Micropropagation of Blackberry (*Rubus spp.*) using concentrations of BA and MS medium *In vitro*

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Summary
This study aims to investigate the optimal protocol to propagate Blackberry (*Rubus spp.*) *In vitro* using MS medium and plant growth regulators. At the establishment stage: the best results after 4 weeks. shows that, the nodes which cultured on media supplemented with 4 mg L^{-1} achieved highest values in response percentage (100%), shoots length (1.36 cm) and the number of leaves (9 leaves) respectively that superior in comparison with control treatment (free of BA), except the number of shoots which achieved the highest value (1.40 shoot) formed from nodes cultured on medium supplemented with 1 mg L^{-1}, and didn't differ significantly with the other BA concentrations. The multiplication stage after eight weeks shows that adding 3 mg L^{-1} had the highest effects on multiplication characteristics (number of shoots and the number of leaves), which achieved 2.10 shoots and 13.80 leaves $shoot^{-1}$, respectively. It was superior in comparison with shoots cultured on media supplemented with 2 mg L^{-1} BA in the number of shoots and the control in the number of leaves; however, the longest shoots were (1.99 cm) from shoots cultured on media supplemented with 1 mg L^{-1}. The rooting stage after four weeks showed that the medium at half strength of salts achieved a significant increase in rooting percentage (68%). Adding IBA at 1.5 mg L^{-1} to the medium had the best effect on rooting percentage (70%). The interaction between IBA concentration and media salt strength showed the highest value in the interaction between half-strength and 1 mg L^{-1} IBA, which achieved 90% of rooted shoots, and it was overcome by most concentrations of IBA supplemented to media with full strength. On the other hand, a significant increase in the number of roots was observed in half-strength medium (11.09 roots $shoot^{-1}$) compared with full-strength salts (7.61 roots $shoot^{-1}$). In addition, the concentration of 1.5 mg L^{-1} IBA produced the highest significant number of roots (12.41), surpassing the other concentrations. As well as, the best interactions were between 1.5 mg L^{-1} IBA and half-strength medium, which achieved the highest number of roots (13.32 roots), overcoming all IBA concentrations interacting with full-strength salts. Furthermore, no significant effect of medium salt strength and IBA concentration supplemented to the medium on root length. During the acclimatization stage, it was observed that all rooted plantlets derived from rooting experiments, acclimatized in the laboratory, and transferred to the greenhouse exhibited a 98% survival rate after two months of being moved outdoors in field conditions.

Keywords: Blackberry (*Rubus* spp.), MS, BA, micropropagation, *in vitro*.

Introduction

American blackberries (*Rubus* spp.) are a group comprising over 100 genera and 750 species. belong to the Rosaceae family. Some species are cultivated for fruit production and others as ornamental plants.

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These plants exhibit diverse characteristics [43]. Some varieties feature thorny stems. They are cultivated commercially for their taste, flavor, and nutritional. They produce biennial stems known as "canes." In the first year a new stem called a primocane. The plant shrubs grow in a semi erect or trailing habit which grows vigorously reaching to 3 to 6 meters in length, and sometimes up to 9 meters under specific conditions. The stems remain vegetative during the initial phase. In the second season the primocane develops to a productive stem. These primocanes produce large palmately compound leaves consisting of three, five, or seven leaflets [18]. Blackberries are typically eaten fresh or flash frozen, or used in processed products such as jams, syrups, wines, teas, and juices. Many health benefits associated with blackberry fruits or their products are attributed to their metabolites, these metabolites directly and indirectly influence processing methods, like shelf life, and consumer satisfaction [23]. Much people use plant stems fibers to make rope, while Britons used the spiny stems as boundaries or barriers [6];[17]. blackberry shrubs can help prevent soil erosion in poor or arid areas. Additionally, a purple to pale blue dye extracted from the fruit [20]. Their potential health benefits include dietary fiber, vitamins A, C (ascorbic acid), and E, potassium, and calcium, as well as phenolic compounds [12].

[13] noted that blackberry is a native American plant, as a shrub Widespread across Europe, Asia, and North and South America. This type of blackberry is found in Iraqi Kurdistan, where it is cultivated for its fruit [19]. They grow best in fertile well drained soils with adequate moisture, in areas characterized by mild to relatively cold winters and moderate summer conditions. blackberry plants can be propagated by various methods, including seeds, which exhibit deep dormancy after drying and require storage in a cool environment for months or even years, leading to a significant reduction in seed viability. Root cuttings, stem cuttings and grafting [16]. They well grow in temperate climates and demonstrate a high capacity to adapt to diverse environmental conditions and are grown in many regions worldwide. Plants require a chilling period of 300 to 900 hours to stimulate flower bud formation following the dormancy phase [1]. While vegetative propagation of blackberries requires Spacious planting areas and poses challenges because of annual weed growth. Likewise, propagation by layering demands time and effort for rooting [9]. Europe and the United States have employed Micropropagation techniques widely [11]. Tissue culture is a modern biotechnological technique that has played a significant role in benefiting humanity. Micropropagation is a method used to propagate different herbaceous and woody plant species. particularly in the field of plant production. This method offers several advantages, in most cases free of virus, insect infections and disease, producing large numbers of healthy plants within a short time and at any time of the year, that are genetically similar to the mother plant [5]; [44]. Furthermore, induce the lateral bud growth and regeneration of adventitious shoots are prerequisites for any plant improvement program aiming at development through *In vitro* techniques and genetic engineering. Plant genetic engineering and biotechnology techniques are particularly valuable for fruit trees, as they offer the potential to reduce the time required for traditional breeding programs [31]. Propagation techniques have become methods of highly significant quality and safety compared to any other production method, as the plant material obtained through them. This is achieved

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through the selection of culture media and various combinations of plant growth regulators for ex vivo plant propagation [21]. This research aims to investigate the objective of establishing an optimal and effective protocol for the *In vitro* micropropagation of blackberry, and the effect of various growth regulators supplemented to MS media, as well as improve lateral shoot formation by culturing nodes on media containing varying concentrations of cytokinins, followed by the continued multiplication of the resulting shoots. Rooting the shoots produced from nutrient media with varying salt and auxin concentrations, and studying the impact on the characteristics of the resulting roots. [33] conducted experiments on propagation of blackberry *Rubus* sp. cultivars 'Čačanska bestrna' and 'Čačanska crna' *In vitro*. Cultured on MS medium supplemented with BA (0.5 and 1.0 mg L^{-1}) combined with IBA or NAA (0.1 mg L^{-1}) and GA_3 (0.1 mg L^{-1}). Results showed that the highest number of new shoots (2.45) for the 'Čačanska bestrna' cultivar was obtained on medium supplemented with 1.0 mg L^{-1} BA combined with 0.1 mg L^{-1} of both IBA and GA_3 . However, the highest shoot length (0.89 cm) and shoot length (0.59 cm) for the same cultivar were achieved on medium containing 0.5 mg L^{-1} BA combined with 0.1 mg L^{-1} of both IBA and GA_3 , respectively. No significant differences were observed in the studied parameters for the 'Čačanska crna' cultivar. Another study by [29] investigated the effects of BA and GA_3 on blackberry *Rubus* sp. axillary buds cultured on MS medium supplemented with BA (0, 2, and 3 mg L^{-1}) and GA_3 (0, 0.2, 0.5, and 1 mg L^{-1}). The results indicated that the addition of BA and GA_3 significantly increased both shoot number and shoot length. After two weeks, increasing the GA_3 concentration improved shoot growth and elongation, specifically, the combination of 0.5 mg L^{-1} GA_3 and 2 mg L^{-1} BA achieved the highest shoot length (5.87 cm), a result that was significantly superior to most other treatments. The highest shoot number was achieved by treatments containing 2 mg L^{-1} BA, which produced 3.33 shoots per explant, interacting with 0.5 or 1 mg L^{-1} GA_3 . In contrast, the control treatment (0 mg L^{-1} BA and 0 mg L^{-1} GA_3) achieved the lowest values for both shoot number and shoot length (1.0 shoot, 1.2 cm). The effect of MS and WPM culture media investigated by [13] without the addition of growth regulators on the development of *Rubus fruticosus* L. (American blackberry) nodes. They observed a maximum response rate of 91.67% for nodes grown on both MS and WPM media; however, the highest values of shoot and leaf number and length were achieved for nodes cultured on MS medium, reaching 1.83 shoots explant^{-1} and 7.33 leaves and 31.67 mm, respectively. [35] investigated the effects of three culture media free of plant growth regulators (MS, B5, and WPM) on blackberry *Rubus* sp. cv. 'Karaka Black' node explants, on the number of shoots, shoot length, and number of leaves. They observed no significant differences in the number of shoots, with an average of 1.00 shoot recorded across all media types. However, the MS medium significantly outperformed the others in shoot length (0.90 cm), followed by the WPM medium (0.50 cm), while the B5 medium achieved the shortest shoot length (0.17 cm). The number of leaves in the WPM medium achieved the highest value (3.33 leaves), significantly differed with MS and B5 media, which recorded 2.67 and 1.67 leaves, respectively. In the multiplication stage after eight weeks, the media supplemented with 0.4 mg L^{-1} BA yielded the highest number of shoots (5.33 shoots)

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and the longest shoots (2.73 cm), as well as the highest number of leaves (22.22 leaves). [28] reported that culturing shoots derived from the multiplication on MS, WPM, and DKW media supplemented with NAA at concentrations of 0.0, 0.1, 0.2, and 0.5 mg L^{-1} resulted in the highest values of root induction (an average of 5.4 roots $shoot^{-1}$) when 0.2 mg L^{-1} NAA was added to the WPM medium. [26] successfully cultured blackberry *Rubus fruticosus* L. on MS and WPM media supplemented with BA (0, 1, 1.5, 2, and 2.5 mg L^{-1}). After four weeks of culture, shoot tips on the WPM medium exhibited a superior response, as assessed based on response percentage, number of shoots, length of the longest shoot, and number of leaves compared to the explants cultured on the MS medium. The results indicated that the addition of BA to the culture medium led to a significant increase in the percentage of response (100%), which was achieved at all BA concentrations compared to the control treatment (0 mg L^{-1} BA), which recorded 80%. It was also observed that the number of shoots increased with higher concentrations, it was 1.0 shoot for all concentration treatments, compared to 0.8 shoots in the control treatment. The length of the longest shoot, at 1.5 mg L^{-1} concentration, yielded the greatest length (5.1 cm), followed by the 1 mg L^{-1} concentration (4.9 cm), whereas the control treatment produced the shortest length (3.7 cm). The number of leaves achieved the highest mean of 8.4 leaves per explant at 2.5 mg L^{-1} BA, followed by 1.5 mg L^{-1} (8.0 leaves). In a study by [15] to propagate blackberry (*Rubus fruticosus* L.) cv. APF-19OT on three basal media (WPM, MS, and 1/2MS). He found that the MS medium achieved significant superiority over the other media tested. Recorded the highest response (63.33%), followed by the WPM medium, which achieved (52.22%), while the 1/2MS medium (half-strength salts) showed the lowest response rate (30.00%). The shoot length of explants grown on the MS medium achieved the greatest length (1.41cm), followed by the WPM medium (1.13 cm), whereas the 1/2MS medium resulted in the shortest length (0.86 cm). In the rooting experiment, he investigated the effect of NAA concentrations (0.5, 1.0, and 1.5 mg L^{-1}) in half-strength MS medium on the rooting percentage and number of roots. The results showed that the highest rooting percentage (97.78%) and the highest number of roots (5.11 roots) were achieved with treatment 1.0 mg L^{-1} NAA added to half-strength MS medium; the resulting roots were of appropriate thickness.

Materials and Methods:

This study was conducted at the University of Mosul, College of Agriculture and Forestry, Department of Forestry Science, in the Laboratory of Plant Tissue and Cell Culture, from November 2025 to May 2026. It aims to determine the optimal conditions to propagate blackberry plants. Several experiments were carried out during each stage of the propagation process (initiation, multiplication, and rooting), as follows:

Preparation of nutrient media:

MS medium was used in this study and obtained from the American company (Caisson Lab). The contents of the can were macronutrients, micronutrients, vitamins, amino acids, and myo-inositol; the mix sufficient for preparing one liter was added to a one-liter glass flask containing 500 mL of distilled water and dissolved using a hot plate magnetic stirrer. During the dissolution process, sucrose was added at a concentration of 30 g L^{-1}; subsequently, the medium was divided into treatment solutions according to the requirements of each experiment. The pH was adjusted to 5.7–5.8 using 1 N sodium hydroxide (NaOH) or diluted hydrochloric

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acid (HCl), and agar powder was added to the prepared medium at a concentration of 6 g L^{-1}. Once prepared, the nutrient medium was dispensed into 125 mL glass bottles, with 20 mL per bottle. The bottles were then covered with heat-resistant aluminum foil and sterilized in an autoclave at 121°C and a pressure of 1.04 kg cm^2 for 20 minutes.

Preparation and sterilization of explants:

Explants of newly grown stem cuttings (15-20 cm in length) were collected from blackberry donor plants (*Rubus* spp.) one year old, grown in the greenhouse. After being transported to the laboratory, the leaves were removed, and the cuttings were immersed in a solution of the fungicide Beltanol 1 mL L^{-1} (v/v) for 30 minutes. Then, they were washed under running tap water for 30 minutes to remove dust and adhering particles. The explants were transferred to a laminar airflow cabinet for surface sterilization. The explants were soaked in a sodium hypochlorite (NaOCl) solution (commercial bleach) containing 5.6 g of chlorine (Cl) at a 10% (v/v) concentration for 15 minutes. Subsequently, the explants were washed four times with sterile distilled water and then cut into single-node segments (1–2 cm in length) and cultured on solid media.

Establishment stage:

Media supplemented with BA (6-benzyladenine) at concentrations (0, 1, 2, 3, 4 mg L^{-1}) were cultured. The growth parameters (percentage of nodal response, number of shoots, shoot length (cm), and the number of leaves formed from shoots were measured after four weeks. The data were recorded after four weeks. The Experiments were conducted in ten replicates, with one node per replicate.

Multiplication stage:

Shoots that were established were used for multiplication experiments. 1 cm length of shoots was selected. To study the effect of adding BA at (0, 1, 2, 3, 4 mg L^{-1}) to the medium on shoot multiplication characteristics (Number of shoots, shoot length (cm), and number of leaves (leaves per shoot). The data were recorded after eight weeks. The Experiments were conducted in ten replicates, with one shoot per replicate.

Rooting Stage:

The experiments were conducted as a factorial in two factors: the 1st factor was the salt concentration of MS, and the 2nd factor was the IBA concentration. Shoots (1–2 cm in length) were obtained from explants at the multiplication stage to study the effects of salt concentrations of MS media at (half and full strengths), while maintaining constant levels of sucrose) in combination with IBA concentrations (0, 0.5, 1, 1.5, and 2 mg L^{-1}). The study aimed to evaluate their impact on rooting characteristics, using 10 replicates per treatment (one shoot per replicate). The data taken after four weeks included rooting percentage, number of roots per shoot, and root length (cm).

Acclimatization Stage:

The plantlet stage was acclimatized by thoroughly washing their roots under running tap water to remove adhering agar, followed by soaking in a solution of the fungicide Beltanol (the same concentration above) for 3 minutes to protect against fungal infections. Subsequently, the plantlets were planted in multi-cell seedling trays (26 × 18 cm) comprising 24 cells, each cell (4×4 cm) with a depth of 5 cm, using a growing medium consisting of a peat moss and perlite mixture (5:1). Following transplantation, the plantlets were incubated under the same laboratory conditions, and the growing medium was watered as needed during the acclimatization period. For the first two weeks, the plantlets were covered with transparent plastic to maintain high humidity; the covering was gradually removed until it was completely taken off in the third to fourth week. The

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plants were transferred to the greenhouse has been ready for growth under outdoor conditions.

Statistical Analysis:

All study experiments were conducted as simple experiments using a Completely Randomized Design (CRD), except the rooting stage experiment, which was conducted as a factorial experiment. Means were compared using Duncan's Multiple Range Test at a 5% probability level for all treatments [7], and statistical analysis was performed using SAS software (Version 9) [37].

Results:

Establishment stage:

In this stage, we investigated the effect of BA concentration on nodes established after 4 weeks. The results in table (1) shows that, the nodes which cultured on media supplemented with 4 mg L^{-1} achieved highest values in establishment characteristics response percentage (100%), shoots length (1.36 cm) and the number of leaves (9 leaves) respectively that superior in comparison with control treatment (free of BA), except the number of shoots which achieved the highest value (1.40 shoot) formed from nodes cultured on medium supplemented with 1 mg L^{-1}, and didn't differ significantly with the other BA concentrations **Fig. (1)**.

Table (1): Effect of BA concentration on explant establishment of Blackberry *Rubus* spp. After 4 weeks.

BA Conc. mg L^{-1}	Explant Response %	No. of Shoots	Shoots Length(cm)	No. of Leaves
0	50 b	1.20 a	0.91 b	4.70 d
1	70 ab	1.40 a	1.07 ab	5.90 dc
2	70 ab	1.10 a	1.25 a	7.00 bc
3	90 ab	1.20 a	1.32 a	7.70 ab
4	100 a	1.10 a	1.36 a	9.00 a

Means followed by the same letter for each factor or their interaction do not differ significantly according to Duncan's Multiple Range Test at the 0.05 level.



Fig 1: nodes established on MS medium after 4 weeks: (A) 0 mg L^{-1} BA, **(B)** 1 mg L^{-1} BA, **(C)** 2 mg L^{-1} BA, **(D)** 3 mg L^{-1} BA, **(E)** 4mg L^{-1} BA.

Multiplication stage:

The data in Table (2) show the effect of BA supplemented at different concentrations to MS medium after eight weeks, where adding 3 mg L^{-1} had the highest effects on multiplication characteristics (number of shoots and the number of leaves), which achieved 2.10 shoots and 13.80 leaves $shoot^{-1}$, respectively. It was also superior in comparison with shoots cultured on media supplemented with 2 mg L^{-1} BA in the number of shoots and the control in the number of leaves, **(Fig. 2)** however, it didn't differ significantly in shoot length, which achieved the highest value (1.99 cm) from shoots cultured on media supplemented with 1 mg L^{-1}.

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Table (2): Effect of BA concentration on shoot multiplication of Blackberry *Rubus* spp. after 8 weeks.

BA Conc. mg L^{-1}	No. of Shoots	Shoot Length (cm)	No. of Leaves
0	1.60 ab	1.98 a	9.60 b
1	2.00 a	1.99 a	13.70 a
2	1.30 b	1.58 a	10.70 ab
3	2.10 a	1.86 a	13.80 a
4	2.00 a	1.76 a	13.20 a

Means followed by the same letter for each factor or their interaction do not differ significantly according to Duncan's Multiple Range Test at the 0.05 level.

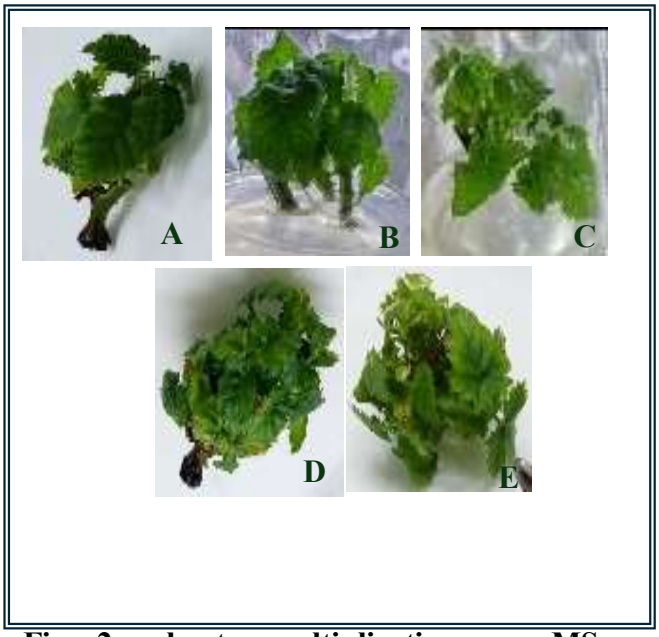


Fig 2: shoots multiplication on MS medium after 8 weeks: (A) 0 mg L^{-1} BA, **(B)** 1 mg L^{-1} BA, **(C)** 2 mg L^{-1} BA, **(D)** 3 mg L^{-1} BA, and **(E)** 4mg L^{-1} BA.

Rooting Stage:

The data in Table (3) and **Figure (3)**, show the effect of salt concentration and IBA on rooting percentage after 4 weeks. It is evident from the results that the medium at half strength of salts achieved a significant increase in rooting percentage (68%) in comparison with the full strength. Adding IBA at 1.5 mg L^{-1} to the medium had the best effect on rooting percentage (70%) and was superior to the control treatment (free of IBA). The interaction between IBA concentration and media salt strength, the highest value in the interaction between half-strength and 1 mg L-1 IBA, which achieved 90% of rooted shoots, and it was overcome by most concentrations of IBA supplemented to media with full strength, which achieved the lowest percentage (20%) in the medium with full strength and free of IBA.

Table (3): Effect of MS salt conc. interacted with IBA conc. on the rooting percentage (%) of blackberry *Rubus* spp. after 4 weeks.

IBA mg L^{-1}	Full strength	Half strength	Effect of IBA
0	20 d	30 cd	**25 b**
0.5	20 d	70 a-c	**45 ab**
1	20 d	90 a	**55 ab**
1.5	60 a-d	80 ab	**70 a**
2	40 b-d	70 a-c	**55 ab**
Effect of medium	**32 b**	**68 a**	

Means followed by the same letter for each factor or their interaction do not differ significantly according to Duncan's Multiple Range Test at the 0.05 level.

A significantly higher number of roots was observed in half-strength medium (11.09 roots $shoot^{-1}$) compared with full-strength salts (7.61 roots $shoot^{-1}$) (Table 4). In addition, the concentration of 1.5 mg L^{-1} IBA produced the highest significant number of roots (12.41), surpassing the other concentrations. As well as, the best interactions were between 1.5 mg L^{-1} IBA and half-strength medium, which achieved the highest number of roots (13.32 roots), overcoming all IBA concentrations interacting with full-strength salts, which achieved the lowest number of roots (4.00

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roots) from shoots cultured in medium in full-strength free of IBA **(Fig. 3)**.

The data in Table (5) show no significant effect of media salt strength and IBA concentration supplemented to the medium on root length. The highest root length was observed on half-strength medium (0.82 cm) and at 0.5 mg L^{-1} of IBA (0.87 cm). On the other hand, the interaction between the medium salt strength and IBA concentrations, the highest root length was 0.94 cm from shoots cultured on media in half-strength supplemented with 0.5 mg L^{-1} IBA, superior to the interaction between medium in half-strength with 1mg L^{-1} IBA and medium in full-strength free of IBA (control), which achieved the lowest root length (0.67 and 0.62 cm), respectively.

Table (4): Effect of MS salt conc. interacted with IBA conc. The number of roots of blackberry *Rubus* spp. after 4 weeks.

IBA mg L^{-1}	Full strength	Half strength	Effect of IBA
0	4.00 f	9.00 c-e	**6.50 c**
0.5	6.30 ef	11.14 a-c	**8.72 b**
1	7.35 de	12.00 ab	**9.68 b**
1.5	11.50a-c	13.32 a	**12.41 a**
2	8.88 c-e	10.00 b-d	**9.44 b**
Effect of medium	**7.61 b**	**11.09 a**	

Means followed by the same letter for each factor or their interaction do not differ significantly according to Duncan's Multiple Range Test at the 0.05 level.

Table (5): Effect of MS salt conc. interacted with IBA conc. on the root length of blackberry *Rubus* spp. after 4 weeks.

IBA mg L^{-1}	Full strength	Half strength	Effect of IBA
0	0.62 b	0.92 a	**0.77 a**
0.5	0.79 ab	0.94 a	**0.87 a**
1	0.82 ab	0.67 b	**0.75 a**
1.5	0.78 ab	0.81 ab	**0.79 a**
2	0.76 ab	0.74 ab	**0.75 a**
Effect of medium	**0.75 a**	**0.82 a**	

Means followed by the same letter for each factor or their interaction do not differ significantly according to Duncan's Multiple Range Test at the 0.05 level.

Acclimatization Stage:

During the acclimatization stage, it was observed that all rooted plantlets derived from rooting experiments, acclimatized in the laboratory, and transferred to the greenhouse exhibited a 98% survival rate after two months of being moved outdoors in field conditions **(Fig. 4)**.

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Fig 3: Rooted shoots culture on MS media in full and half strength of salts after 4 weeks.
(A) 0 mg L^{-1} IBA in full strength, (**B)** 0.5 mg L^{-1} IBA in full strength, (**C)** 1 mg L^{-1} IBA in full strength, (**D)** 1.5 mg L^{-1} IBA in full strength, (**E)** 2 mg L^{-1} IBA in full strength, (**F)** 0 mg L^{-1} IBA in half strength, (**G)** 0.5 mg L^{-1} IBA in half strength, (**H)** 1 mg L^{-1} IBA in half strength, (**I)** 1.5 mg L^{-1} IBA in half strength, (**J)** 2 mg L^{-1} IBA in half strength.



Fig. 4: Acclimation of plantlet produced from rooting stage: (A) plantlet after one month, **(B)** plantlets after two months.

Discussion:
The concentration of BA 3 mg L□¹ added to the culture medium yielded the best significant results in the node percentage of response (Table 1). The positive effect of 1 mg L^{-1} BA on increasing the number of shoots, while 4 mg L^{-1} BA achieved the highest number of leaves, may be attributed to the efficacy of cytokinins in stimulating bud break, proliferation, and branching, which ultimately resulted in a greater number of leaves. [40] noted the regulatory role of cytokinins in leaf formation; they promote cell division in growing tips and subsequent differentiation into leaves by breaking apical dominance and stimulating the outgrowth of lateral buds. However, the BA effect on the response percentage and shoot length was compared to the control (Table 1). This variation may be attributed to the specific role of BA in promoting plant cell division by synthesizing RNA, proteins, and enzymes within the cell. Thereby, BA reduces apical dominance, which stimulates lateral branching [8].

as well as with the findings of [16], who reported that the optimal BA concentration for raspberry propagation was 0.5 mg L^{-1} (the lowest concentration tested); this concentration yielded the highest number of shoots. Similarly, [25] explained that the highest number of shoots was obtained from media supplemented with 1 mg L^{-1} BAP, although these shoots were shorter than those grown at other BA concentrations. The multiplication experiment table (2) shows that 3 mg L^{-1} of BA achieved the best results in shoot number. While the highest shoot length and number of leaves were achieved in 1 mg L^{-1} BA. These results might refer back to what was explained by [32], who

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noted that the plant parts propagated *In vitro*, exhibit vigorous growth as a result of regaining their biological capacity and revert to a juvenile phase for cell division. The effect of BA on increasing the number of shoots may be attributed to its role in reducing the effectiveness of apical dominance and stimulating the vascular differentiation of lateral buds, thereby facilitating their growth and branching. Alternatively, this role of BA in mitigating the inhibitory influence of auxins on lateral buds, which otherwise prevents bud opening and growth, promotes their development. This occurs through BA's significant contribution to the production of nucleic acids and amino acids; it enhances the synthesis of transfer RNA (tRNA) and reduces nucleic acid degradation by inhibiting the activity of the enzyme ribonuclease (RNase) [22]. [40] explained the effect of cytokinins, which play a regulatory role in leaf formation from shoots by promoting cell division in growing tips, followed by elongation and differentiation into leaves, as well as the formation of other stem tissues, in breaking apical dominance and stimulating bud break. BA exerts a positive effect by stimulating cell division, a result of enhancing nucleic acid production and protein synthesis. Cytokinins are structurally incorporated into nucleic acids as nitrogenous compounds (adenine, guanine, thymine, uracil, and cytosine), which is likely the primary reason for their influence on cell division [2]. This aligns with [42] findings that cytokinins regulate protein synthesis by activating the production of certain proteins while inhibiting others; they increase the plant's content of polyribosomes, the mRNA encoding the Rubisco enzyme (responsible for carbon dioxide reduction during photosynthesis), and the polypeptide associated with chlorophyll. The movement of cytokinins from growing tips to lateral buds is stimulated by the presence of auxin, thereby facilitating the formation and growth of a greater number of buds. This aligns with the findings of [38] and [14], who noted that a hormonal balance between the culture medium and the plant explant enhances explant proliferation. Specifically, the high endogenous auxin content of the shoot tips, which serve as primary sites of auxin synthesis, combined with the BA concentrations added to the medium, likely established an optimal hormonal balance for shoot tip proliferation. [39] and [22], noted that the interaction between cytokinins and auxins, wherein each activates the other. Specifically, cytokinins accelerate the synthesis of DNA, mRNA, and tRNA by regulating their functional coordination on ribosomes during translation, whereas auxins accelerate the synthesis of rRNA. Tissue culture experiments confirm that supplementing culture media with both auxins and cytokinins promotes rapid cell division and growth in apical meristems and cambial tissue. [27] indicated that the initiation of leaf buds, as well as the growth and activity of lateral buds, depend on an appropriate ratio of cytokinins to auxins. These results align with [24], who cultured explants of the *Rubus* spp. cultivar 'Chester Thornless' on media supplemented with 2 mg L^{-1} BA during the multiplication stage; this treatment yielded the greatest shoot length (2.2 cm) eight weeks after culture initiation. The results from the rooting experiments using MS medium at either half-strength or full-strength salt concentrations demonstrate a significant superiority of the half-strength medium in rooting percentage and root number (Tables 3 and 4); however, the salt concentration did not significantly affect root length (Table 5). On the other hand, IBA concentrations added to the medium at 1.5 mg L^{-1} resulted

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in a significant increase in the rooting percentage and the number of roots (Tables 3 and 4), whereas no significant differences were found in root length. Furthermore, the interaction between half-strength MS salts and 1 mg L^{-1} IBA yielded the best result for rooting percentage (Table 3). The addition of 1.5 mg L^{-1} IBA to the half-strength medium significantly outperformed most interaction treatments in comparison with the full-strength medium in the number of roots (Table 4). While most interaction treatments did not differ significantly from one another regarding root length (Table 5). It was observed that the addition of IBA at various concentrations stimulated root formation in shoots produced from multiplication stage, with root numbers increasing with IBA concentration. This response may be attributed to the effect of the half-strength salt medium in enhancing rooting characteristics (specifically, rooting percentage and root number); the significant increase in the rooting percentage of shoots cultured on half-strength MS medium compared with half strength because of reduced the nitrogen to carbohydrate ratio to a level that provided optimal conditions for rooting compared to the other media tested [36]; [4]. [3] indicated that the rooting response of shoots may be attributed to optimal conditions created by the interaction between the media's salts and the auxin concentration added for rooting, which subsequently promoted root initiation and elongation. This increase may be attributed to IBA activity resulting from hydrolysis and the translocation of carbohydrates and proteins at the cellular level to the bases of the shoots, which increase cell elongation and division under favorable environmental conditions and stimulates the activity of root-primordium cells [30]. The effect of IBA on root formation and development may be attributed to its role in stimulating cell division and elongation in the initial stages of growth and development for any plant organ as well as its role in promoting xylem differentiation. Xylem plays a key role in transporting nutrients from the growth medium to the leaves, where minerals are processed and converted into organic compounds (carbohydrates, lipids, and proteins) that are subsequently utilized in vital processes driving plant growth and development [27]. [34] reported that the differentiation of cortical ray parenchyma cells into root primordia depends on the type and concentration of auxin. Furthermore, [10] and [41] indicated that adding IBA to the medium stimulates cell division, leading to the formation of root primordia.

Conclusion:

From the results, we concluded that to propagate blackberry *Rubus* spp. In MS, supplementing media with 3 mg L^{-1} of BA for both (initiation and multiplication stages), which achieved the best results. The best strength of salt and IBA concentration for all rooting characteristics was the half strength, interacting with 1.5 mg L^{-1} IBA.

Recommendation:

Due to the results of this study, we recommend studying the effect of other cytokinins like Kin, either alone or in combination with auxins or gibberellins, for establishing nodes or multiplying shoots. Use another type of auxin, such as NAA and IAA, in higher concentrations to stimulate the rooting of shoots In vitro.

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