative propagation. In particular, IBA combined with BS was associated with enhanced development of the root system and overall plantlet biomass under the conditions tested.

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