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Effects of Plant Growth Regulators, Culture Media, and Light on Bulblet Production of Lily 'Fujian' Using Scaling Technique

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Abstract

Lily (*Lilium* spp.) is one of the most important flower in ornamental plants industry. This flower is one of the geophyte plants, therefore lilies cultivation was done with bulb. Between different lily bulb producing methods, scaling is a cost-effective and short-term technique. Lily bulb producers have always various challenges in different stage of production. Aiming to focus on solving these challenges, two separate experiments were performed to optimize bulblet production, as the first stage of bulb production. In the first experiment, four concentrations of two types of auxin (indole-3- butyric acid and naphthalene acetic acid at concentrations of 250 and 500 mg/L) and a control treatment as the first factor and effects of three culture media, including coco peat, coco peat + perlite (1:1) and black peat moss, as the second factor were examined. Results indicated that although the highest number of bulblets per scale (2.07) was observed in concentration of 500 mg/L of indole-3- butyric acid, but the maximum bulblet's weight (0.81 g) and bulblet's diameter (1.32 cm) were produced under control treatment. Regarding culture media effects, the highest number of bulblet per scale (2.02) maximum bulblet's weight (0.71 g) and bulblets' diameter (1.21 cm) were obtained in peat moss media. In the second experiment, above mentioned concentrations of the plant growth regulators as a first factor and also the presence or absence of light as a second factor was examined. Results showed that present or absent of light has not significant effects on all evaluated traits except bulblet's diameter. Bulblet diameter significantly increased in dark situation. Extract of this study indicated, in lily bulbs production, with using PGR treatment can improve some quantity and quality traits.

Keywords: Geophyte, Indole-3-butyric acid, Naphthalene acetic acid, Peat moss.

INTRODUCTION

Lilium spp. (lily) is one of the most important bulbous flowers in the world. It has belonging to the Liliaceae family (Marinangeli *et al*., 2003; Li *et al*., 2019). Lilies have several applications as a cut flower, outdoor and potted flower (Nongdhar *et al*., 2019) and also medicinal properties (Huang *et al*., 2017). Lily has a strategic role in cut flower industry due to their incomparable beauty, variety of flower color and flower shape (Howlader *et al*., 2020). Lily is commercially cultivated through the bulb. Due to extensive cultivations of lily in the world so the area under cultivation of lily bulbs has been increasing among pioneer countries. Lily bulb cultivation area in the Netherlands has been increased from 5,000 ha in 2008 to 6,000 ha in 2019 (Statistics portal, 2021).

Commercial propagation of lily bulbs occurs through methods such as scaling, bulbil, and tissue culture (Bakhshaie *et al*., 2016). Among these In the meantime, scaling is the most common technique (Hatamzadeh *et al*, 2015), in which 3-4 growth cycles are required to bulb production (Park, 1994).

Some studies have suggested that plant growth regulators (PGRs) and different culture media effects on propagation of lily bulb. Tang *et al*. (2020) reported that types and concentrations of PGRs had different effects on bulblet production in lilies. The using of indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) on lily bulb's propagation through the scaling method increased number of bulblet production (Gupta *et al*., 2016) bulblet's uniformity, bulblet's regeneration and bulblet's rooting (Masoodi and Nayeem, 2018).

On the other hand, culture media has also significantly affected on the quantity and quality of lily bulblet production (Gupta *et al*. 2010). Positive effects of cocopeat and soil+coco peat (1:1) on the lily bulblet production through the scaling method have been reported (Lyngdoh *et al*., 2015).

Furthermore, limited studies have investigated the influence of the presence or absence of light on lily bulb propagation with scaling method. In this regard, Niimi *et al*. (1999) revealed that the regeneration of bulblets in light conditions was better than that in darkness conditions.

Considering the effectiveness of various factors on lily bulblet's production through the scaling technique, the present study was done to investigate the effects of PGRs, culture media and light conditions to achieve a proper instruction for the lily bulb propagation.

MATERIALS AND METHODS

In the present study, bulbs (*Lilium* spp. cv. Fujian) were selected from Orienpet (OT Hybrid) section. It should be noted that the uniformity and health indices of the bulbs were involved in their selection for scaling. Sampling were done with similar features in color, shape, size (20-22 cm), and root quality were preferred. Also, outer scales of bulb (1-2 layers) with the same features, were selected. After washing scales with raw water, to continue the experiment, scales disinfected with chemical fungicides like benomyl (1×1000 ratio) and carbendazim (1×1000 ratio). Eventually damaged scales were removed.

Auxin and culture media experiments

In order to evaluate the effects of treatments containing auxin and culture media, an experiment was conducted using a factorial experiment based on the completely randomized design with four concentrations of two types of auxin (IBA and NAA at concentrations of 250 and 500 mg/L) and also a control treatment as the first factor and three culture media (coco peat, coco peat + perlite, and black peat moss) as the second factor in three replications. In this experiment a total of 360 samples (eight scales per replicate) cultivated in trays with cell volumes of 90 ml. Since lily scales was very sensitive to high media moisture and plant pathogens, the media moisture during the experiments was constantly monitored. To apply the first factors (Auxin), scales were immersed for 60 minutes in auxin solution and immediately cultured in the desired media. Finally, the trays containing scales were stored at 23±2 °C during the day and 18±2 °C at night at a relative humidity of 75 ± 5%.

Auxin and light experiments

In order to evaluate the effects of auxin and light factors on lily bulblet's production through the scaling technique, experiment was conducted using a factorial experiment based on the completely randomized design with four concentrations of two types of auxin (similar to the above experiment) and a control treatment as the first factor and two light conditions (presence and absence conditions) as the second factor in three replications. In this experiment a total of 240 samples (eight scales per replicate) cultivated in trays with cell volumes of 90 ml. Similarly, scales were immersed in different concentrations of IBA and NAA for 60 minutes to evaluate the effects of hormonal treatments and immediately cultured in the desired substrates. The dark treatment was applied by drawing a black curtain on the trays containing scales.

Statistical analysis

The data were analyzed by SAS software (version 9.4) and the Duncan's Multiple Range Test was used at $P<0.05$ to compare the mean of the data.

RESULTS

Effects of auxin concentrations and culture media

Data analysis showed (Table 1) the number of bulblets per scale, bulblet weight, bulblet diameter, root number and number of micro scales per bulblet significantly affected by auxin treatments. On the other hand, culture medium had significant effects on the all evaluated traits (except root number). Results also revealed that the interaction of auxin concentrations with culture media had significant effects on number of bulblets per scale, bulblet weight, the number of roots and on the trait of bulblet diameter. It was while interaction between auxin concentrations with culture media had no significant effects on the number of micro scales per bulblet.

Analysis of the results from the comparison of means values effect of auxin (Table 2) showed that the highest number of bulblets per scales (2.07) was obtained in the treatment of indole-3-butyric acid 500 mg/L, while the highest bulb weight (0.81 g) and bulb diameter (1.32 g) were obtained in the control treatment. The highest number of roots (4.37) and the highest number of micro scales (4.16) were obtained in the treatment of indole-3- butyric acid 250 mg/L, which in the highest number of micro scales was a no significant difference with the control treatment, 500 mg /L indole-3- butyric acid and 500 mg/L naphthalene acetic acid.

Table 1. Analysis of variance of the effect of auxin and media culture on traits of bulblet lily.

S.o.V	df	MS				
		No. of bulblet per scale	Bulblet weight	Bulblet diameter	Root No.	No. of micro scales per bulblet
Auxin (A)	4	0.46**	0.23**	0.16**	1.38**	0.45*
Culture media (C)	2	0.94**	0.24**	0.16**	0.14ns	3.09**
A×C	8	0.33**	0.05**	0.04*	0.63**	0.17 ns
Error	28	0.05	0.00	0.01	0.09	0.11
CV (%)		12.96	15.18	10.85	8.01	8.71

ns, *, **: Non significant, significant at the 5 and 1% probability levels, respectively.

Table 2. Means comparison of the effect of auxin on some traits of lily bulblet.

Auxin (mg/L)	No. of bulblet per scale	Bulblet weight (g)	Bulblet diameter (cm)	Root No.	No. of micro scales per bulblet
0	1.50^{c}	0.81^{a}	1.32^{a}	3.87^{bc}	4.05^{ab}
250 IBA	1.91^{b}	0.66^{b}	1.17^{b}	4.37^{a}	4.16^{a}
500 IBA	2.07^{a}	0.50^{c}	1.05^{cd}	4.03^{b}	3.80^{ab}
250 NAA	1.78^{b}	0.48^{c}	1.11^{bc}	3.61^{cd}	3.73^{b}
500 NAA	1.69^{b}	0.42^{c}	0.96^{d}	3.35^{d}	3.82^{ab}

*In each column, means with the similar letter(s) are not significantly different (P<0.05) using the Duncan's multiple range test.

According to the results of comparison of mean values (Table 3), the highest number of bulblets per scale (2.02) was obtained in the peat moss culture medium while had no significant difference with the number of bulblets per scales obtained from the cocopeat media (1.85). Also, bulblet weight (0.71 g) and the highest values for micro scales per bulblet (4.38 pcs) were obtained under the application of the peat moss media. Both of cocopeat and peat moss treatments had more effectiveness than cocopeat + perlite. Finally, culture media had non-significant effects on the bulblet's root number.

Table 3. Effect of substrate on some traits of bulblet production with scale propagation.

Media	No. of bulblet per scale	Bulblet weight (g)	Bulblet diameter (cm)	Root No.	No. of micro scales per bulblet
Cocopeat	1.85^{a}	0.55^{b}	1.15^{a}	3.96^{a}	3.79^{b}
Cocopeat + perlite (1:1)	1.47^{b}	0.47^{c}	1.00^{b}	3.76^{a}	3.51^{c}
Peat moss	2.02^{a}	0.71^{a}	1.21^{a}	3.82^{a}	4.38^{a}

*In each column, means with the similar letter(s) are not significantly different (P<0.05) using the Duncan's multiple range test.

Table 4. Interaction effects of auxin and substrate on some traits of bulblet lily.

Auxin (mg/L)	Substrate	No. of bulblet per scale	Bulblet weight (g)	Bulblet diameter (cm)	Root No.	No. of micro scales per bulblet
	Cocopeat	1.83^{bced}	0.90^{a}	1.46^{a}	3.86^{bc}	4.10^{a}
Control	Cocopeat+ perlite	1.33^{gf}	0.70^{bc}	1.20^{b}	4.26^{ab}	3.80^{ab}
	Peatmoss	1.53^{defg}	0.83^{ab}	1.30^{ab}	3.50^{c}	4.26^{a}
	Cocopeat	1.96^{bcd}	0.56^{cd}	1.13^{bc}	4.16^{ab}	4.03^{a}
250 IBA	Cocopeat+ perlite	1.10^{gf}	0.53^{cde}	1.10^{bcd}	4.66^{a}	3.76^{ab}
	Peatmoss	2.06^{bc}	0.90^{a}	1.30^{ab}	4.30^{ab}	4.70^{a}
	Cocopeat	2.16^{b}	0.45^{def}	1.00^{cd}	4.06^{bc}	3.80^{ab}
500 IBA	Cocopeat+ perlite	1.40^{efg}	0.56^{cd}	1.10^{bcd}	4.16^{ab}	3.53^{ab}
	Peatmoss	2.66^{a}	0.46^{def}	1.03^{cd}	3.86^{bc}	4.06^{a}
	Cocopeat	1.90^{bcd}	0.43^{def}	1.10^{bcd}	4.20^{ab}	3.46^{ab}
250 NAA	Cocopeat+ perlite	2.10^{b}	0.30^{gf}	0.90^{d}	2.93^{d}	3.00^{ab}
	Peatmoss	2.00^{bc}	0.66^{c}	1.26^{ab}	3.70^{bc}	4.50^{a}
	Cocopeat	1.40^{efg}	0.36^{ef}	1.03^{cd}	3.50^{c}	3.56^{ab}
500 NAA	Cocopeat+ perlite	1.63^{cdef}	0.20^{g}	0.70^{e}	2.80^{d}	2.90^{abc}
	Peatmoss	1.83^{bcde}	0.70^{bc}	1.16^{bc}	3.76^{bc}	4.40^{a}

*In each column, means with the similar letter(s) are not significantly different (P<0.05) using the Duncan's multiple range test.

Results related to the interaction of auxin concentrations with culture media (Table 4) showed that the maximum number of bulblets per scale (2.66) was obtained in peat moss media when IBA at a concentration of 500 mg/L had been used. The thickest bulblet diameter (equal to 1.46 cm) was recorded for the interaction of cocopeat media at the same time with control treatment. It was while this trait had no significant differences with bulblet diameter obtained by the interaction of peat moss culture medium with control treatment and also concentration of 250 mg/L of IBA and NAA.

Effects of auxin concentrations and light conditions

Analysis of auxin and light condition data (Table 5) showed number of bulblets per scales, bulblet weight, diameter of bulblets, root numbers and micro scales per bulblet were affected significantly by auxin concentrations. On the other hand, presence or absence of light factor only had a significant effect on the bulblet diameter, so that the other traits were not affected by this factor. The interaction between auxin concentrations and light factor also had significant effects only on the number of bulblets per scale.

Analysis of the results from the comparison of means values effect of auxin in second experiments (Table 6) showed that the highest number of bulblets per scale was achieved under the concentration of 500 mg/L and 250 mg/L of IBA. Also, results indicated that the highest bulblet fresh weight (0.66 g) was obtained in control treatment. In addition, although the highest root number per bulblet (4.35 roots per bulblets) was counted for the concentration of 250 mg/L of IBA, there was no significant difference with the number of roots per bulblet treated with 250 mg/L of IBA (Table 6). Furthermore, the highest number of micro scales per bulblet (4.16 No.) was obtained under the concentration of 250 mg/L of IBA but had not significant difference with the control and the concentration of 500 mg/L of IBA treatments (Table 6).

Table 5. Analysis of variance of the effect of auxin and light on some traits of bulblet lily.

S.o.V	df	MS				
		No. of bulblet per scale	Bulblet weight	Bulblet diameter	Root No.	No. of micro scales per bulblet
Auxin (A)	4	0.22 **	0.08 **	0.04 *	1.13 **	0.86 **
Light (L)	1	0.09 ns	0.02 ns	0.09 **	0.15 ns	0.08 ns
A×L	4	0.37 **	0.00 ns	0.00 ns	0.07 ns	0.08 ns
Error	20	0.03	0.00	0.01	0.09	0.04
CV (%)		10.25	17.73	10.05	8.00	5.76

*, ** and ns: significant at P<0.05, P<0.01 and insignificant, respectively.

Table 6. Means comparison of the effect of auxin on some traits of lily bulblet.

Auxin (mg/L)	No. of bulblet per scale	Bulblet weight (g)	Bulblet diameter (cm)	Root No.	No. of micro scales per bulblet
0	1.52 c	0.66 a	1.15 a	3.65 c	3.86 ab
250 IBA	2.05 a	0.48 b	1.10 a	4.35 a	4.16 a
500 IBA	2.08 a	0.46 bc	1.01 a	4.11 ab	3.95 ab
250 NAA	1.85 b	0.40 bc	0.93 b	3.76 bc	3.30 c
500 NAA	1.66 b	0.36 c	1.04 ab	3.12 d	3.27 c

*In each column, means with the similar letter(s) are not significantly different (P < 0.05) using the Duncan's multiple range test.

Checking the effects of light on the evaluated traits showed (Table 6), presence or absence of the light has no significant difference on number of bulblet per scale, bulblet weight, bulblet root number and number of micro scales per bulblet. Even though bulblet diameter significantly increased under light condition.

Investigating the interaction of light with auxin concentrations on the number of bulblets showed (Table 8) that the highest number of bulblets per scale (2.26 pcs) was obtained at a concentration of 250 mg/L of NAA and the presence of light factor, which had a no significant difference with the number of bulblets produced under another treatments.

Table 7. Means comparison of the effect of light on some traits of lily bulblet.

Light	No. of bulblet per scale	Bulblet weight (g)	Bulblet diameter (cm)	Root No.	No. of micro scales per bulblet
Light presence	1.94[a]	0.50[a]	1.10[a]	3.73[a]	3.80[a]
Light absence	1.82[a]	0.46[a]	0.99[b]	3.92[a]	3.68[a]

*In each column, means with the similar letter(s) are not significantly different (P < 0.05) using the Duncan's multiple range test.

Table 8. Interaction effects of light and substrate on some traits of bulblet lily.

Auxin (mg/L)	Light	No. of bulblet per scale	Bulblet weight (g)	Bulblet diameter (cm)	Root No.	No. of micro scales per bulblet
Control	Light presence	1.56 [c]	0.66 [a]	1.16 [a]	3.63 [a]	3.86 [a]
	Light absence	2.10 [a]	0.66 [a]	1.13 [a]	3.66 [a]	3.86 [a]
250 IBA	Light presence	2.16 [a]	0.53 [a]	1.15 [a]	4.43 [a]	4.26 [a]
	Light absence	2.00 [ab]	0.43 [a]	1.06 [a]	4.26 [a]	4.06 [a]
500 IBA	Light presence	2.00 [ab]	0.46 [a]	1.06 [a]	3.90 [a]	3.86 [a]
	Light absence	2.10 [a]	0.46 [a]	0.96 [a]	4.33 [a]	4.03 [a]
250 NAA	Light presence	2.26 [a]	0.43 [a]	1.00 [a]	3.70 [a]	3.53 [a]
	Light absence	1.43 [c]	0.35 [a]	0.86 [a]	3.83 [a]	3.06 [a]
500 NAA	Light presence	1.70 [bc]	0.40 [a]	1.13 [a]	3.00 [a]	3.30 [a]
	Light absence	1.50 [c]	0.30 [a]	0.90 [a]	3.30 [a]	3.25 [a]

*In each column, means with the similar letter(s) are not significantly different (P<0.05) using the Duncan's multiple range test.

DISSCUSION

Plant growth regulators

Some studies have shown that the production of bulblets per scales affected by various biological factors such as scales position on the bulb, cultivar, scales size and bulb physiological activity levevs and non-biological factors such as temperature, light, humidity, culture media and also chemical treatments such as PGRs (Matsuo *et al*., 1987; Xiaona *et al*., 2013). Evidence showed that PGRs have been widely used to produce and propagate bulbs of geophytes using the scaling technique under *ex vitro* conditions (van Tuyl and Arens, 2012). In this regard Sun *et al*. (2008) found that the use of IBA significantly increased bulb propagation rate of hyacinth through the scaling method. However, Zhang *et al*. (2013) investigated the effects of different concentrations of PGRs (IBA, NAA, and GA_3) on the proliferation of twin scales in amaryllis bulbs in *ex vitro* condition and revealed that PGRs had no significant effects on bulblet proliferation, but enhanced root formatting in scales. The result of present study showed that the use of different concentrations of auxin compounds (NAA and IBA), had significant effects on bulblet production per scale. The results of the present study are in line with the findings of Memar Moshrefi *et al*. (2004).

Several research discus about the effectiveness of auxin-based compounds in the production or propagation of bulblet per each scale. For example, Chung *et al*. (1995) and Gupta *et al*. (2016) reported that IBA was more effective than NAA in producing bulblets. However in some other studies (Dhiman and Sindho, 2007; Masoodi and Nayeem, 2018), there are reports contrary to the results of previous research. The present study showed that different concentrations of IBA had greater impacts on bulblet production compared to NAA. Some studies have suggested that functional differences in PGRs might depend on plant cultivars. The cultivar used in the present study was one of the new hybrids of two large groups of Eastern lilies and Trumpet lilies, which has not been reported for its scaling so far. Accordingly, the production of bulblets induced by PGRs in this plant may be cultivar-dependent.

In the present study, the highest values for diameter and fresh weight of bulblets were obtained under the control treatment, which contradicted the observations of Sharma *et al*. (2007) and Ramos *et al*. (2020). This finding can be due to the greater impacts of cultivar and plant hormones on bulblets. It is also possible that the use of PGRs is involved in changing the balance of plant hormones in bulbs and adverse effects on the production of bulblets. Furthermore, the phenomenon of organogenesis, which includes two stages of differentiation and elongation, is affected by plant hormones and PGRs, but differentiation does not necessarily lead to elongation and cell growth. On the other hand, it seems that not only increasing the fresh weight of bulblets depends on endogenous hormones but also the protein content of the scales (Memar Moshrefi *et al*., 2004).

Generally, the separation of scales from the maternal tissue causes a series of biochemical phenomena in plants that are closely related to the differentiation of adventitious organs (Niimi, 1985). In this regard, wounding in plant tissues and scales also activates the process of protein synthesis. Experiments have shown that actinomycin and cycloheximide, which inhibit protein synthesis, were involved in inhibiting bulblet differentiation. This finding confirms that mRNA transcription and synthesis of new proteins are essential for the differentiation of bulblets. It seems that separating scales from the maternal bulbs and differentiating the bulblets are associated with changing the ratio of plant hormones (especially auxin and cytokines). Some studies have also shown that the measured protein content of bulblets in the control treatment (no PGR applied) was lower than the treatments containing PGRs (Memar Moshrefi *et al*., 2004). Another study showed that PGRs had no significant effects on increasing fresh weight and diameter of bulblets compared to the control treatment, which was consistent with our findings (Bakhshaie *et al*., 2010; Akcal and Kahreman, 2016).

Culture media

The effects of different culture media on the growth of geophyte plants were considered in some studies (Narendra *et al*., 2018; Rajera and Sharma, 2007). Some studies demonstrated that selecting a suitable culture medium (in terms of moisture preservation and adequate ventilation) is of particular importance in plant growth and plant cell differentiation (Suh and Lee, 1996). In the present study, results revealed that the highest values in traits of the number of bulblet per scale, fresh weight of bulblet, bulblet diameter, and scale rot were achieved under the application of the peat moss media, while the lowest amounts of the mentioned traits were observed for the combined medium of coco peat + perlite (1:1).

In general, limited studies have been reported on the effects of peat moss on scaling lilies. Sun *et al*. (2008) investigated the effects of peat moss and sawdust culture media on lily. They reported that the highest propagation rate, root number, bulblet size, and bulblet germination percentage were obtained under the application of sawdust culture medium. Also, Sun *et al*. (2009) investigated the effects of peat moss and sawdust on the *Lilium davidii* and showed that sawdust had the highest propagation coefficient. In general, the latter result does not agree with the findings of the present study and may be due to different culture media and cultivars in both experiments.

Since peat moss was less efficient than coco peat in most of the evaluated traits, coco peat could be used for commercial productions at a lower cost due to its properties almost identical to peat moss. Some studies have confirmed the positive effects and high efficiency of coco peat culture medium compared to other culture media (Dhiman and Sindho, 2007; Gupta *et al*., 2010).

Light factor

The role of light has been identified in the production and stimulation of plant physiological responses, e.g., seed germination, flowering, tuber/root formation, and dormancy (Dole and Wilkins, 2005). Few evidence has been reported for the effects of darkness and light periods on the regeneration stage of lily scales. Niimi (1995) showed that light treatments (especially continuous light) increased the number of bulblets in *Lilium rubellum*. Also, light periods in *Lilium longiflorum* significantly increased the number of bulblets compared to darkness conditions (van Le *et al*., 1999). The formation of corm in gladiolus was also greater in light treatment than in darkness (Dantu and Bhojwani, 1995). In this research light did not effect on most of the studied traits in 'Fujian' cultivar. According to Stimart and Asher (1978), the findings of the present study could be attributed to the physiological responses of different cultivars to different light periods.

CONCLUSION

This experiments indicated, between artificial auxin compounds, indole-3- butyric acid has a more effectiveness increasing on number of bulblets and percentage of bulblet production than naphthalene acetic acid. The number of bulblets and percentage of bulblet production are the most important factor in increasing the quantity of bulb production of lily. Among the culture media, the peat moss culture medium was recognized as the most suitable medium for culturing scales by increasing the production of number of bulbs and reducing the decay of scales. Presence or absence of light did not have a significant effect on the production and quality of the bulbs.

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