Original Article

Effect of Phenylacetic Acid (Paa) and Adenine Sulfate on In Vitro Propagation of Eustoma Grandiflorum

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Abstract

The study was conducted in the Plant Tissue Culture Laboratory, Department of Horticulture and Landscape Gardening, College of Agricultural Engineering Sciences, University of Baghdad, from October 2024 to January 2025. It aimed to determine the effect of BA, PAA, IAA, IBA, and adenine sulfate (SA) on the tissue culture propagation of *Eustoma grandiflorum*. The study involved three experiments, i.e., multiplication, rooting and acclimatization. The results showed that the 1 mg L^{-1} BA treatment combined with 0.5 mg L^{-1} PAA was superior, giving the highest average number of shoots per explant at 18, while the 0.5 mg L^{-1} BA and 0.5 mg L^{-1} IAA concentration gave the highest at 5.6 shoots per explant. As for branch length, the 0 mg L^{-1} BA concentration interacting with 1 mg L^{-1} PAA gave the highest average length, reaching 4 cm., while the control treatment (0 mg L^{-1} BA and 0 mg L^{-1} IAA) gave the maximum shoot length of 2 cm. For adenine sulfate, the concentration of 80 mg L^{-1} highest of shoots (22 shoots. explant^{-1}), shoot length (2.4 cm), and leaf chlorophyll content (2.20). The best medium for shoot rooting was the ½ MS prepared with 0.5 mg L^{-1} PAA, as it gave the highest rooting percentage of 70% and the highest average number of roots of 8.5 roots. shoot^{-1}, while the ½ MS prepared with 0.5 mg L^{-1} IBA gave a rooting percentage of 50% and 5 roots. shoot^{-1}. As for acclimatization, the highest acclimatization rate, 100%, was achieved using a medium of peat moss and perlite at 1:1 size ratio.

Keywords: Eustoma grandiflorum, In vitro SA, BA, IBA, IAA, PAA, micro propagation

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Introduction

Lisianthus (*Eustoma grandiflorum*) is one of the world's most important cut flowers. It belongs to the Gentianaceae family and is an annual or perennial herbaceous plant, sometimes perennial, depending on the species (29). It is native to central North and South America, where it grows wild, as well as in the prairies of Nebraska and Texas (23,27). Spain, the Netherlands, France, Portugal, and Italy are the leading producers. The plants are characterized by the beauty of their flowers, their diverse colors and shapes, similar to those of roses, and their ability to remain on the plant (26). The flower life span (vas life) is up to two weeks, and the plant may continue to produce flowers for up to five weeks (8,21). In general, a greater stem diameter is associated with longer vas live of cut flowers. Therefore, flower life is related to the diameter and strength of the stem (22, 13). It can be used as cut flowers or potted plants (29). It is classified among the top ten cut flowers traded in the flower market, and therefore demand for it has increased in recent years.

The plant is propagated in two ways. The first is vegetatively by terminal cuttings, which are limited in number and are difficult to obtain (12). The second method, sexual propagation by seed, is flawed by its slow germination and weak seed growth, as well as its small size, which makes it difficult to handle (28). Furthermore, plants produced by sexual propagation are heterogeneous in their growth, flowering time, and quality (18). Therefore, plant propagation using tissue culture has become increasingly popular as it produces plants that are difficult to propagate and produces large

numbers of homogeneous plants from a small plant section within a short period of time and in a small area, in addition to extending the season (12). One of the most important factors influencing plant growth in tissue culture is growth regulators, as their effect is determined by the balance between the added growth regulators and the plant hormones within the plant (30).

The most widely used growth regulators are auxins and cytokinin's, and the interaction between them is the most important for regulating plant growth and development in tissue culture, because auxins work synergistically with cytokinin's (4). Among the auxins used in tissue culture, PAA (phenylacetic acid) is a natural auxin derived from phenylalanine. It is found in many plants, and most organs accumulate much higher levels of PAA than IAA. For example, in the mouse ear plant, the PAA content ranges from 200 to 3500)pmol/g FW) fresh weight, depending on the organ, which is higher than IAA, which is 50)pmol/g FW) fresh weight (24, 31,16). In general, PAA levels in plants are several times higher than IAA. The main activity of PAA auxin is to stimulate lateral roots, promote root growth, and stimulate callus formation. and elongation) (25). In addition, it has an antimicrobial and antibacterial role Using high concentrations of PAA increases the plant's protection against pathogens (25, 32,14).

Among the compounds that influence the multiplication and growth of tissue shoots is adenine sulfate ($C_{10}H_{12}N_{10}SO_4$), which is added with cytokinin's and serves as a precursor to the cytokinin biosynthesis pathway (20).

Based on the above, the aim of this research was to study the effect of BA, PAA, IAA, and adenine sulfate on shoot multiplication, and to compare it with the auxin IBA on shoot rooting.

Materials and Methods

The study was conducted in the Plant Tissue Culture Laboratory, Department of Horticulture and Landscape Architecture, College of Agricultural Engineering Sciences, University of Baghdad, from October 2024 to January 2025. Flowers of the *Eustoma grandiflorum* (Lisianthus) white variety were taken from a private flower shop. MS medium from HiMedia was used, weighing 4.9 g L^{-1}, and 30 g L^{-1} of sucrose was added. Growth regulators, auxins and cytokinin's, were prepared in the form of stock solutions. The pH was then adjusted to 5.7 ± 1 by adding drops of NaOH or HCl. The volume was then increased to 1000 ml, and agar-agar, weighing 7 g L^{-1}, was added to solidify the medium. The medium was then sterilized in an autoclave at 121 °C. for 15 minutes and a pressure of 1.04 kg cm^{-2}. The agricultural tools were sterilized in a dry heat oven for four hours. Distilled water was sterilized in an autoclave at 121 °C for 30 minutes before use.

Initiation stage

The plant parts (single node) were planted after sterilizing them with commercial bleach (6%) at a concentration of 5 ml 100 ml for 10 minutes, then planted on MS medium supplied with 0.5 mg L^{-1} BA with 0.1mg L^{-1} PAA, and after 6 weeks they were transferred to the multiplication stage.

Multiplication stage

Effect of the interaction between BA, PAA, and IAA, each individually, on the multiplication of plant shoots.

The resulting shoots were taken from the best treatment from the emergence stage and grown on MS medium supplemented with different concentrations of BA (0, 0.5, 1, and 2) mg L^{-1}, in combination with the auxins PAA and IAA at concentrations of 0, 0.25, 0.5, 1, and 1.5 mg L^{-1} (10), each separately. After six weeks, the number and length of shoots were calculated.

Effect of adenine sulfate on shoot multiplication.

The resulting shoots were taken from the best treatment during the multiplication phase and planted on MS medium supplemented with different concentrations of adenine sulfate (0, 40, 80, 120, and 160 mg L^{-1}) (19). After six weeks, the number and length of shoots were counted, and chlorophyll pigment was measured.

Rooting stage

1. Effect of MS medium strength, PAA concentrations, and their interaction on shoot rooting.

The resulting shoots were taken from the best treatment during the multiplication phase and planted on MS medium at full and half strength supplemented with different concentrations of PAA (0, 0.1, 0.3, 0.5, and 1 mg L^{-1}), plus 30 g L^{-1} of sugar.

2. Effect of MS medium strength, IBA concentrations, and their interaction on shoot rooting.

The resulting shoots were taken from the best treatment during the multiplication phase and planted on MS medium at full and half strength, supplemented with

different concentrations of PAA (0, 0.1, 0.3, 0.5, and 1) mg L^{-1}, supplemented with 30 g L^{-1} sugar. The results were then obtained 6 weeks after planting, representing the rooting percentage and root number, as follows:

Rooting percentage = number of rooted shoots/total number × 100

The plants were incubated for all experiments in a growth chamber at 23 ± 2 °C for a light: darkness period of 16:8 hours at a light intensity of 1000 lux.

Statistical analysis

All results were analyzed using a completely randomized design (CRD) with one and two factors, and 10 replications for each treatment. The results were analyzed using the GenStat statistical program to study effects of different factors and their interactions on the studied traits. Averages were compared using the least significant difference (LSD) at a 5% probability level.

Results and Discussion

Multiplication Stage

Effect of BA, PAA, and their interaction on the average shoot number of lisianthus plants in vitro

Table 1 shows that BA concentrations significantly increased shoot number, with the 0.5 mg L^{-1} concentration being superior to the others, giving the highest average of 11.14 shoots. explant^{-1}, which differed significantly from the other concentrations. The 1 mg L^{-1} concentration produced the best result, giving the highest average number of shoots, reaching 11.37 shoots. explant^{-1}, which differed significantly from the other concentrations. For the two-way interaction between BA and PAA, the results show that the interaction treatment of 1 mg L^{-1} BA and 0.5 mg L^{-1} PAA excelled, giving the highest average number of shoots at 18 shoots. explant^{-1}, which differed significantly from the other treatments (**Figure 1**).

Table 1. Effect of BA, PAA, and their interaction on the average number of shoots of the lisianthus plants (shoot. explant^{-1}) after 6 weeks of culture on the MS medium *in vitro*

BA (mg. l^{-1})	PAA (mg. l^{-1})					average BA
	0	0.25	0.5	1	1.5	
0	2.00	2.00	2.00	1.00	1.00	1.60
0.5	5.00	12.40	11.00	15.00	12.30	11.14
1	5.00	11.20	18.00	15.50	11.00	12.14
2	3.00	11.00	11.20	14.00	8.00	9.44
LSD			1.49			LSD 0.67
average NAA	3.75	9.15	10.55	11.37	8.07	
LSD			0.74			

$LSD_{0.05}$ BA: 0.67; $LSD_{0.05}$ PAA: 1.49; $LSD_{0.05}$ interaction: 0.74

Effect of BA, PAA, and their interaction on the average shoot length of lisianthus plants in vitro

The results in **Table 2** show that the different concentrations of BA significantly reduced average shoot lengths. The 0 mg L^{-1} BA concentration (the control treatment) produced the highest average shoot length of 2.78 cm, which differed significantly from the other concentrations. As for the PAA concentrations, the 1 mg L^{-1} concentration significantly excelled in the 1 mg L^{-1} concentration, producing the highest average shoot length of 2.88 cm. This did not significantly differ from the 1.5 mg L^{-1} concentration, which produced an average shoot length of 2.68 cm, though it did for the other concentrations. For the interaction between BA and PAA concentrations, the 0 mg L^{-1} BA and 1 mg L^{-1} PAA concentrations outperformed the 0 mg L^{-1} BA and 1 mg L^{-1} PAA, producing the highest average shoot length of 4 cm, which significantly excelled from the other treatments.

Table 2. Effect of BA, PAA and their interaction on the shoot length rate of lisianthus plant (shoot of explant^{-1}) after 6 weeks of culture on the MS medium *in vitro*

BA (mg. l^{-1})	PAA (mg. l^{-1})					average BA
	0	0.25	0.5	1	1.5	
0	2.0	2.2	2.7	4.0	3.0	2.78
0.5	1.5	2.2	1.8	3.0	3.3	2.36
1	1.4	2.0	1.6	2.5	2.6	2.02
2	1.1	2.0	2.3	2.0	1.8	1.84
LSD 0.05			0.75			0.34
average PAA	1.50	2.10	2.10	2.88	2.68	
LSD 0.05			0.37			

$LSD_{0.05}$ BA: 0.75; $LSD_{0.05}$ PAA: 0.34; $LSD_{0.05}$ interaction: 0.37

Figure 1. Multiplication of lisianthus plant shoots grown on MS medium supplemented with 0.5 mg L^{-1} BA and 1.5 mg L^{-1} PAA concentrations after 6 weeks of culture

Effect of BA and IAA and their interaction on the average number of shoots of the lisianthus plant in vitro

The results in **Table 3** show a significant difference in BA concentrations on the number of shoots produced. The 0.5 mg L^{-1} concentration excelled, giving the highest average number of shoots (5.22 shoots. explant^{-1}, which did not differ much from the 1 mg L^{-1} concentration which gave 4.98 shoots. explant^{-1}, but significantly different from the other treatments. As for effect of IAA, the 0.25 mg L^{-1} concentration was superior producing the highest average number of shoots (4.28 shoots. explant^{-1}), not significantly different from the 4.05 shoots. explant^{-1} for the 0.5 mg L^{-1} concentration, and the 0.5 and 1 mg L^{-1} concentrations at 4.05 and 3.93 shoots. explant^{-1}, respectively but differing considerably from the other concentrations.

For the interaction treatments, that of the 0.5 mg L^{-1} BA and IAA interaction was superior giving the highest average number of shoots at 5.6 shoots per explant^{-1}. This was not significantly different from most of the treatments (0.5 mg L^{-1} BA in interaction with all IAA concentrations, as well as 1 mg L^{-1} BA in interaction with all IAA concentrations except 1.5 mg L^{-1}), while differing significantly from the others.

Table 3. Effect of BA, IAA and their interaction on the number of shoots of the lisianthus plant (shoot. explant^{-1}) after 6 weeks of culture on the MS medium *in vitro*

BA (mg. l^{-1})	IAA (mg. l^{-1})					average BA
	0	0.25	0.5	1	1.5	
0	2.0	2.0	1.0	1.0	1.0	1.40
0.5	5.0	5.0	5.6	5.4	5.1	5.22
1	5.0	5.5	5.1	4.8	4.5	4.98
2	3.0	4.6	4.5	4.5	4.0	4.12
LSD 0.05			0.80			0.36
average IAA	3.75	4.28	4.05	3.93	3.65	
LSD 0.05			0.40			

$LSD_{0.05}$ BA: 0.80; $LSD_{0.05}$ IAA: 0.36; $LSD_{0.05}$ interaction: 0.40

Effect of BA, IAA, and their interaction on the average shoot length of lisianthus plants in vitro

Table 4 shows significant differences between BA concentrations on average shoot length. The concentration above 0 mg L^{-1} produced the highest average of 1.74 cm, which differed significantly from the other concentrations. As for IAA concentrations and the interaction coefficients between BA and IAA, there were no significant differences between the treatments.

Table 4. Effect of BA, IAA, and their interaction on the average shoot length of lisianthus plants (1 explant shoot) after 6 weeks of culture on the MS medium *in vitro*

BA (mg. l^{-1})	IAA (mg. l^{-1})					average BA
	0	0.25	0.5	1	1.5	
0	2.0	1.5	1.7	1.8	1.7	1.74
0.5	1.5	1.3	1.0	1.2	1.2	1.24
1	1.4	1.0	1.2	1.4	1.2	1.24
2	1.1	1.0	1.2	1.2	1.1	1.12
LSD 0.05			NS			0.19
average IAA	1.50	1.20	1.28	1.40	1.30	
LSD 0.05			NS			

$LSD_{0.05}$ BA: 0.19; $LSD_{0.05}$ IAA: NS; $LSD_{0.05}$ interaction: NS

The increase in the number of shoots is due to effect of cytokinin's, which stimulate cells to divide and differentiate by stimulating the synthesis of proteins and enzymes necessary for the process of division and cell differentiation. The higher number of shoots is attributed to effect of cytokinin's and the growth of lateral buds through their effect on the development of the vessels between the axillary buds and the main stem. This results in vegetative shoots (4). This depends on the internal balance between the auxin/cytokinin level. The higher the cytokinin level, the higher it breaks the apical dominance of auxin and stimulates the emergence of buds. The higher ratio of auxins to cytokinin's inhibits the growth buds. More cytokinin are used in BA tissue culture because they are stable compounds that do not decompose easily and are highly efficient, which leads to the expansion of the transporting vessels in the phloem tissues, stimulates cell division, increases DNA production, and prevents chlorophyll decomposition (17).

Increases in shoot lengths were observed. It was noted that the medium contains auxin, which inhibits lateral bud growth and stimulates apical bud growth and cell elongation. The role of auxins, including PAA auxin, interacts with cytokinin's. Auxin elongates cells and activates the division process by increasing cell wall flexibility and cell permeability, which increases cell size

and expansion (32). PAA auxin is superior to NAA, IAA, and IBA because it contains a single indole ring (6). The side chain is shorter and directly connected to the ring, increasing the effectiveness and activity of auxin, unlike other auxins which contain two rings and have longer apical chains. This is consistent with what was noted by (3.31)

Effect of adenine sulfate on the average number and length of shoots and leaf chlorophyll content (%) in lisianthus plants in vitro

The results in Table 5 shows significant differences in sulfate concentrations. Adenine (SA) concentration of 80 mg L^{-1} gave the highest average shoot count (22 shoots per explant), not significantly different from the 40 mg L^{-1} concentration's 19 shoots per explant, while differing significantly from the other concentrations. As for shoot length, the 0 mg L^{-1} concentration gave the highest average length of 3.3 cm, which did not significantly differ from the 40 mg L^{-1} concentration, which gave an average length of 3 cm, while differing significantly from the other concentrations. As for leaf chlorophyll content, the 80 mg L^{-1} concentration gave the highest at 2.20, significantly different from the other concentrations.

Table 5. Effect of adenine sulfate on the average number of shoots (shoot. explant^{-1}), shoot length (cm), and leaf chlorophyll content (%) of lisianthus plants after 6 weeks of culture on the MS medium *in vitro*

SA (mg. l^{-1})	Number of branches	Branch length	Chlorophyll
0	18	3.3	1.40
40	19	3.0	1.80
80	22	2.4	2.20
120	17	1.5	1.40
160	16	1.3	1.20

$LSD_{0.05}$: Number of branches = 3.80; Branch length = 1.13; Chlorophyll = 0.99

The increase in shoot number is attributed to the role of adenine sulfate, which is added with cytokinin's and serves as a precursor to the cytokinin biosynthesis pathway (17, 19). Sulfates contain adenine, the same nitrogenous base as cytokinin's. The presence of adenine plays a role in protein synthesis; adding adenine sulfate to the medium increase's natural endogenous hormones, including auxins which help stimulate lateral roots. As for the importance of adenine sulfate in the chlorophyll content of leaves, sulfur is the main component of amino acids, cystine and methionine, which are considered the basic building blocks for proteins associated with chlorophyll found leaf plastids. This agrees with (5,15, 20).

Rooting Stage

Effect of MS salt strength, PAA concentrations, and their interaction on rooting percentage

Table 6 shows the effect of different PAA concentrations on rooting percentage. The 0.5 mg L^{-1} concentration produced the highest rate of 60%, which differed significantly from the other concentrations. The control treatment failed to form roots. Regarding effect of salt strength, the use of ½ strength MS salt increased the rooting percentage by 40%, significantly different from MS strength salt, which gave 24%. For the interaction treatments, 0.5 mg L^{-1} PAA and ½ strength MS salt gave the highest rooting percentage, reaching 70%, the 0.1 mg L^{-1} PAA with MS strength salt concentration produced the lowest rooting percentage of 10%, while the control treatment (0 with all MS strengths) did not give any rooting percentage.

Table 6. Effect of MS salt strength, PAA concentrations, and their interaction on the rooting percentage of the lisianthus plants after 6 weeks of culture on the MS medium *in vitro*

PAA (mg. l^{-1})	MS salt strenght		average PAA
	MS ½	MS	
0	0	0	0
0.1	20	10	15
0.3	50	30	40
0.5	70	50	60
1	60	30	45
LSD 0.05	7.7		5.5
average MS	40	24	
LSD 0.05	3.5		

$LSD_{0.05}$ PAA: 7.7; $LSD_{0.05}$ MS: 5.5; $LSD_{0.05}$ interaction: 3.5

Effect of MS salt strength, PAA concentrations, and their interaction on the number of roots and shoots in lisianthus plants

The results in **Table 7** show that the different concentrations of PAA significantly affected root number. The 0.5 mg L^{-1} concentration gave the highest average root number, reaching 6.85 roots per shoot, significantly different from the other concentrations. The control treatment failed to produce roots. Regarding effect of MS salts, the ½ strength MS salt gave the highest average root number, reaching 4.4 roots per shoot, which differed significantly from MS strength salt, which produced 3.6 roots per shoot. For the dual interaction between PAA and MS salts, the 0.5 mg L^{-1} PAA concentration with ½ strength MS salt gave the highest average root number, reaching 8.5 roots per shoot, which differed significantly from the other treatments.

Table 7. Effect of MS salt strength, PAA concentrations and their interaction on the number of roots (root/$shoot^{-1}$) of the lisianthus plants after 6 weeks of culture on the MS medium *in vitro*

PAA (mg. l^{-1})	MS salt strenght		PAA average
	MS ½	MS	
0	0.00	0.00	0.00
0.1	2.00	3.00	2.50
0.3	6.30	2.10	4.20
0.5	8.50	5.20	6.85
1	5.20	5.00	5.10
LSD 0.05	0.85		0.60
average MS	4.40	3.60	
LSD 0.05	0.38		

$LSD_{0.05}$ PAA: 0.85; $LSD_{0.05}$ MS: 0.60; $LSD_{0.05}$ interaction: 0.38

Effect of MS salt strength, IBA concentrations, and their interaction on rooting percentage

The results in **Table 8** show the effect of different concentrations of IBA on rooting percentage. The 0.5 mg L^{-1} concentration gave the highest response rate of 40%, which differed significantly from the other concentrations. Meanwhile, the control treatment failed to produce roots. Regarding the effect of salt strength, ½ strength MS salt increased the rooting percentage by 26.4%, which differed significantly from the MS strength salt treatment at 16%. For the interactions, the 0.5 mg L^{-1} concentrations of IBA and ½ strength MS salt

gave the highest rooting percentage of 50%, while the control treatment (0 mg L^{-1} with all MS strengths) did not produce any rooting percentage.

Table 8. Effect of MS salt strength, IBA concentrations and their interaction on the rooting percentage of the lisianthus plants after 6 weeks of culture on the MS medium *in vitro*

IBA (mg. l^{-1})	MS salt strenght		IBA average
	MS ½	MS	
0	0	0	0
0.1	10	10	10
0.3	30	20	25
0.5	50	30	40
1	40	20	30
LSD 0.05	6.5		4.6
average MS	26.4	16	
LSD 0.05	2.9		

$LSD_{0.05}$ IBA: 6.5; $LSD_{0.05}$ MS: 4.6; $LSD_{0.05}$ interaction: 2.9

Effect of MS salt strength, IBA concentrations, and their interaction on the number of roots and shoots in the lisianthus plants

Table 9 shows the effect of different concentrations of IBA on root number. The 0.5 mg L^{-1} concentration gave the highest average root number, reaching 4.1 roots. $shoot^{-1}$, which differed significantly from the other concentrations. Meanwhile, the control treatment failed to produce roots. Regarding the effect of MS salts, the ½ strength MS salts gave the highest average root number, reaching 2.44 roots. $shoot^{-1}$, which differed significantly from the MS strength salts, which produced 1.84 roots. $shoot^{-1}$. For the bi-interaction, the 0.5 mg L^{-1} IBA concentration with ½ strength MS salts gave the highest average root number, reaching 5 roots. $shoot^{-1}$, which differed significantly from the other treatments.

Table 9. Effect of MS salt strength, IBA concentrations and their interaction on the number of roots (root/$shoot^{-1}$) of the lisianthus plants after 6 weeks of culture on the MS medium *in vitro*

IBA (mg. l^{-1})	MS salt strengh		average IBA
	MS ½	MS	
0	0.00	0.00	0.00
0.1	1.00	1.50	1.25
0.3	3.00	2.00	2.50
0.5	5.00	3.20	4.10
1	3.00	2.50	2.85
LSD 0.05	0.74		0.52
average MS	2.44	1.84	
LSD 0.05	0.33		

$LSD_{0.05}$ IBA: 0.74; $LSD_{0.05}$ MS: 0.52; $LSD_{0.05}$ interaction: 0.33

Figure 2. Rooted lisianthus plant shoots after planting on ½ MS medium with 0.5 mg L^{-1} PAA added after 6 weeks

The reason for the rooting of the shoots is due to the auxins increasing the rooting rate. Studies show that auxins play a major role in determining rooting type. They have a significant role in the meristematic division of the cambium-generating layer, thus forming root primordia and root formation. Adding auxins to the nutrient medium increases ionic exchange and enzyme activity, which increases the rate of branched root formation (**9**). The concentration of endogenous auxins added to the nutrient medium affects the division of root-originating cells. It also stimulates the emergence of adventitious roots through its phylogenetic effect in the loss of differentiation of specialized parenchyma cells and their return to meristematic cells. This is achieved through the process of differentiation of the root primordia, which continue to grow and develop, forming adventitious roots. These results are consistent with (**3**).

The auxins used are PAA, which is one of the auxins that are effective in increasing the rooting percentage and reducing the rooting period. It is considered one the effective forms of auxins in rooting numbers of plant species (**1**). The reason for the failure of the shoots to root when planted on MS medium devoid of PAA auxin is that the shoots resulting from the multiplication stage have insufficient auxin levels to stimulate rooting and increase the concentration of cytokinin. The superiority of MS salts over half the strength is due to the increased ratio of carbohydrates to nitrogen (C:N) in the medium, increasing the carbon content and decreasing the nitrogen content in the shoots, thus increasing the rooting rate (**2**,**7**), as seen in **Figure 2**.

Acclimatization Stage

Acclimatization was carried out by planting rooted shoots in plastic pots using different media of compost, peat moss, and perlite. The ratios of peat moss were: compost 1:1 volume: volume and peat moss; compost, perlite 1:1:1 volume: volume and peat moss; perlite 1:1 volume: volume. **Table 10** show that the best survival rate (100%) was for plants grown on peat moss and perlite. This is because peat moss provides a good medium for root growth and spread, in addition to providing essential elements and helping to retain moisture. Perlite also prevents root suffocation by increasing the aeration of the medium (**11**, **18**).

Table 10. Survival rate of acclimatized plants using different media of peat moss, perlite, and compost after 6 weeks of acclimatization

Medium Components	Survival Rate (%)
Peat moss: river soil 1:1	90
Peat moss: Perlite 1:1	100
Peat moss: river soil: Perlite 1:1:1	90

LSD: NS

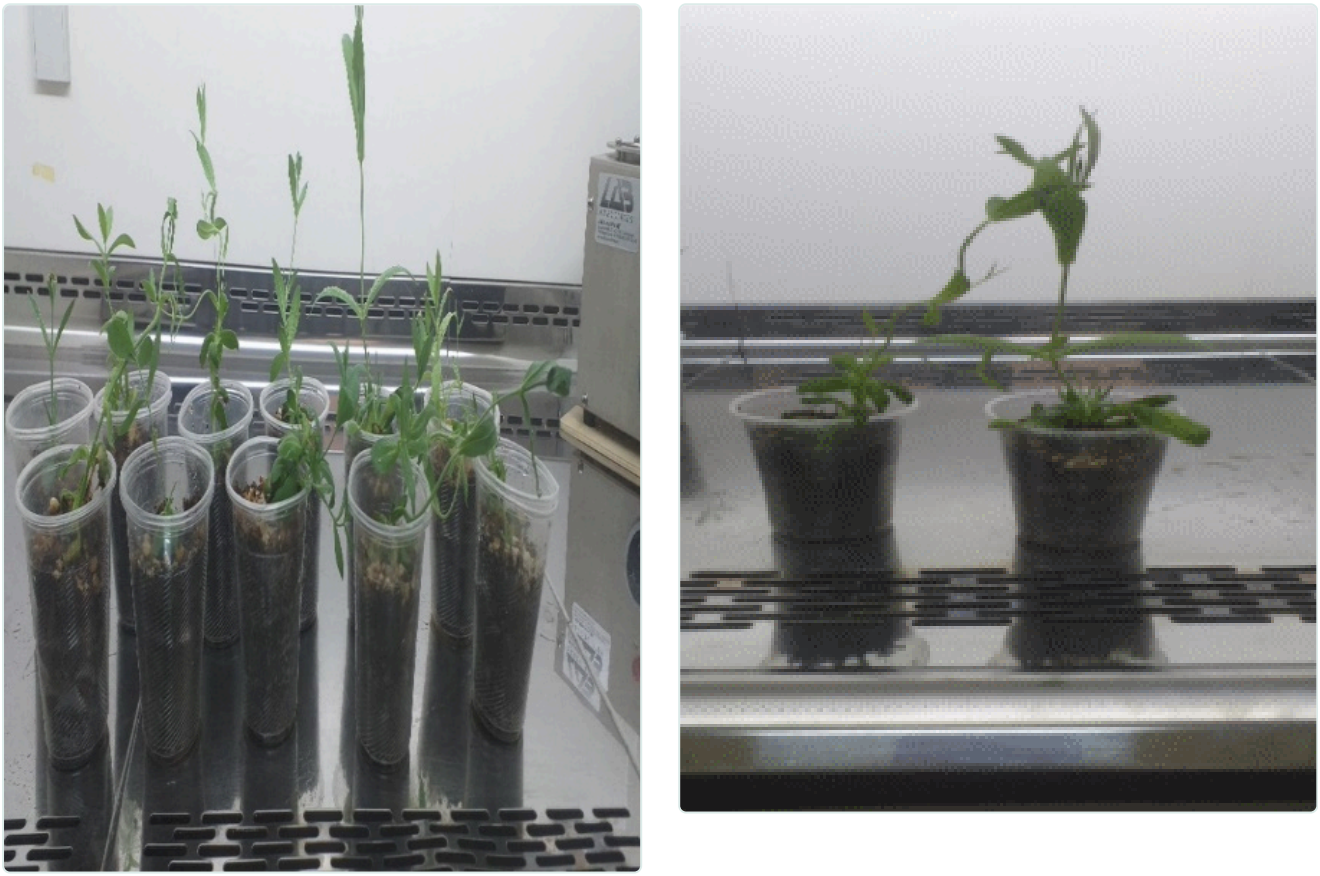

Figure 3. Acclimatization stages of the lisianthus plant

Conclusion

This study showed that the highest number branches (18 branches/plant part) was on the MS medium supplemented with 1 mg L^{-1} in combination with 0.5 mg L^{-1} PAA. Adding adenine sulfate at 80 mg L^{-1} concentration gave the highest number of branches, reaching 22 branches/ plant part, with chlorophyll content reaching 2.20. The highest rooting percentage (90%) and the number of roots (8.5 root/plant), and root length (7.5 cm) was attained by cultivating them on a medium supplemented with 0.5 mg L^{-1} PAA. For survival rates, the possibility of increasing the survival percentage of acclimatized lisianthus plant by 100% was on a medium containing peat moss and perlite at a ratio of 1:1.

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مقالة أصيلة

تأثير حامض فينيل أسيتيك (PAA) وكبريتات الأدينين على الاكثار النبات خارج الجسم الحي Eustoma grandiflorum

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الخلاصة

نفذت الدراسة في مختبر زراعة الانسجة النباتية/ قسم البستنة وهندسة الحدائق/ كلية علوم الهندسة الزراعية – جامعة بغداد من شهر تشرين الأول 2024 حتى كانون الثاني 2025 لمعرفة تاثير BA وPAA وIAA وIBA وكبريتات الادنين (SA) في اكثار نبات الاستوما *Eustoma grandiflorm* نسيجيا. اشتملت الدراسة على ثلاثة تجارب هي التضاعف والتجذير والاقلمة. اظهرت النتائج تفوق المعاملة 1 ملغم لتر$^{-1}$ BA بالتداخل مع 0.5 ملغم لتر$^{-1}$PAA بإعطائها اعلى معدل عدد افرع بلغ 18 فرع جزء نباتي$^{-1}$ في حين اعطت معاملة التداخل بين 0.5 ملغم لتر$^{-1}$ BA مع 0.5 ملغم لتر$^{-1}$ IAA اعلى معدل عدد افرع بلغ 5.6 فرع جزء نباتي$^{-1}$. اما بالنسبة لطول الافرع فقد أعطت معاملة التداخل بين 0 ملغم لتر$^{-1}$ BA مع 1 ملغم لتر$^{-1}$ PAA أعلى معدل طول بلغ 4 سم، في حين أعطت معاملة التداخل بين 0 ملغم لترBA مع 0 ملغم لتر$^{-1}$ IAA اعلى معدل طول بلغ 2 سم. اما بالنسبة لكبريتات الادنين فقد اعطى التركيز 80 ملغم لتر$^{-1}$ عدد أفرع بلغ 22 فرع جزء نباتي$^{-1}$ وطول فرع بلغ 2.4 سم ومحتوى كلوروفيل في الأوراق 2.20 على التوالي. وان افضل وسط للتجذير الافرع كان باستخدام ½ MS المجهز ب PAA بتركيز 0.5 ملغم لتر$^{-1}$ اذ اعطى اعلى نسبة تجذير بلغت 70% واعلى معدل عدد جذور بلغ 8.5 جذر فرع$^{-1}$، في حين اعطى ½ MS المجهز 0.5 ملغم لتر$^{-1}$ IBA نسبة تجذير 50% وعدد جذور بلغ 5 جذر فرع$^{-1}$. اما بالنسبة الاقلمة فقد تم الحصول على اعلى نسبة اقلمة بلغت 100% عند الوسط بتموس، برلايت وبنسبة 1:1 حجم: حجم

الكلمات المفتاحية: الاكثار الدقيق