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DEVELOPING A COST-EFFECTIVE MICROPREDATION PROTOCOL FOR TWO CARNATION (*Dianthus Caryophyllus* L.) VARIETIES UNDER *IN VITRO* CONDITIONS

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ABSTRACT:

A successful attempt was made through this investigation to develop a cost-effective protocol for two varieties of carnation (*Dianthus caryophyllus* L.) ornamental plants named Clove Pink and Scarlet. Healthy and vigorous cultures were established by inoculating axillary buds on MS medium after a successful disinfestation process by sterilizing in 2.5% NaOCl for 10 minutes. The microshoots were multiplied by testing different benzyladenine (BA) and kinetin concentrations. Luckily, the best multiplication parameters were achieved with MS medium free of growth regulators, which will reduce the propagation costs. The higher number of shoots (11.60 shoots/ explant), leaves (59.80 leaves/ explant), and longest shoots (10.90 cm) when grown on MS medium without Plant growth regulators (PGRs), Additionally, the clove pink variety on MS medium with 1.5 mg l^{-1} BA and Scarlet variety grown on MS-free of PGRs respectively. A 100% percent rooting was achieved for both varieties when 1.5 mg l^{-1} Naphthalene acetic acid (NAA) was added to the culture media. The same NAA concentration for scarlet variety microshoots resulted in the highest number of roots (26.2 roots/explant) and the longest roots (9.0 cm). Successful acclimatization was observed by transferring the well-rooted plantlets to greenhouse conditions while putting them in peat moss.

KEYWORDS: Tissue Culture, Culture Media Carnation, *Dianthus Caryophyllus* L., Cost-Effective Protocol, Micropropagation

1. INTRODUCTION

Carnation (Dianthus caryophyllus L.) is considered as a widely favored type of cut flower globally, celebrated for its diverse color palette, exceptional longevity, and various forms Carnation the wild carnation is found in the Mediterranean countries of mainly to Europe and Asia, with a few species in north Africa and in southern Africa, and one species (D. repens) in arctic North America such as Portugal, Spain, Italy, Croatia, Albania, Greece and Turkey. Pharmacological studies revealed that the plant possessed anticancer, antiviral, antibacterial, antifungal, insecticidal, repellent, antioxidant, reno-protective, anesthetic and analgesic effects. The current review highlights the chemical constituents and pharmacological effects of Dianthus caryophyllus and belongs to the taxonomic family Caryophyllaceae. It has been extensively cultivated for the last 2,000 years. It is an herbaceous perennial plant growing to 80 cm tall. The leaves are glaucous greyish green to blue-green, slender, up to 15 cm long (Ali *et al*. 2008, Kanwar and Kumar 2009). The majority of decorative plants in the Kurdistan Region of Iraq are usually imported from abroad, especially from Turkey and the Netherlands. This import cost a huge amount of local and foreign currency. This research was designed to be an initial step towards mass production of carnation ornamental plants in the Duhok governorate and then distributed to the local nurseries to meet the local market demands. Plant tissue culture techniques have been employed to produce virus-free plants, facilitate commercial mass production, enable genetic engineering, and enhance somaclonal variation (Brar *et al*., 1995). Elements like the culture environment and plant growth regulators (PGRs) are chemicals that regulate plant development and are active at low doses. Natural regulators, which are produced by the plant itself, exist alongside synthetic regulators; the latter are known as phytohormones or plant hormones, and explant type significantly influence *in vitro* shoot regeneration in carnation (Kanwar and Kumar, 2009). The explants usually used in the literature were axillary buds and shoot tips (Salehi 2006). Tissue culture-derived plants maintain true-to-type characteristics, as the micropropagation process occurs without a callus phase (Brar *et al.,* 1995). Earlier research has shown that optimal multiplication rates for carnations occur within a cytokinin concentration range of 0.5 to 3.0 mg l^{-1}, varying by variety. Various cytokinins, including benzyladenine (BA), Kinetin, thidiazuron (TDZ), and 2-isopentenyladenine (2ip), have been utilized (Brar *et al*. 1995, Kovac 1995, Mujib, Pal 1995, Salehi 2006 and Ali *et al*. 2008).

For rooting of carnation explants, most of the previous studies found that IBA was the most frequently auxin used to enhance its *in vitro* rooting (Ahmadian *et al.*, 2017; Samir, 2017). For carnation acclimatization, Ahmadian *et al*. (2017), noted that after carefully washing the plantlets were taken from the rooting medium, potted in a 1:1 blend of coco peat and perlite, and then placed directly in a greenhouse. During the initial days, the plants were misted to maintain a relative humidity of 90%. This research aims to create a dependable and efficient

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micropropagation method for carnation ornamental plants., thereby establishing a local source for the mass propagation of this vital cut flower in the Duhok Governorate.

2. MATERIALS AND METHODS

This research was conducted in the plant tissue culture lab within the Horticulture Department at the College of Agricultural Engineering Sciences at the University of Duhok. Two varieties of carnations, clove pink and scarlet, were selected for the experiment. Plant samples were collected from mature plants at a local nursery in Duhok City during early spring.

Explant disinfestations were done by thoroughly washing under tap water for 40 minutes, then rinsing in 2.5% NaOCl, followed by three washes with sterilized distilled water, each lasting 5 minutes (Eshoa, and Danial, 2023). The culture medium utilized was MS medium with added supplements. with growth regulators according to the developmental stage. The pH was adjusted to 5.7 after incorporating 30 $g.l^{-1}$ of sucrose as a source of carbon and 7 $g.l^{-1}$ of agar to solidify the culture medium. The whole culture medium was sterilized by autoclave for 20 minutes at 121°C and 1.04 $kg.m^2$ pressure. Three explants were inoculated in each culture vessel, and 5 vessels represented each treatment. The cultures were maintained in a growth room at a temperature of 25±2 °C under a light cycle of 16 hours of light and 8 hours of darkness with 100 feet. $candle^{-1}$ light intensity.

At the establishment stage, the axillary buds were excised and cultured as explants in an MS medium free of growth regulators. The produced microshoots were transferred to the multiplication culture medium enriched with varying concentrations of BA and kinetin (0.0, 1.5, 2.0, 2.5, and 3.0 mg. l^{-1}). After one month, the number of shoots and leaves / explant, as well as the average shoot length was recorded. For the rooting phase, the microshoots were moved to rooting culture media containing various concentrations of IBA and NAA (0.0, 0.5, 1.0, 1.5, and 2. $0mg.l^{-1}$). After another four weeks, the rooting percentage, the number of roots per explant, and the average root length were assessed by way of rooting parameters. The well-rooted sprouts were gradually moved to the greenhouse for acclimatization by putting them in peat moss. The experiment was arranged as factorial based on completely randomized design (CRD) After 6 weeks of incubation, were recorded per explants. Data were analyzed using SAS software Ver.9.1. The means of different treatments were compared using Duncan's Multiple Range Tests at the 5 % probability level using a computerized program of SAS (SAS, 2001 and Duncan, 1955).

3. RESULTS AND DISCUSSION

Overall, the results of this experiment showed that this new ornamental plant can be micro-propagated through tissue culture techniques, facilitating mass production locally to satisfy nursery demand. During the establishment stage, healthy and vigorous cultures were obtained following an effective disinfestation process (Figure 1).

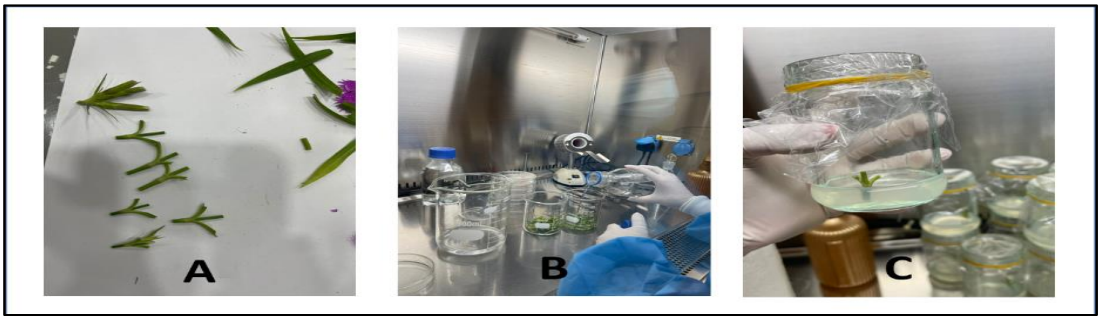

Figure 1: Initiation Stage of Carnation explants MS medium. A; Explants preparation. B; Explants disinfestation. C; Initially cultured explant on MS medium

At shoot multiplication stage, Table (1) shows that clove pink variety was superior upon scarlet variety, producing an average of 4.78 leaves per explant. On other hand, MS medium free of PGRs gave the highest Number reached to 7.60 leaves/ explant as compared to the all concentrations of BA and kinetin. Also, clove pink variety with MS free of PGRs produced the highest number of shoots, averaging 11.60 shoots / explant.

Table1: Effect of BA and Kinetin on the number of shoots per explant of carnation plant after 4 weeks in culture on MS medium

Cytokinins (mg. l^{-1})	Number of shoots/ explants		Means of BA (mg. l^{-1})
	Clove pink variety	Scarlet variety	
BA (0.0)	11.60 a	3.60 e	11.60 a
BA (1.5)	5.20 c	8.20 b	5.20 c
BA (2.0)	5.00 c	5.80 c	5.00 c
BA (2.5)	3.40 e	3.00 e	3.40 e
BA (3.0)	4.00 d	4.20 d	4.00 d
	Means of Kinetin (mg. l^{-1})		
Kinetin (1.5)	2.20 f	1.60 g	2.20 f
Kinetin (2.0)	3.80 e	2.40 f	3.80 e
Kinetin (2.5)	3.00 e	2.80 f	3.00 e
Kinetin (3.0)	4.80 c	1.60 g	4.80 c
Means of Verities	4.78 a	3.69 b	

Different letters in the same column indicate significant differences between means according to Duncan's new multiple range test at P≤ 0.05.

Table (2) shows the impacts of BA and kinetin on the count of leaves in carnation explants. The results show that clove pink variety was also superior upon scarlet variety by producing 35.29 leaves/ explant. The treatment with 1.5 mg. l^{-1} BA was the most effective, resulting in 46.70 leaves per explant. Furthermore, the clove pink variety treated with 1.5mg. l^{-1} BA showed significantly higher results than the other treatments, except for the 2.0 mg. l^{-1} BA treatment with the same variety (Figure 2)

Table 2: Effect of BA and Kinetin on the number of leaves per explant of carnation plant after 4 weeks in culture on MS medium

Cytokinins (mg. l^{-1})	Number of leaves/explants		Means of BA (mg. l^{-1})
	Clove pink variety	Scarlet variety	
BA (0.0)	21.6 d	22.4 d	22.00 c
BA (1.5)	59.80 a	33.60 c	46.70 a
BA (2.0)	58.40 a	34.60 c	46.50 a
BA (2.5)	39.80 b	19.40 d	29.60 b
BA (3.0)	42.00 b	22.40 d	32.00 b
	Means of Kinetin (mg. l^{-1})		
Kinetin (1.5)	23.20 d	9.40 f	16.30 cd
Kinetin (2.0)	31.20 c	8.60 f	19.80 cd
Kinetin (2.5)	13.00 e	13.40 e	13.20 d
Kinetin (3.0)	27.60 cd	8.00 f	17.80 cd
Means of Verities	35.29 a	19.09 b	

Different letters in the same column indicate significant differences between means according to Duncan's new multiple range test at P≤ 0.05

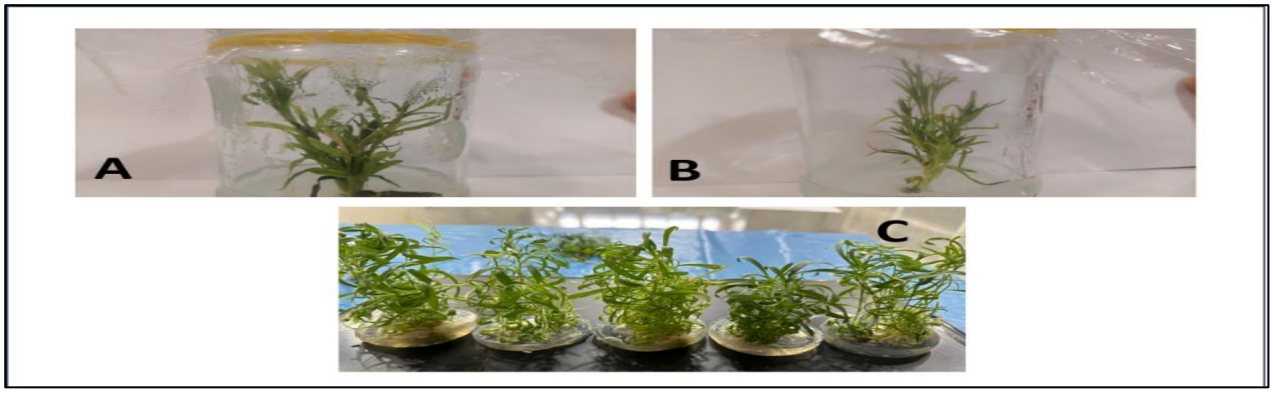

Figure 2: Shoot Multiplication Stage of Carnation. A; Clove pink Variety after four weeks in MS culture medium. B: Scarlet Variety after four weeks in MS culture medium. C; Clove pink Variety from left to right;(0.0,1.5,2.0,2.5 and 3.0 mg. l^{-1} BA)

Table 3 illustrates the impact of benzyl adenine and kinetin on the average length of shoot. Both varieties showed no significant differences in this regard. Notably, the control treatment without cytokinins produced the longest shoots, measuring 8.00 cm, which were significantly longer than those from the other treatments. The scarlet variety, under control conditions, achieved the longest length at 10.90 cm.

Table3: Effect of BA and Kinetin on the mean length of shoots of carnation plant after 4 weeks in culture on MS medium

Cytokinins (mg. l^{-1})	Mean length of shoots (cm)		Means of BA (mg. l^{-1})
	Clove pink variety	Scarlet variety	
BA (0.0)	5.10 d	10.90 a	8.00 a
BA (1.5)	7.60 b	5.65 d	6.63 b
BA (2.0)	5.70 d	5.70 d	5.70 c
BA (2.5)	4.10 e	6.20 c	5.15 c
BA (3.0)	5.70 d	4.30 e	5.00 c
	Means of Kinetin (mg. l^{-1})		
Kinetin (1.5)	3.30 f	5.00 de	4.15 d
Kinetin (2.0)	4.80 e	4.80 e	4.80 bc
Kinetin (2.5)	4.80 e	4.40 e	4.60 bc
Kinetin (3.0)	5.90 d	3.40 e	4.65 bc
Means of Verities	5.23 a	5.60 a	

Different letters in the same column indicate significant differences between means according to Duncan's new multiple range test at P≤ 0.05

The capacity of cytokinins to free lateral buds from the domination of terminal buds without removing the apical bud is the main factor responsible for their advantageous function in shoot multiplication. The cytokinns have no influence on shoot multiplication on this type of plant because it is a positive result because it contains a sufficient concentration of cytokinins, which leads to no response to the hormone. Its occurrence is rather species specific occurring easily in some species (Gonbad *et al,.*2014)

This affect enhances the flow of nutrients and water that support growth of lateral buds by improving the creation of vascular tissues in the buds (Toma and Tamer, 2016). Additionally, cytokinins play a critical role in boosting RNA, protein, and enzyme activity within the cells, further supporting bud growth (Fadaladeen *et al*., 2022). The genetic makeup of the cultured explants also affects their response due to its influence on endogenous hormone levels, which may explain the superiority of the control treatment (Pandey *et al.,* 2024).

At the rooting stage, different concentrations of two auxins were tested (Figure 3). The effects of NAA and IBA are seen in table (4) on the rooting percentages of the microshoots produced during the multiplication stage. In general, both varieties rooted well, with a high rooting percentage reaching 92.67% and 91.12% for Clove pink and scarlet varieties, respectively. However, the addition of 1.0 mg. l-1 NAA proved to be especially effective, resulting in a 100% success rate for rooting. Accordingly, Clove pink and scarlet varieties treated with the same NAA concentration gave the highest rooting percentage (100%).

Table 4: Effect of IBA and NAA on the rooting percentage of carnation plant after 4 weeks in culture on MS medium

Auxins (mg. l^{-1})	Rooting Percentage		Means of IBA (mg. l^{-1})
	Clove pink variety	Scarlet variety	
IBA (0.0)	90	90	90.00
IBA (0.5)	90	95	92.50
IBA (1.0)	90	95	92.50
IBA (1.5)	93	70	81.50
IBA (2.0)	93	100	96.50
	Means of NAA (mg. l^{-1})		
NAA (0.5)	95	70	82.50
NAA (1.0)	100	100	100.00
NAA (1.5)	93	100	96.50
NAA (2.0)	90	100	95.00
Means of Verities	92.67	91.12	

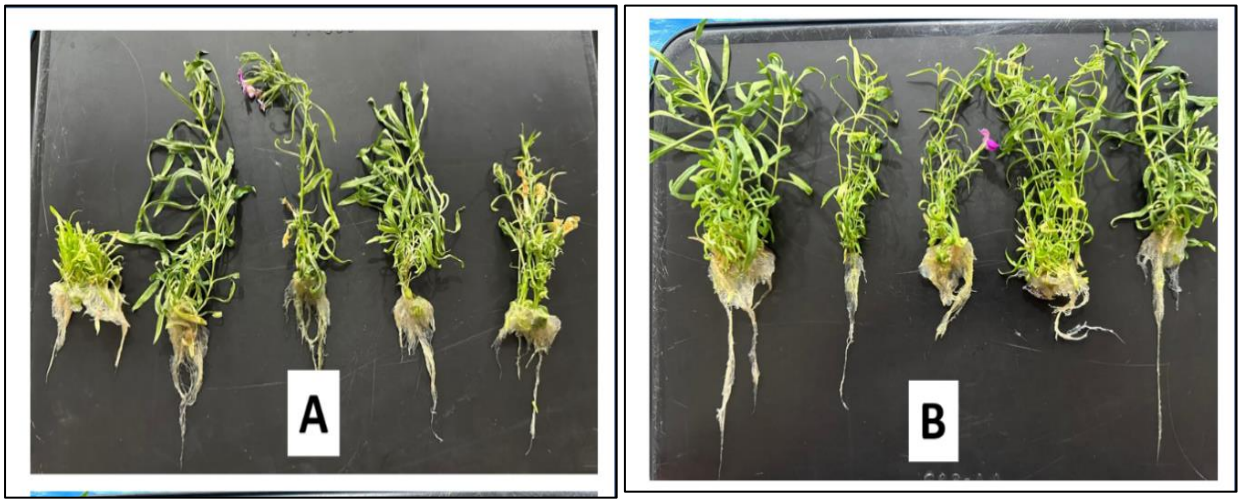

Figure 3: Root Formation Stage of Carnation explants on MS medium. A; Rooting of Clove pink Variety after four weeks in MS culture medium enriched with 0.0,0.5,1.0,1.5 and 2.0 mg. l^{-1} NAA. B; Rooting of Scarlet Variety after four weeks in MS culture medium enriched with 0.0,0.5,1.0,1.5 and 2.0 mg. l^{-1} NAA

Table 5 presents the effects of IBA and NAA on the number of roots per explant. The scarlet variety outperformed the clove pink variety, producing an average of 15.47 roots per explant. Adding 2.00 mg. l-1 IBA and 1.5 mg. l-1 of NAA resulted in the highest root counts, with 21.60 and 20.10 roots per explant, respectively, both significantly surpassing the other auxin concentrations. The best dual combination treatment was the superior IBA and NAA concentration with the scarlet variety by recording 25.40 and 26.20 roots per explant, respectively.

Table 5: Effect of IBA and NAA on the number of roots per explant of carnation plant after 4 weeks in culture on MS medium

Auxins (mg. l^{-1})	Number of roots per explant		Means of IBA (mg. l^{-1})
	Clove pink variety	Scarlet variety	
IBA (0.0)	13.00 d	8.40 f	10.70 cd
IBA (0.5)	8.20 f	24.20 a	16.20 b
IBA (1.0)	14.80 d	10.20 e	12.50 c
IBA (1.5)	20.60 b	15.00 c	17.80 b
IBA (2.0)	17.80 c	25.40 a	21.60 a
	Means of NAA (mg. l^{-1})		
NAA (0.5)	3.20 f	15.80 c	8.00 e
NAA (1.0)	13.80 d	13.60 d	13.70 c
NAA (1.5)	14.00 d	26.20 a	20.10 a
NAA (2.0)	9.40 e	14.00 d	11.70 d
Means of Verities	12.76 b	15.47 a	

Different letters in the same column indicate significant differences between means according to Duncan's new multiple range test at P≤ 0.05

Table 6 details the impact of IBA and NAA on the average root length of carnation microshoots after four weeks in culture. Again, the scarlet variety excelled, producing the longest average root length at 6.86 cm, compared to just 4.37 cm for the clove pink variety. Treatments with 1.5 and 2.0 mg. l-1 IBA as well as 1.5 mg. l-1 NAA also yielded long roots, measuring 6.75, 6.95, and 7.15 cm, respectively. The optimal dual treatment of 2.0 mg l-1 IBA and 1.5 mg. l-1 NAA to the Scarlet variety was the best dual combined treatment, recording 8.60 cm and 9.00 cm, respectively.

Table 6: Effect of IBA and NAA on the mean length roots per explant of carnation plant after 4 weeks in culture on MS medium

Auxins (mg. l^{-1})	Mean length of roots (cm)		Means of IBA (mg. l^{-1})
	Clove pink variety	Scarlet variety	
IBA (0.0)	5.30 e	6.50 cd	5.90 b
IBA (0.5)	2.60 h	7.00 c	4.80 c
IBA (1.0)	3.70 g	4.30 ef	4.00 c
IBA (1.5)	5.50 e	8.00 b	6.75 a
IBA (2.0)	5.30 e	8.60 a	6.95 a
	Means of NAA (mg. l^{-1})		
NAA (0.5)	3.80 g	6.40 d	5.10 bc
NAA (1.0)	4.60 ef	7.40 c	6.00 b
NAA (1.5)	5.30 e	9.00 a	7.15 a
NAA (2.0)	3.20 g	4.50 ef	3.85 d
Means of Verities	4.37 b	6.86 a	

Different letters in the same column indicate significant differences between means according to Duncan's new multiple range test at P≤ 0.05

The findings of this study confirm that auxins play an important part in the rooting process by promoting the initiation of adventitious roots from cultured shoots (Toma, 2019). Generally, Propagation technology *in vitro* is more costly as opposed to traditional Methods for propagating plants, with the cost of premixed growth media varying depending on the type and additional ingredients.

During the acclimatization stage, all produced carnation plantlets successfully underwent a 100% hardening process by being grown in pots filled with peat moss in a greenhouse (Figure 4). No abnormal growth was observed in the plantlets under greenhouse conditions.

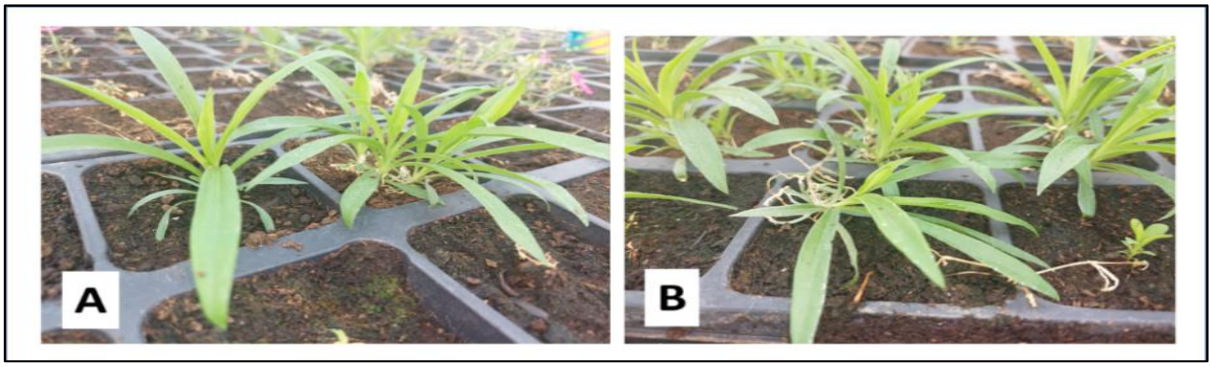

Figure 4: Acclimatization Stage of Carnation plantlets after two weeks under greenhouse conditions in peatmoss..A,;love Pink variety.B; Scarlet Variety

Conclusion

As a general conclusion, this study demonstrated that MS medium without growth regulators yielded favorable results during the multiplication stage. Additionally, culture media with very low auxin concentrations showed strong rooting performance. This suggests a cost-effective protocol as the commercial widespread propagation of this significant ornamental plants.

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Author Contributions:

L.R.M., worked on Methodology, Formal Analysis. R.S.T., Supervision, Writing Original Draft, Visualization. F.K.Y., Data analysis.

Ethical Statement

Not required. This study did not involve any human or animal experimentation.

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