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Research Article

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EFFECTS OF GROWTH REGULATOR ON INVITRO PROPAGATION OF *LILIUM* USING BULB SCALE

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ABSTRACT

Lilium spp. is the most significant decorative plant and has high demand in the floriculture market. We studied the impact of different growth regulators on culture initiation, shoot proliferation, and root formation of *Lilium* for disease-free plants from in vitro propagation. The explant basal scale was used for culture initiation, and varying concentrations of BAP (6-Benzylaminopurine) alone and in combination with IAA (Indole-3-acetic acid) showed different responses. It was observed that MS media supplemented with BAP at 1.5 mg/l and IAA at 1.0 mg/l exhibited the maximum number of shoots, i.e., 4.3 shoots and 7 cm length of shoots, which were attained in four weeks. Several concentrations of BAP and Kinetin (KIN) supplemented with MS medium alone or in combination with BAP with IAA, IBA, and NAA were used for shoot proliferation. The results revealed that all treatments resulted in 100% shoot development, with significant variability in the number of shoots and shoot length. MS supplemented with 1.5 mg BAP and 1.0 mg KIN showed the maximum 7.4 number of shoots, with an average shoot length of 7.7 cm. The maximum 11 roots observed in MS supplemented with 1.5mg IBA and the longest roots 4.4cm found in MS with 2mg IAA, and the rooted plantlets were hardened in peat moss media, had the highest ex-vitro survival rate. These results highlight the significance of optimized combinations of growth regulators for improving micropropagation efficiency in the horticulture industry.

Keywords: Lily, Horticultural crop, Micropropagation, Phytohormones, Tissue culture.

INTRODUCTION

Lilium belongs to the Liliaceae family. The lily has gained significant popularity as a cut flower worldwide (Askari, Visser, & De Klerk, 2018) and is abundantly grown in a broad spectrum of regions across the globe. In the international floriculture industry, it holds the 4th spot among the main ten cut flowers (Khan, Jaskani, Iqbal, & Rafiq, 2015). There are two methods of propagation for *Lilium* cultivars: 1- vegetative propagation (formation of shoots and root bulbs, as well as propagation on bulb scales, tissue culture, and aerial axillary stalk bulbs) and 2- sexual propagation, which involves the development of seeds. Using seed has several limitations, the most significant of which are the duration of time it takes for crops to reach the proper bulb size to produce a flowering stalk, as well as the presence of undesired variation and seasonal availability (Gochhayat, Beura, & Rout). Although vegetative propagation results in the production of three to four bulbs per bulb, depending on the size and variety, there is a poor level of effectiveness in multiplication by bulb (Kaur, Thakur, & Sharma, 2006). The vegetative approach is susceptible to causing issues, especially the accumulation of viruses and the deterioration of species, which ultimately results in a decrease in quality. The process of in vitro propagation, not only allows for the rapid proliferation of plants, but it is also possible to produce virus-free plant material from the meristem or uninfected tissues (Huong, Gioi, & Van Thang, 2017). It has been reported that a significant number of primary lilies and commercial lilies have been successfully propagated in vitro (Li, Xia, Liang, & Yang, 2019). Through the process of in vitro propagation, not only is there a continual supply of bulblets, but it is also possible to obtain plants that are perfectly true to type and free of disease (Gochhayat, Beura, & Subudhi, 2017). However, to make in vitro culture a commercially feasible production method, it is necessary to design unique preparation procedures for each cultivar and crop (Rafiq *et al.*, 2021). Therefore, the industry of floriculture in the present day requires the construction of an effective protocol for their enhancement in terms of their rapid multiplication and marketable qualities. Plant regeneration has been accomplished in lilies using a wide variety of explants, ranging from bulb scales to other parts of the plant. Therefore, the role of auxin and cytokinin is essential in the formation of shoots, bulblets, and roots (Bhandari, Aswath, Bakshi, & Goshwami, 2019). The current research was carried

out to optimize an in vitro propagation protocol and identify the suitable concentrations of cytokines and auxins for mass production of the lily plant.

MATERIALS & METHODS

Site Location: The current study was carried out in the Tissue Culture Laboratory, National Institute for Genomics and Advanced Biotechnology (NIGAB), NARC (33.67°N, 73.13°E) in Islamabad, Pakistan.

Sterilization: *Lilium* (Richmond) bulb scales were removed and disinfected by immersing them in a fungicide solution for 20 minutes. They were washed 6-7 times with tap water before being sterilized with 15% sodium hypochlorite (NaOCl) and a few drops of Tween-20, followed by an autoclaved distillation water wash.

Treatments: The explants were cultured in MS medium supplemented (0.5, 1.0, and 1.5mg) alone or in combination with IAA (BAP 0.5 mg/L + IAA 0.25 mg/L, BAP 1.0 mg/L + IAA 0.5 mg/L, and BAP 1.5 mg/L + IAA 1.0 mg/L) using different concentrations along with 30 g/L sucrose, 2.2 g/L Gallen gum (*Pseudomonas elodea* produces gellan gum, a water-soluble anionic polymer made up of tetrasaccharide, a repeating unit of monomers. A common use for gellan gum as a food ingredient is to texturize, bind, or stabilize processed foods), and a pH of 5.8. For shoot proliferation, BAP and Kinetin (1.0, 1.5, and 2 mg/l) were used separately, and combinations of BAP (1.0, 1.5, and 2 mg/l) with IAA, IBA (Indole-3-butyric acid), NAA (1-Naphthaleneacetic acid), and Kinetin (0.5, 1.0, and 1.0 mg/l) were added to MS medium to investigate their efficacy. For root formation, MS medium was supplemented with (1.0, 1.5, and 2.0 mg/L IAA, IBA, and NAA, respectively). Each treatment contains three replications per explant.

Growing Conditions: The cultured explants were maintained in a growth room at 25°C with a 16-hour photoperiod under a 16:8 h fluorescent light at an intensity of 100 µmol m^{-2} per second. The cultures were transferred every thirty days to a fresh medium.

Data Recording and Statistical Analysis: After thirty days, data were recorded: the parameters were: i. Day to bud initiation; ii. frequency of shoot proliferation (%); iii. number of shoots; iv. shoot length (cm); v. number of roots; and vi. length of roots (cm). All data was analyzed using CRD. ANOVA (analysis of variance) was used for the statistical analysis based on mean values per explant. The differences between treatments were determined using the comparative LSD multiple range test (P = 0.05).

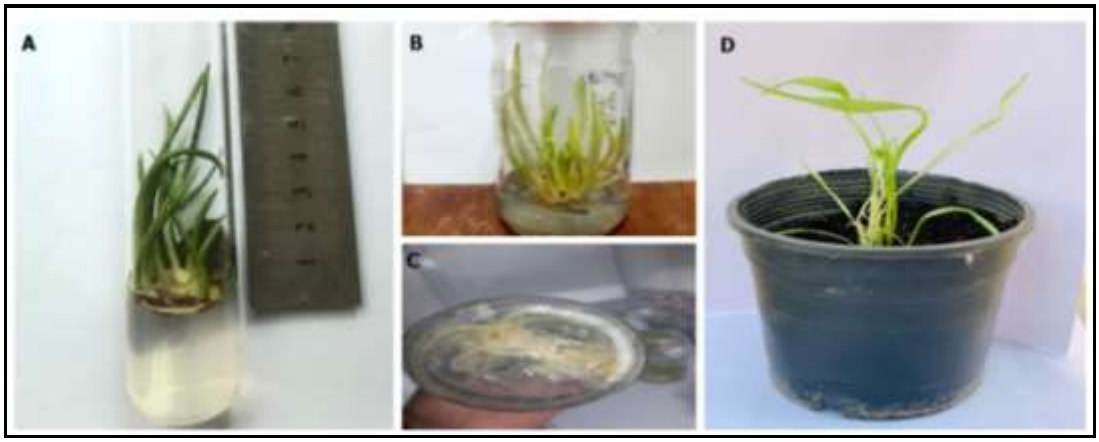

Figure 1. (A) Bulb scale of *Lilium* used as an explant for culture establishment; (B) After five weeks of inoculation, MS medium with 1.5mg BAP and 1.0 mg KIN produced numerous shoots. (C) An in vitro-cultivated shoot is utilized as an explant for root formation.; (D) After four weeks, the plantlets were acclimatized in peat moss media. The scale is equal to 1 cm.

RESULTS

Culture Establishment: The culture establishment of *Lilium* was examined under several treatments using varied concentrations of BAP (6-benzyl amino purine) and IAA (3-indole-3-acetic acid), as shown in (Table 1). The results revealed that there are notable differences between treatments in terms of shoot length, number of shoots, and day-to-bud initiation. The treatment (T6), MS supplemented with 1.5 mg/l BAP and 1 mg/l IAA, had the maximum number of shoots per bulb scale, with an average of 4.3 shoots per scale. In terms of average shoot length, treatment (T6) showed the longest shoots with an average of 7 cm as shown in (Figure 1; A), followed by treatment (T5) with an average shoot length of 4.5 cm and 3.3 shoots recorded. However, the treatment T1 MS supplemented with BAP 0.5 mg/l produced a minimum number of shoots with an average of 1.3 and a minimum shoot length of 2.3 cm recorded in T2 per bulb scale. According to these findings, the combination of BAP and IAA considerably increased shoot elongation when compared to the other treatments. Moreover, the combination of BAP and IAA significantly reduced the time for bud initiation. The earliest bud initiation was observed in treatments (T4) to bud initiation of 23 days, followed by (T5) to bud initiation of 24 days.

Table. 1: Effect of different growth regulators supplemented with MS medium on culture establishment in *Lilium*

Treatment	BAP	IAA	Number of shoot/bulb scale	Length of shoot	Day to bud initiation
T1	0.5mg/l		1.3±0.5^{c}	2.3±0.5^{c}	40
T2	1mg/l		2±1^{c}	2.2±0.4^{c}	30
T3	1.5mg/l		2.3± 0.5^{bc}	2.6± 0.5^{c}	28
T4	0.5mg/l	0.25mg/l	2.±1^{c}	2.9± 0.17^{c}	23
T5	1mg/l	0.5 mg//l	3.3± 0.5^{ab}	4.5±0.15^{b}	24
T6	1.5mg/l	1mg/l	4.3± 0.5^{a}	7±0.8^{a}	31
MEAN			2.5	3.7	29.3
LSD ($p < 0.05$)			1.3260	0.9744	3.7
Different letters (a, b, and c) above the column differ at $p < 0.05$. BAP = 6-Benzylaminopurine, IAA = Indole-3-acetic acid.					

Shoot Proliferation: The study aims to evaluate the effect of growth regulators on the rapid multiplication of shoots and growth in *Lilium*. The findings showed a trend of 100% shoot development frequency in all the treatments, demonstrating the significant effect of the selected growth regulators on shoot proliferation in cultured explants. Additionally, when concentrations of kinetin (KIN) increased with different concentrations, the results showed distinct shoot lengths, as shown in (Table 2). The treatment T3 (MS+KIN, 2 mg/L) had the longest shoot length of 6.9cm and produced 4.5 shoots. While the treatments that used 6-benzyl aminopurine (BAP) demonstrated uniform growth of shoot frequency at various amounts. The treatment T5 MS supplemented with BAP (1.5 mg/L) produced the longest shoots, measuring 6.5 cm, and the maximum number of shoots was 6.9, as observed in T6 MS supplemented with BAP (2 mg/L), as shown in (Table 2). We observed different responses when we combined BAP with different types of auxins (IAA, IBA, and NAA). The longest shoots (7.3 cm) were produced by Treatment T13 MS supplemented with (BAP+NAA, 1 mg+0.5 mg/L). The maximum number of shoots 7.3 was recorded by Treatment T14 MS supplemented with (BAP+NAA, 1.5+1.0 mg/L). Moreover, the treatment T17 MS supplemented with (BAP+KIN, 1.5 mg+1 mg/L) showed significant results, as shown in (Table 2), which had the longest shoot length of 7.7 cm and a maximum number of shoots of 7.4 as compared with all other treatments (Figure 1; B-C).

Table. 2: Effect of different growth regulators supplemented with MS medium on shoot proliferation in *Lilium.*

Treatments	BAP	IAA	IBA	NAA	KIN	Shoot formation (Frequency, %)	No. of Shoots	Length of shoot (cm)
T1					1mg/L	100	5±0.45^{efg}	6.3±0.6^{def}
T2					1.5mg/L	100	4.5±0.5^{g}	6.7±0.3^{cde}
T3					2mg/L	100	4.5±0.4^{fg}	6.9± 0.1^{bcd}
T4	1mg/L					100	6±0.5^{cd}	5.3±0.6^{gh}
T5	1.5mg/L					100	5±0.4^{efg}	6.5±0.4^{cde}
T6	2mg/L					100	6.9±0.45^{ab}	4.9±0.4^{h}
T7	1mg/L	0.5mg/L				100	6.5±0.5^{bc}	6.4±0.5^{de}
T8	1.5mg/L	1mg/L				100	5.16±0.2^{efg}	6.2±0.4^{ef}
T9	2mg/L	1mg/L				100	5.3±0.5^{def}	7.3±0.5^{ab}
T10	1mg/L		0.5mg/L			100	5.43±0.51^{de}	7±0.1^{bc}
T11	1.5mg/L		1mg/L			100	5.43±0.51^{de}	6.7±0.2^{cde}
T12	2mg/L		1mg/L			100	6±0.5^{cd}	5.4±0.4^{gh}
T13	1mg/L			0.5mg/L		100	6.4±0.5^{bc}	7.36±0.1^{ab}
T14	1.5mg/L			1mg/L		100	7.3±0.5^{a}	7.1±0.2^{abc}
T15	2mg/L			1mg/L		100	5.4±0.3^{de}	5.7±0.2^{fg}
T16	1mg/L				0.5mg/L	100	6.5±0.5 ^{bc}	6.8±0.2 ^{bcd}
T17	1.5mg/L				1mg/L	100	7.4±0.3 ^{a}	7.7±0.2 ^{a}
T18	2mg/L				1mg/L	100	6±0.2 ^{cd}	5.7±0.3 ^{fg}
MEAN						100	6.44	5.82
LSD ($p < 0.05$)							0.81	0.62

Different letters (a, b, and c) above the column differ at $p < 0.05$. BAP = 6-Benzylaminopurine, IAA = Indole-3-acetic acid, NAA = Naphthaleneacetic acid, IBA = Indole-3-butyric acid, KIN = Kinetin.

Root Formation and Hardening: The root formation of *Lilium* was examined using varied concentrations of auxin (1mg, 1,5 mg, and 2 mg/l) of IAA (Indole-3-acetic acid), Naphthaleneacetic acid (NAA), and Indole-3-butyric acid (IBA) in each treatment, as shown in (Table 3). The results revealed that the highest frequency of root formation observed in T3 MS medium supplemented with IAA (2 mg/l), produced nine roots and the longest root length with an average of 4.4cm as compared to other treatments with IAA. The lowest frequency of root formation was in T1 (IAA 1 mg/l), with an average of 3 roots and a length of 2.5 cm, Additionally, T6 MS medium supplemented with NAA (2 mg/l) produces seven roots and a length of 2 cm. While the MS supplemented with NAA (1 mg/l) produced the minimum number of roots with the lowest root length of 1.6cm per explant, However, the treatment T4 MS medium supplemented with IBA 1.5 mg/l showed the highest frequency of root formation, with a significantly high 11 number of roots, and an average root length of 3.8cm per explant. Moreover, the treatment T7 MS medium supplemented with IBA (1 mg/l) recorded 5.9 number of roots and 3cm of root length per explant, as shown in (Table 3). After five weeks, rooted plantlets were hardened in peat moss-containing media exhibited the highest ex vitro survival rate of 95% as shown in (Figure 1; D).

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Table. 3: Effect of different growth regulators supplemented with MS medium on root formation in *Lilium.*

Treatment	IAA	NAA	IBA	Number of roots	Length of roots (cm)
T1	1mg/L			3 ± 0.5^{f}	2.5 ± 0.2^{cd}
T2	1.5mg/L			5 ± 0.4^{e}	3 ± 0.3^{bc}
T3	2mg/L			9.1 ± 0.3^{b}	4.4 ± 0.5^{a}
T4		1mg/L		5 ± 0.4^{e}	1.6 ± 0.4^{e}
T5		1.5mg/L		5.1 ± 0.7^{e}	2 ± 0.1^{de}
T6		2mg/L		7 ± 0.5^{cd}	2 ± 0.1^{de}
T7			1mg/L	5.9 ± 0.9^{de}	3 ± 0.4^{bc}
T8			1.5mg/L	11 ± 1^{a}	3.5 ± 0.4^{b}
T9			2mg/L	7.1 ± 0.7^{c}	3.4 ± 0.5^{b}
MEAN				6.4	2.8
LSD ($p < 0.05$)				1.1403	0.6586

Different letters (a, b, and c) above the column differ at $p < 0.05$. IAA = Indole-3-acetic acid, NAA = Naphthaleneacetic acid, IBA = Indole-3-butyric acid.

DISCUSSION

The primary growth regulators in plants, auxin and cytokinin, regulate several characteristics of plant growth and development (Kakani, Li, & Peng, 2009). In plant tissue cultures, these growth regulators are required to promote cell division and explant differentiation. (Zeng *et al.*, 2007). The first stage of this technique is to design an effective and reproducible micropropagation protocol that produces disease-free *Lilium* plants. The culture establishment of *Lilium* was examined through multiple treatments combining various concentrations of BAP (6-benzylaminopurine) and IAA (3-indole-3-acetic acid), each of which exhibited a different combination of growth regulators as shown in (Table 1). We evaluated the parameters such as the number of shoots per bulb scale, the length of the shoots, and the time taken for bud initiation. This study used the basal scale as an explant as shown in (Figure 1; A). More effective explants with higher values for establishment factors were found in basal-scale segments confirmed by (Rafiq *et al.*, 2021). The combination of BAP 1.5 mg/l and IAA 1 mg/l exhibited the shortest time to bud initiation and optimal growth in both the number of shoots and the length of the shoot. Our findings with (Song, Lee, Yi, Lee, & Yoon, 2021) reported that multiple shoots were produced in MS media with 0.4 mg/L of BA hormone and 0.3 mg/L of IAA. While earliest shoot induction BAP 0.5 mg/l and 2,4-D 0.5 mg/l were used by Gochhayat et al. The most effective medium for small bulb proliferation was MS + 6-BA 1.5 mg/L + NAA 0.5 mg/L (Su Jiang *et al.*, 2014). Shoot development in the culture medium is induced only by the amounts of cytokinin present (Wang, Zhao, Zhuang, Wang, & Chen, 2008) Any species that intends to multiply rapidly must have the ideal cytokinin level for proper shoot proliferation. In the present study for shoot proliferation, the result showed that after a 4-week culture establishment, the highest shoot formation frequency was observed in MS supplemented with 1.5mg BAP and 1mg KIN as shown in (Table 2; Figure 1. B). Of all the cytokinins used in the study (Youssef, Shaaban, Ghareeb, & Taha, 2019), found that BA (6-Benzylaminopurine) was the most effective for multiple shoot induction. The optimal medium for small bulb multiplication was MS + 6-BA 0.5 mg/L + NAA 0.25 mg/L + KT 0.25 mg/L, as reported by (Su Jiang *et al.*, 2014), which supports our study. Many authors have reported that MS media containing 2.0 mg/L 6-BA and 0.2 mg/L NAA was the most effective medium for the proliferation of plantlets

from three distinct genotypes of lily (Cui Qi & Jia GuiXia, 2014). According to (Pandey, Singh, & Mamta Sharma, 2009), maximum proliferation has been recorded by micropropagation of Lilium using bulb scales as explants cultivated on MS medium supplemented with 0.50 mg/1 NAA and 2.0 mg/1 BAP. The *Lilium* "Batavus" and *Lilium* "Hyde Park" varieties exhibited better in vitro responses, especially when grown with 1.0 and 1.5 mg L1 BAP (Lapiz-Culqui *et al.*, 2022). In the present study, different concentrations of IAA, NAA, or IBA-supplemented MS medium were utilized to promote root development. For root formation, the highest frequency was found in MS supplemented with 1.5mg IBA, and the longest roots were found in MS supplemented with 2mg IAA. (Zhao, 2010) reported that Indole-3-acetic acid (IAA), the major auxin present in higher plants, has a significant impact on the development and growth of plants. Both plants and certain plant diseases can produce IAA, which regulates plant growth. The higher number of roots and percentage found in IAA at 0.5 mg/L (Taha, Sayed, Farahat, & El-Sayed, 2018) support our study. The culture medium with 2.0 mg L^{-1} IBA produced the highest percentage of rooted shoots (85%), as reported by (Kumar & Dogra, 2020). (Sindhu, Chandanie, & Dhiman, 2016) also reported that the half-strength MS media with 1.0 mg/L of IBA showed superior results in the rooting of Asiatic *Lilium* cv. Pollyanna micro shoots also demonstrated that auxin application, particularly IBA, increases rooting percentage and quality. (Arbaoui, Soufi, & Bettaieb, 2017) found similar results, finding that 1.50 mg/l IBA was the optimal concentration for various rooting properties. Earlier studies by (Tang, Liu, Gituru, & Chen, 2010) and (Liu, Yuan, & Liu, 2011), in *Lilium* also supported the current research, which indicates that IBA and IAA are the ideal mediums for *Lilium* rooting. A high quantity of auxin has been demonstrated to have an inhibited impact, which results in a reduction in the number of roots in a plant (Novak, Luna, & Gamage, 2014). In the present study, plants harden in peat moss media for a high survival rate under controlled conditions as shown in Figure 1; D). Our study relates to (Kaur *et al.*, 2006), those who observed that the hardening of Lilly in vitro-rooted bulblets in peat moss provided 100% survival.

CONCLUSION

The bulb scale was used as an explant and placed in an MS medium supplemented with different combinations of growth regulators for culture establishment, shoot proliferation, and root formation. The early bud initiation from the bulb scale induced a greater number of shoots in MS supplemented with (1.5 mg/l BAP and 1 mg/l IAA). The maximum number of shoots were produced in MS supplemented with (BAP+KIN, 1.5 mg+1 mg/L). The highest root formation frequency and number of roots were recorded in MS supplemented with IBA 1.5 mg/l. while the longest roots were observed in MS supplemented with 2 mg of IAA per explant. After that, plants harden in peat moss media with a high humidity level for a high survival rate under controlled conditions.

AUTHOR'S CONTRIBUTIONS

Conceived idea/funding: Haider Ali and Iqbal Hussain, Writeup and Review: Kazim Ali, Muhammad Zeeshan, Mumtaz Hussain, Amber Imtiaz, Hina Hafeez, Sadia Sarwar, and Ghulam Muhammad Ali, analysis /tools: Haider Ali, Amber Imtiaz, Saima Noor, Supervision: Iqbal Hussain.

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Not applicable.

INFORMED CONSENT STATEMENT

Not applicable.

COMPLIANCE WITH ETHICAL STANDARDS

This manuscript contains no studies with humans/animals performed by any authors.

Data Availability Statement: All the relevant data are within the paper.

Conflicts of Interest

The authors declare no conflict of interest.

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