In Vitro Micropropagation of Eustoma grandiflorum

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Abstract

The experiment was conducted at the Central Plant Tissue Culture Laboratory of the Department of Horticulture and Landscape Architecture-College of Agriculture-University of Kirkuk, with the aim of propagating the Eustoma grandiflorum cultivar "Rosita White" using different concentrations of the auxin phenylacetic acid (PAA) and the cytokinin (BA) and to determine the optimal concentration for increasing vegetative growth indices as well as fresh and dry weights. The tissue culture experiments were conducted according to a completely randomized design (CRD) with ten replicates, studying the effect of different concentrations of PAA (0, 0.5, 1, 1.5, 2) $mg.L^{-1}$ in combination with cytokinin BA at a concentration of 2.0 $mg.L^{-1}$, The results of the replication experiment showed that the treatment with 1.0 $mg.L^{-1}$ PAA combined with BA at a concentration of 2.0 $mg.L^{-1}$ yielded the highest average number of branches, number of leaves, and fresh and dry weights, amounting to 14.00 branches, per $plant^{-1}$, 38.0 leaves per $branch^{-1}$, 1.410 g, and 0.237 g. Meanwhile, the control treatment yielded the highest average branch length of 2.38 cm.

Keywords: *Eustoma grandiflorum*, in vitro, phenyl cetic acid.

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1-introduction and Literature Review

Growth regulators are one of the most important factors influencing plant growth in tissue culture, as they play a role in balancing the added growth regulators with the plant hormones within the plant. Among the most commonly used growth regulators are auxins and cytokinins, and the interaction between them is considered the most important in regulating plant growth and development in tissue culture, because auxins act synergistically with cytokinins [2]. One of these auxins is phenylacetic acid (PAA), a natural auxin derived from phenylalanine and found in many plants. It has a wide range of industrial, pharmaceutical and agricultural applications. PAA plays an essential role as a plant hormone in the growth and development processes such as cell expansion, elongation and root stimulation. It is naturally occurring in a wide range of organisms including plants, fungi and bacteria [12]. Therefore, the objective of this study was to:

To study the effect of different concentrations of the auxin PAA in the presence of BA on the vegetative growth characteristics and fresh and dry weights of Lisianthus plants. The sources reviewed highlighted the importance of growth regulators in the process of multiplication; [6] in a study conducted on chickpea plants that the highest response rate reached 100%, with 44.5 branches per plant part, was obtained on MS medium supplemented with 1.0 $mg.L^{-1}$ PAA and 2.0 $mg.L^{-1}$ BA. In date palm study, the researcher [3], found that MS medium enriched with 5.0 $mg.L^{-1}$ BA and 0.5 $mg.L^{-1}$ PAA was the best for the branching rate. The researcher Saleh in 2026 studied the Lisianthus plant and found that the use of a combination of 2.0 $mg.L^{-1}$ BA and 1.0 $mg.L^{-1}$ PAA to reach an average length of 1.8 cm and the branching rate of 14.0

Eustoma grandiflorum is a herbaceous ornamental plant belonging to the Gentianaceae family and is known as prairie gentian. Some of its varieties are biennial, completing their life cycle in two years: in the first year, they grow vegetatively, while in the second year, they bloom and produce seeds. It ranges in height from 15 to 60 cm, and its leaves are bluish-green and slightly succulent. It has large, funnel-shaped flowers that grow on a single stem or branched stems. The flowers come in a variety of colors and are borne on long, clustered stems; the sepals fuse only near the base and are much smaller than the petals [9,14]. The flowers come in several colors, such as white, blue, pink, purple, and bicolored. Lisianthus flowers are gaining immense popularity in the cut flower industry due to their rose-like appearance, lack of thorns, and long vase life [4].

Eustoma grandiflorum is most commonly propagated in two ways: the first is vegetatively via tip cuttings; however, this method has the drawback that the number of cuttings that can be obtained is limited and small, while the second is sexually via seeds; this method is criticized because seed germination is slow, growth is weak, and the seeds are small, making them difficult to handle [15]. This has led researchers to use tissue culture technology for the propagation of Lisianthus, which is characterized by the ability to produce plants that are difficult to propagate and to generate large numbers of uniform plants from a small plant part in a short period of time and within a limited, small space, as well as the ability to propagate across all genotypes without restriction. [7] In addition to precise propagation used on a commercial scale, it is one of the vegetative methods for plant propagation [1,11].

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calculated manually in the cultivation chamber.

- Shoot length was measured using a graduated ruler.
- The number of leaves, fresh weight and dry weight was measured after drying the samples in an oven at a temperature of 60–70 °C until the weight stabilized.

4 .Results and Discussion

1.4 Effect of Different PAA Concentrations in the Presence of BA at 2.0 mg L^{-1} on the Number of Branches per Vegetative Part of Eustoma grandiflorum Four Weeks after Planting:

The data presented in Table 1. show The addition of PAA to the culture media for *Eustoma grandiflorum* propagation resulted in an increase in the average number of branches formed the concentration of 1.0 $mg.L^{-1}$was statistically significantly higher than the control treatment, yielding the highest average number of branches at 14.00 branches per plant part, compared to the control treatment, which yielded 5.00 branches per plant part.

branches per plant part.

3 .Methodology

The experiment was held in the Central Plant Tissue Culture Laboratory of the Department of Horticulture and Landscape Architecture-College of Agriculture—University of Kirkuk during April 2026 to September 2026, for propagation (in vitro) Eustoma grandiflorum & Lisianthus (variety Rosita White).

1.3 Initiation Stage

1.1.3 Source of Plant Parts:

Lisianthus flowering was obtained from a flower nursery of a single node flower stem. The plant parts (single nodes) were collected and washed under running water for 15 minutes then disinfected with commercial disinfectant containing 10 mL of the disinfectant per 100 mL of distilled water for 10 minutes, after that they were washed three times with sterilized distilled water before being planted on (Murasing and Skoog-MS) medium. For the propagation experiment, they were grown in an 8/16-hour light/dark cycle with a light intensity of 1,000 lux in a growth chamber[16].

2.3 Multiplication Stage

1.2.3 Effect of Different Concentrations of the Auxin PAA in the Presence of the Cytokinin BA on the Growth and Multiplication of Shoots

The shoots produced during the germination stage were planted in MS medium supplemented with different concentrations of PAA (0, 0.5, 1, 1.5, 2) $mg.L^{-1}$ in the presence of cytokinin BA at a concentration of 2.0 $mg.L^{-1}$. except for the comparison treatment,

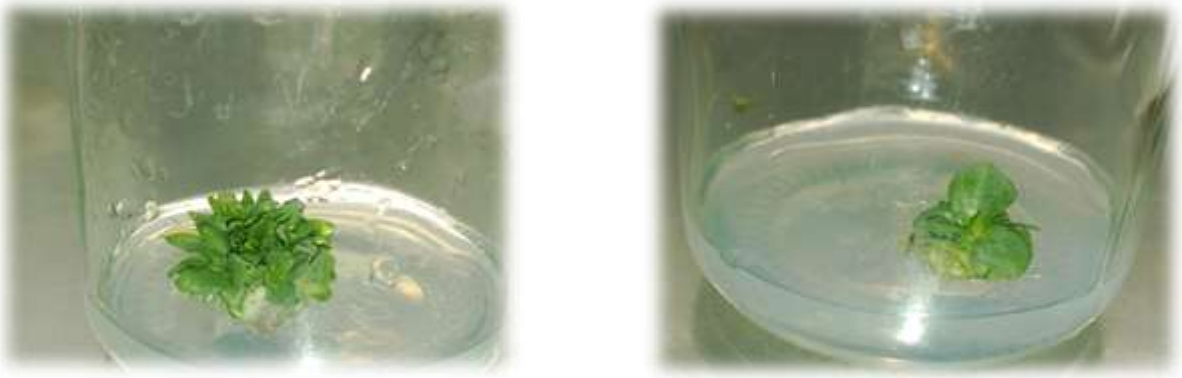
After four weeks, the following results were recorded:

- The number of shoots, which was

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Table 1. Effect of different concentrations of PAA in the presence of BA at a concentration of 2.0 mg.L^{-1} on the branching rate (branches per vegetative part) of Eustoma grandiflorum in vitro four weeks after planting.

PAA mg.L^{-1}	Number of Branches
0.0	5.00
0.5	7.00
1.0	14.00
1.5	9.00
2.0	6.00
LSD 0.05	4.11



(A) (B)

Figure 1. (A) Shows Lisianthus shoots grown on growth regulator-free MS medium (control treatment). (B) Shows the proliferation of Lisianthus shoots grown on MS medium supplemented with 2.0 mg.L^{-1} BA and 1.0 mg.L^{-1} PAA four weeks after planting.

The results in Table 2. indicate that PAA at a concentration of 1.0 mg.L^{-1} was statistically superior to the control treatment in terms of average number of leaves, with an average of 38.0 leaves per branch, whereas the control treatment had an average of 18.0 leaves per branch.

2.4 Effect of Different PAA Concentrations in the Presence of BA at 2.0 mg. L□¹ on the Average Number of Leaves of Eustoma grandiflorum Four Weeks after Planting.

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Table 2. Effect of different concentrations of PAA in the presence of BA at a concentration of 2.0 mg.L^{-1} on the leaf number of Eustoma grandiflorum in vitro four weeks after planting.

PAA mg.L^{-1}	Number of Leave
0.0	18.0
0.5	26.0
1.0	38.0
1.5	29.0
2.0	21.0
LSD 0.05	15.43

average branch height for Eustoma grandiflorum, with the control treatment yielding the highest average branch height of 2.38 cm. The height then decreased as PAA concentrations increased, reaching 2.0 mg.L^{-1} which yielded the lowest average branch height of 1.88 cm.

3.4 Effect of Different PAA Concentrations in the Presence of BA at 2.0 mg.L□¹ on the Average Shoot Length (cm) of Eustoma grandiflorum Four Weeks after Planting

The results presented in Table 3. showed the control treatment had the highest

Table 3. Effect of different PAA concentrations in the presence of BA at a concentration of 2.0 mg.L^{-1} on the average branch length (cm) of Eustoma grandiflorum in vitro four weeks after planting.

PAA mg.L^{-1}	Branch Length
0.0	**2.38**
0.5	**2.02**
1.0	**2.12**
1.5	**2.00**
2.0	**1.88**
LSD 0.05	**0.781**

influenced the fresh weight of Eustoma grandiflorum branches. Among the concentrations evaluated, 1.0 mg. L^{-1} PAA produced a significantly higher fresh weight compared with the control treatment. The branches treated with 1.0 mg. L^{-1} PAA recorded an average fresh weight of 1.410 g, whereas the control treatment showed a considerably lower

4.4 Effect of Different PAA Concentrations in the Presence of BA at 2.0 mg L□¹ on the Fresh Weight (g) of Eustoma grandiflorum Four Weeks after Planting

The results presented in Table 4. demonstrate that the addition of PAA to the culture medium significantly

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promoted greater biomass accumulation in the cultured branches under in vitro conditions.

value of 0.408 g. This result indicates that the appropriate concentration of PAA, in combination with 2.0 mg. L^{-1} BA,

Table 4. Effect of different concentrations of PAA in the presence of BA at a concentration of 2.0 mg.L^{-1} on the fresh weight (g) of Eustoma grandiflorum in vitro four weeks after planting.

PAA mg.L^{-1}	fresh weight
0.0	0.408
0.5	0.856
1.0	1.410
1.5	1.095
2.0	0.596
LSD 0.05	0.642

recorded a significantly higher average dry weight than the control treatment. The highest value was observed at 1.0 mg L□¹ PAA, reaching 0.237 g, while the control treatment recorded only 0.056 g. The marked increase in dry matter accumulation under this treatment indicates that the appropriate concentration of PAA, in combination with 2.0 mg.L□¹ BA, promoted biomass development and enhanced dry matter accumulation in the cultured branches during the four-week growth period.

5.4 Effect of Different PAA Concentrations in the Presence of BA at 2.0 mg L□¹ on the Dry Weight (g) of Eustoma grandiflorum Plants Four Weeks after Planting

The results presented in Table 4. revealed that the concentration of PAA in the culture medium significantly affected the dry weight of Eustoma grandiflorum branches under in vitro conditions. The treatment containing 1.0 mg. L□¹ PAA

Table 5. Effect of different concentrations of PAA in the presence of BA at a concentration of 2.0 mg.L^{-1} on the dry weight (g) of Eustoma grandiflorum in vitro four weeks after planting.

PAA mg.L^{-1}	dry weight
0.0	0.056
0.5	0.087
1.0	0.237
1.5	0.105
2.0	0.073
LSD 0.05	0.043

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the added growth regulators, leads to modifications in the different cellular contents of the shoots. In addition to internal changes, which consist of cell division and increase in cell size, the process of shoot cell division is usually accompanied by an increase in the contents required for the maintenance of division and growth, such as carbohydrates, proteins and amino acids. This was observed in the treatment with a concentration of 2. 0 $mg.L^{-1}$ BA with 1.0 $mg.L^{-1}$ PAA.

5 .Conclusions and Recommendations:

1.5 Conclusions:

1 .It was found that the auxin phenylacetic acid (PAA) was effective, in the presence of the cytokinin benzyl adenine (BA), in enhancing vegetative growth characteristics as well as the fresh and dry weights of *Eustoma grandiflorum*.

2.5 Recommendations

1 .Develop plant tissue culture techniques for the in vitro propagation of Eustoma grandiflorum by studying the number of shoots and leaves and the potential to increase the success rate of plant acclimatization to outdoor conditions.

2 .Use the auxin PAA at a concentration of 1.0 $mg.L^{-1}$ in the presence of the cytokinin BA at a concentration of 2.0 $mg.L^{-1}$ to achieve the highest response in vegetative growth parameters and fresh and dry weight of Eustoma andiflorum.

6 .References

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It can be concluded from the results in Tables 1 and 2 that the auxin phenylacetic acid (PAA), in the presence of the cytokinin benzyl adenine (BA), was effective in increasing vegetative growth indices and their impact on fresh and dry weight, as evidenced by an increase in the average number of branches and leaves and the induction of multiplication. This may be attributed to the fact that BA is a stable compound that does not degrade easily and is highly effective at breaking apical dominance, leading to the expansion of vascular vessels in the phloem tissues, stimulating cell division, increasing DNA production, and preventing chlorophyll degradation [10]. As for the auxin PAA, it contains a single ring, and its side chain is shorter and directly attached to the ring, which increases the auxin's effectiveness and activity; it is also more stable and less susceptible to heat and light, and its effect persists even after it degrades within the plant [8,13]. The effectiveness of cytokinins in increasing the number of branches increases in the presence of auxin in the growth medium [17].

As for the increase in the longitudinal diameter shown in Table 3, this may be attributed to the role that auxins play in the longitudinal growth of branches, and cell elongation is due to the balance between auxins and cytokinins added to the growth medium and the hormones naturally present within the plant in stimulating the division process through their role in increasing cell wall flexibility, cell permeability, enzymatic activity, and nutrient transport, which increases cell size and expansion [5]. These results are consistent with what was noted by the researchers [3,16]. The increase in fresh and dry weight of the shoots in Tables 4 and 5 shows that the growth of *Eustoma grandiflorum* shoots in the nutritional medium, which is mainly determined by

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