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OPTIMIZING MICROPROPAGATION AND GROWTH ENHANCEMENT OF LAVANDULA ANGUSTIFOLIA USING TWO CYTOKININS

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Abstract

This experiment was conducted at the Tissue Cultural Lab of the Department of Horticulture and Landscape Engineering, College of Agriculture, University of Anbar to propagate lavender plant in vitro by cultivating single nodes in the Murashige-Skoog (MS) medium. The medium was supplemented with different concentrations of cytokinins, namely 6-benzylaminopurine (BA) and kinetin (Kin). Concentrations of 0.0, 0.5, and 1.0 mg L^{-1} and 0.0, 1.5 and 2.0 mg L^{-1} were used during the initiation and multiplication phases, respectively were tested separately for each growth regulator. The percentages of dead plant parts, non-responsive, and responsive live explants were determined during the initiation phase. In the multiplication phase, the number of leaves, and branch lengths and numbers were evaluated. Rooting of the branches resulting from multiplication was conducted on medium with half-strength salts supplemented with different concentrations of the auxin (0.0, 0.25, 0.5 mg IBA L^{-1}). Rooting percentage, number of roots, and root length were subsequently calculated. Results demonstrated that BA at 1.0 mg L^{-1} increased

responded explants percentages, decreased the non-responding and dead parts. Whereas, kinetin maximized plantlets length by 5 cm at 2 mg L^{-1}. Furthermore, BA and kin at 2 mg L^{-1} augmented leaf and branch numbers per plantlet except for 6-benzylaminopurine which increased branch numbers at 1.5 mg L^{-1}. Enhancement of the culture media via IBA at 0.25 and 0.50 mg L^{-1} increased rooting percentage, root length, and root numbers per plantlet.

Keywords: In vitro, Micropropagation, BA, Kinetin, Lavender.

تحسين الاكثار الدقيق وتعزيز النمو لنبات اللافندر Lavandula angustifolia باستخدام نوعين من السايتوكاينينات

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الخلاصة

نفذت التجربة في مختبر الزراعة النسيجية التابع لقسم البستنة وهندسة الحدائق، كلية الزراعة، جامعة الانبار، لإكثار نباتات اللافندر خارج الجسم الحي باستخدام عقد مفردة على وسط Murashige وSkoog. ارفد الوسط بتركيز مختلفة من السايتوكاينينات وهما BA وKinetin. اذ استخدمت التركيز 0.0 و0.5 و1.0 ملغم لتر$^{-1}$ في مرحلة النشوء واستخدمت التراكيز 0.0 و1.5 و2.0 ملغم لتر$^{-1}$ في مرحلة التضاعف من كلا المنظمين بالتتابع. سجلت بيانات نسبة الأجزاء الميتة والاجزاء الحية غير المستجيبة والمستجيبة في مرحلة النشوء. اما في مرحلة التضاعف فقد سجلت بيانات عدد الأوراق وطول الافرع وعددها. طبقت تجربة تجذير الافرع المنتجة في مرحلة التضاعف في وسط نصف القوة مدعم بتراكيز مختلفة من IBA وهي 0.0 و0.25 و0.5 ملغم لتر$^{-1}$. اذ سجلت بيانات نسبة التجذير وعدد الجذور واطوالها. أظهرت النتائج ان BA بتركيز 1 ملغم لتر$^{-1}$ زاد من الأجزاء المستجيبة وقلل من الأجزاء الميتة وغير المستجيبة. بينما زاد Kinetin من طول النبيتات 5 سم عند التركيز 2.0 ملغم لتر$^{-1}$. كذلك فان المنظمين بتركيز 2.0 ملغم لتر$^{-1}$ قد زادا من عدد الأوراق والافرع لكل نبيت فيما عدا BA

بتركيز 1.5 ملغم لتر$^{-1}$ زاد من عدد الافرع. تعزيز الوسط الزرعي بتركيزين 0.25 و0.50 ملغم لتر$^{-1}$ من IBA زاد من نسبة التجذير وطول الجذر وعدد الجذور بالنبيت.

كلمات مفتاحية: خارج الجسم الحي، الاكثار الدقيق، البنزل أمينوبيورين، الكاينتين، اللافندر.

Introduction

Lavandula angustifolia, a perennial plant member of the Lamiaceae family, is native to the Mediterranean region and raised internationally as a fragrant oil crop (10). It is one of the most important curative and fragrant crops for treating illnesses. *Lavandula angustifolia* Mill is also used in many fields including foods, pharmaceuticals, eco-friendly pesticides, perfumery, and cosmetics.

Thus, lavender plantations and production has risen globally in recent years (11), with much lavender essential oils production concentrated in Europe, the United States, Australia, and North Africa (39). Lavender essential oils comprise mainly monoterpenes such as linalool, 1,8-cineole, and camphor (21). A high concentration of linalool and low concentration of 1,8-cieole in the oil is considered higher-grade essential oils beneficial to the medicinal, pharmaceutical, and cosmetic industries. The commercial value of medicinal and aromatic plants is determined by its essential oil output and composition (9 and 13). (4) noted that the duration of distillation extraction affects the components of essential oil, including cyclohexanol, cyclohexanone, eucalyptol, caryophyllene oxide, caryophyllene, nerol oxide, trans-carveol, and β-ocimene.

Lavenders are widely utilized as ornamental and medicinal plants (34) and can also aid in regenerating fire-damaged areas (15). However, the economic significance of the lavender species is primarily attributed to the quality of its volatile oil which is strictly regulated internationally based on ISO standard and have been extensively exploited for centuries in cosmetic and therapeutic applications. Its volatile oil is widely employed in the production of soap, perfumes, food flavorings, and other items as scents or antibacterial substances (9 and 36). Lavenders have been used therapeutically since the time of the Romans and Greeks, but the recent rise in the popularity of complementary therapies has rekindled interest for lavender and its volatile oils for use as herbal remedies (36).

Several approaches have been implemented for enhancing lavender propagation in vertical farming systems, including the effect of red-to-blue (R:B) LED light ratios (35) and the incorporation of machine learning, which underlines the success of current micropropagation and routing procedures and offers opportunities over ongoing enhancement (31). Multiple procedures have been designed to propagate lavender species in vitro through meristem proliferation or organogenesis. These methods effectively modify existing characteristics and introduce new traits into the chosen species (14 and 19).

The morphogenic calli were triggered by cytokinins rather than indole-3-acetic acid (IAA), with 6-benzylaminopurine and kinetin stimulating the procedure (1 and 38). Furthermore, the auxin type influences callus pigmentation (18 and 20). IBA enhanced root length compared to NAA which enhanced the number of roots per plantlet in

lavender (16). Regarding morph rootlet caps, meristematic, elongational zones, and zones with root hairs were de novo generated roots in vitro via NAA. The root morphogenesis of lavender cultivars was affected by the plant source and media for cultivation (8). Thus, the aim of this study was to investigate the impact of growth regulators (cytokinins) on the development of the lavender plant's single node and to establish a protocol for the multiplication and dissemination of the plant.

Materials and Methods

Surface sterilization of explants: Single nodes were obtained from lavender branches growing in plant houses at the Department of Horticulture and Landscape Engineering, College of Agriculture, University of Anbar, Iraq. They were washed with water and soap and rinsed under running water for 30 mins. In a sterile isolation room, the nodes were surface-sterilized by immersion in 3% sodium hypochlorite solution with continuous stirring for 5 minutes. They were then rinsed thrice with sterile distilled water for 5 minutes each to remove traces of the sterilizing agent (29).

Preparation of the culture medium: The MS medium (25) obtained from Caisson Company was prepared by dissolving 4.43 g/L of medium powder and adding 30 gL^{-1} sucrose. Each growing regulator was prepared and added to the medium. The volume was adjusted to 800 mL with distilled water, and the PH was revised to 5.7 ±0.01 using sodium hydroxide (NaOH). The final volume was adjusted to 1,000 mL with distilled water, and 7 g/L agarose was added. The medium was agitated on a hot plate magnetic stirrer to dissolve the agar completely and then dispensed into culture vessels. These vessels were autoclaved at 121 °C and 1.04 kg/cm² pressure for 20 minutes.

Initiation phase: In this phase, the MS solution was supplemented with benzylaminopurine (BA) and kinetin (Kin) at doses of 0.0, 0.5, 1.0 mg/L. Single nodal explants of 0.5–1 cm length after sterilization were cultured on the medium with each growth regulator applied separately. Each treatment included twenty replicates. The cultured tubes underwent incubation at 25 °C with photoperiods of 16/8 hours light/darkness for 42 days. Data were recorded on the percentage of dead tissues, and non-responsive and responsive live tissues.

Multiplication phase: BA and Kin were added to the MS medium at doses of 0.0, 1.5, 2.0 mg/L. The sterilization method followed the procedure described previously. Single nodes from the initiation phase of 1–1.5 cm lengths were cultured. Each treatment included ten replicates. The cultures underwent incubation at 25 °C under a photoperiod of 16 hours of light and 8 hours of darkness. After six weeks, data were collected on the number of leaves, branch lengths, and branch count.

Rooting phase: MS media was prepared by diluting it to half strength and branches from the multiplication phase were grown on this medium supplemented with IBA concentrations of 0.0, 0.25, 0.5, 1.0 mg L^{-1} for rooting. After six weeks, data were collected on rooting percentage, root length, and the number of roots. Ten replicates were used for each treatment.

Acclimatization phase: Plantlets with developed roots were thoroughly washed with water to remove residual medium. They were transplanted into small pots containing a 1:1 mixture of peat moss and sand sterilized at 121 °C and 1.2 kg/cm² pressure for 20 minutes. The plantlets were covered with plastic for two weeks, with perforations

introduced gradually. The covers were eliminated incrementally and the plantlets transported to a greenhouse under the same soil mixture.

Experimental design and statistical analysis: The trials were laid out applying a completely randomized design (CRD) with 20 replicates for the initiation phase and 10 for multiplication and rooting. Data were analyzed using Origin pro 2025b software to construct graphs, and means were compared using Turkey test at a 0.01 probability level (6).

Results and Discussion

Initiation Phase: Data from the initiation phase showed significant differences in explant responses to varying concentrations of BA and Kin. Explants cultured on media supplemented with 1 mg L^{-1} BA had the lowest percentage of dead parts and the highest responsive parts compared to other concentrations (Fig 1A). Kinetin at 1 mg L^{-1} produced a higher percentage of non-responsive parts than the control (Fig 1B). These findings align with previous studies that BA is more stable and effective due to its cyclic structure and triple-bond side chains (2 and 24).

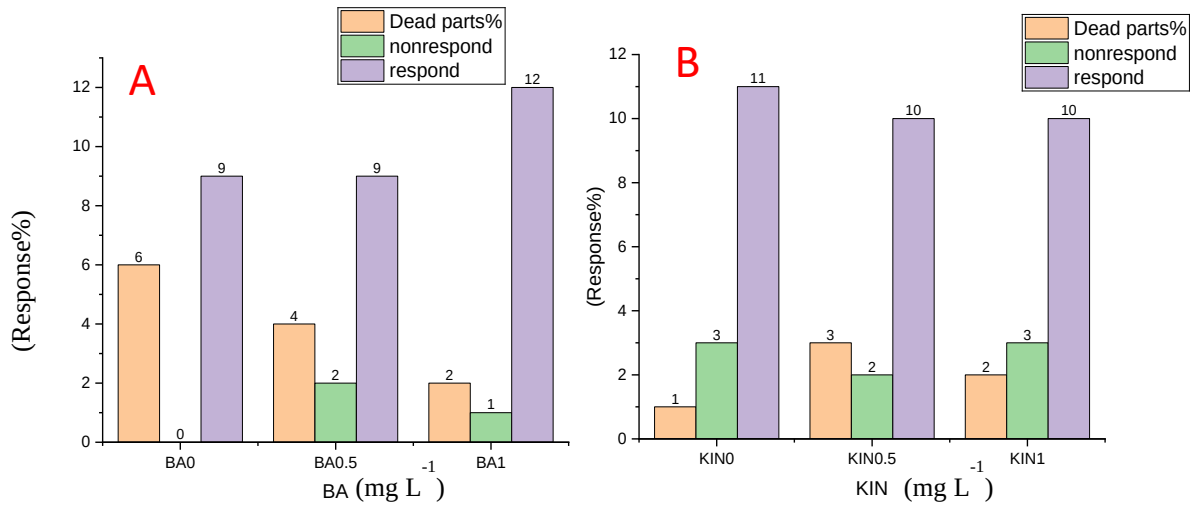

Figure 1: Response of lavender plantlets to BA (A) and kin (B) concentrations.

(Orange: dead parts; green: non-responding; violet: responding).

Multiplication Phase: The addition of BA at 2 mg L^{-1} resulted in a non-significant increase in plantlet lengths (Fig 2A), followed by 1.5 mg L^{-1} compared to control. Conversely, Kin at the same concentration significantly produced the longest plantlets (5 cm) (Fig 2B), followed by 1.5 mg L^{-1} compared to the control.

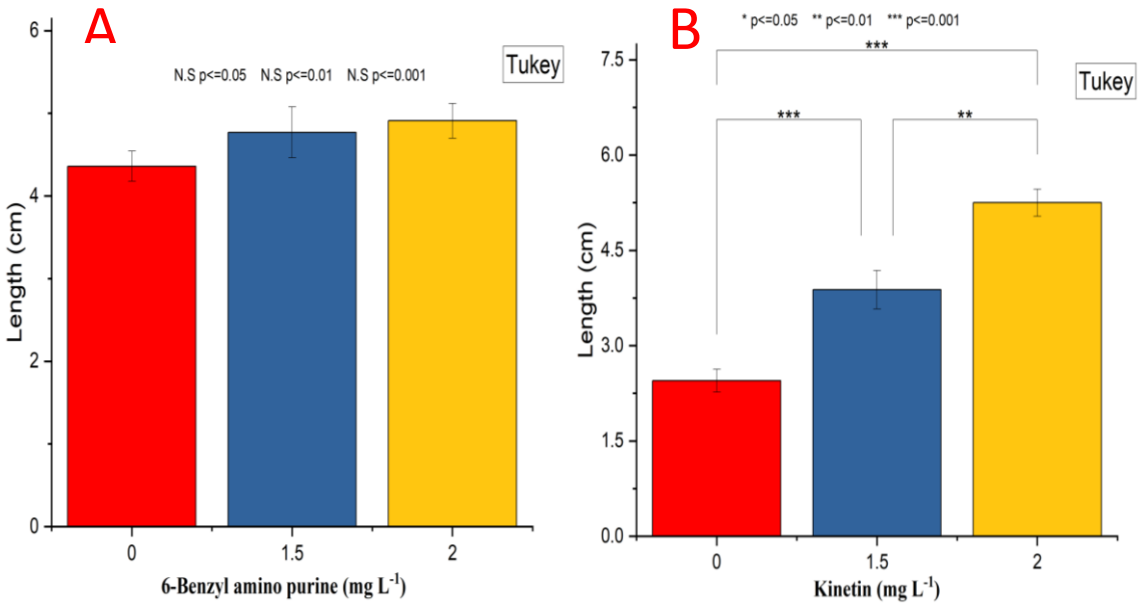

Figure 2: Impact on lavender plantlet lengths of different BA (A) and kin (B) concentrations.

The addition of BA at 2 mg L^{-1} non-significantly increased the number of leaves and branches (Fig 3A, Fig 4A), followed by 1.5 mg L^{-1} compared to the control.

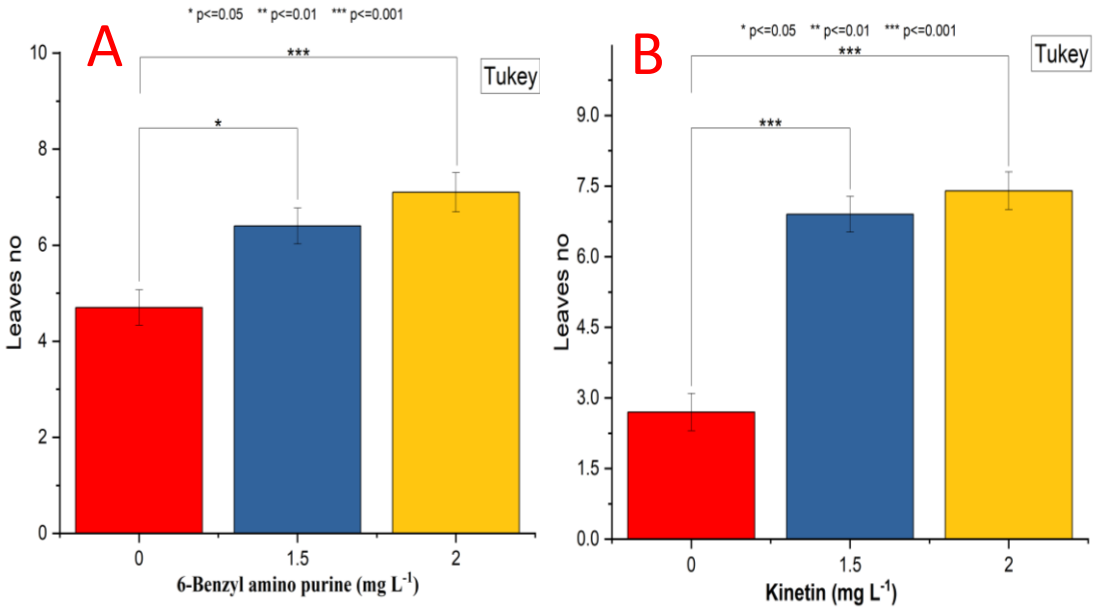

Figure 3: Impact on leaf numbers of lavender plantlets of different BA (A) and kin (B) concentrations.

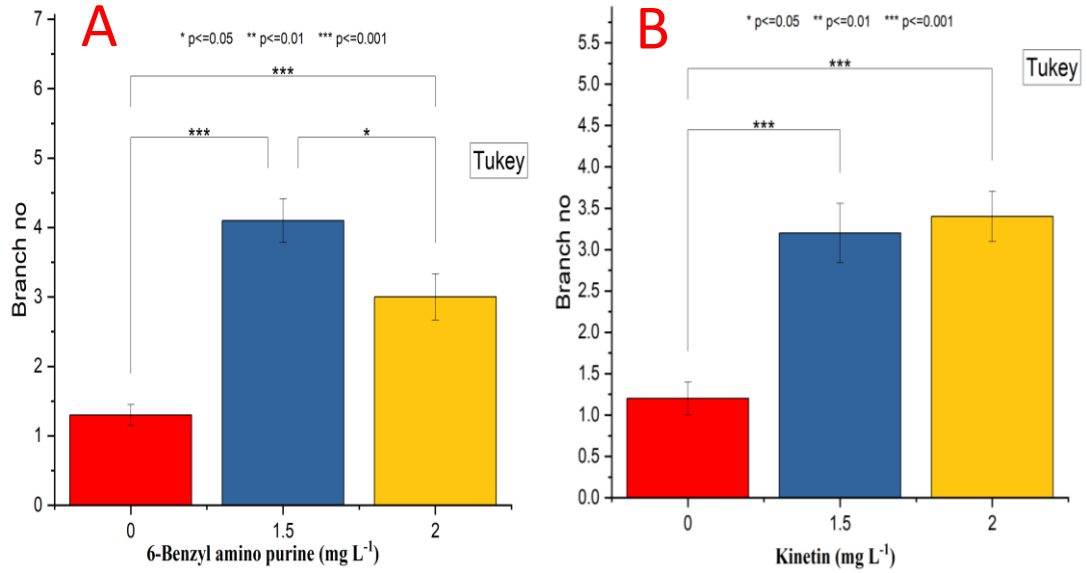

Figure 4: Branch numbers of lavender plantlets treated with different concentrations of BA (A) and kin (B).

Similarly, Kin at 2 mg L^{-1} significantly enhanced these traits (Fig 3B, Fig 4B), emphasizing the role of cytokinins in breaking apical dominance and promoting cell division (17 and 33). These results are consistent with the findings of (7 and 26), who found that the enrichment of culture media with benzyl adenine increased chia plantlets' lengths and branch numbers.

Rooting Phase: IBA at 0.5 mg L^{-1} achieved the highest rooting percentage and root numbers (Fig 5), followed by 0.25 mg L^{-1} compared to control.

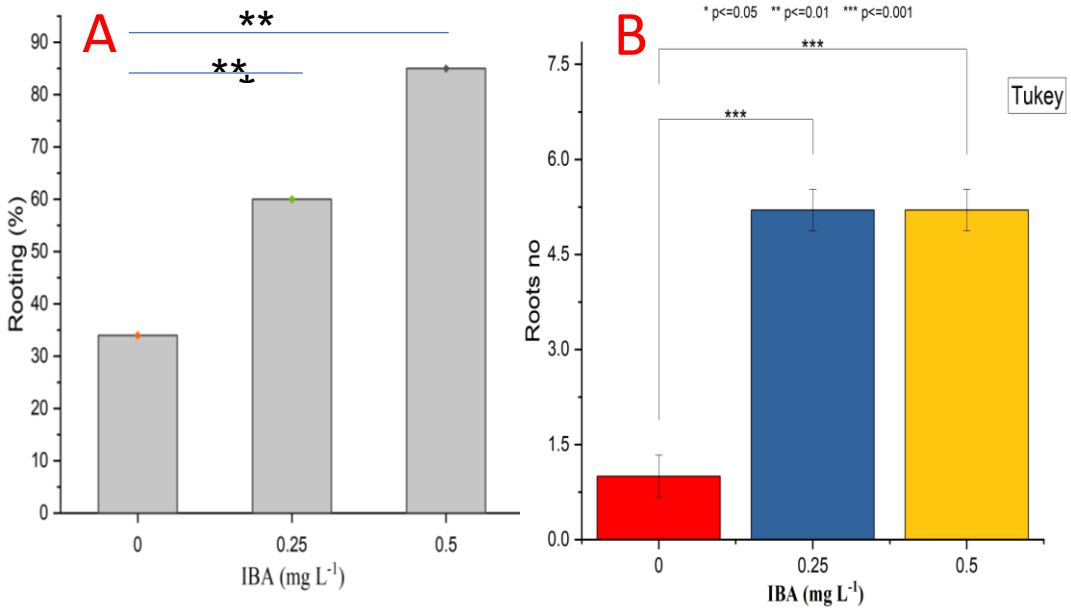

Figure 5: Rooting percentage (A) and root numbers (B) of lavender explants treated with different IBA concentrations.

Likewise, the addition of IBA significantly increased root lengths (Fig 6) especially at the 0.5 mg L^{-1} concentration.

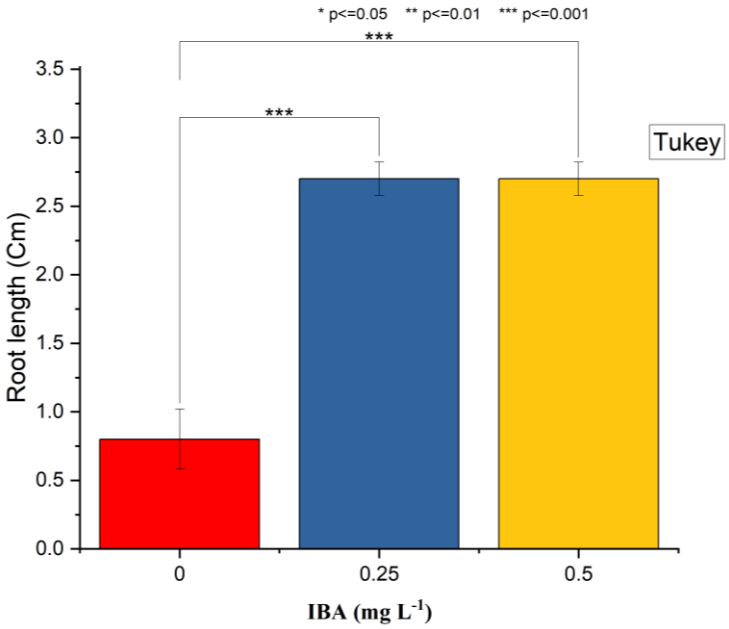

Figure 6: Impact on root length (cm) of lavender plantlet from different IBA concentrations.

Auxins are critical for root initiation and elongation, as they regulate cell wall enzymes and mechanical properties (3 and 5). Several variables contribute to root formation, both external and internal, (30 and 32), particularly IBA auxin. Moreover, the exogenous application of auxins promotes lateral rootlet formation by enhancing auxin transport (27 and 28). IBA improved the average number and length of rootlets in lavender plants grown in vitro (Figs. 5B and Fig 6).

The explanation could be related to PGR IBA, one of the auxins that stimulate root development. (22) demonstrated that nourishing media complemented with IBA was effective in the propagation of rhizogenic roots (12) as well as in rooting plantlets of crops raised in vitro (23 and 37). Meanwhile, (7) illustrated that enriching cultural media with NAA enhanced the number of rootlets and their lengths.

Acclimatization: Plantlets registered an 80% survival rate during acclimatization when transplanted into a peat moss and sand mixture. This finding supports previous research highlighting the importance of substrate sterilization and gradual environmental adaptation.

Conclusions

This study developed a new methodology for in vitro micropropagation of Lavandula angustifolia utilizing single-node explants. The lowest concentrations of BA were effective for the initiation stage while the highest was ideal for the multiplication stage. Additionally, the half-strength IBA in the media proved effective for rooting explants generated from plantlets. Therefore, these growth regulators possess good stimulatory attributes for the propagation of lavender. This protocol can be used to

propagate this plant in Iraq due to its importance in the manufacture of therapeutic ingredients.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Y. S. Sekh: methodology, writing—original draft preparation, A. A. Abdulkareem and A. F. Almehemdi writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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No Data Availability Statement.

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The authors declare no conflict of interest.

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