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Full Length Research Paper

In vitro micro-propagation of Longiflorum-Asiatic (LA) hybrids lily (*Lilium*) cultivar 'eyeliner'

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Bulblets propagation by tissue culture was one of the key techniques in the production of lily (*Lilium*) bulbs. Therefore, *in vitro* micro-propagation of lily bulblets was studied in detail in this paper. L A hybrids lily cultivar 'eyeliner' was selected as the materials. By using the method of orthogonal design, the following were concluded from the research: the optimum treatment and disinfection methods of 'eyeliner' bulb scales was soaking in 1:500 carbendazim solution for 30 min, disinfection in 75% alcohol for 10 to 60 s, disinfection in 2% NaClO solution for 15 min; the optimum medium for bud induction of 'eyeliner' was MS + 0.5 mg·L^{-1} 6-benzyl aminopurine (6-BA) + 0.1 mg·L^{-1} naphlene acetic acid (NAA) + 90 g·L^{-1}sucrose, and 25°C and in darkness; the optimum medium for bulblets induction of 'eyeliner' was 2MS + 1.0 mg·L^{-1} 6-BA + 0.5 mg·L^{-1} NAA + sucrose 90 g·L^{-1} + Paclobutrazol (PP333) 2 mg·L^{-1}; the optimum culture condition for bulblets induction of 'eyeliner' was 20°C, 14 h·day^{-1} lightness + 10 h·day^{-1} darkness. The optimum medium for rooting culture of 'eyeliner' was ½ MS + 0.8 mg·L^{-1} NAA + 3 g·L^{-1} activated charcoal, 20°C, 14 h·day^{-1} lightness + 10 h·day^{-1} darkness.

Key words: Lily bulb, orthogonal experiment, *in vitro* micro-propagation.

INTRODUCTION

Bulblets propagation by tissue culture was one of the key technologies of lily (*Lilium*) bulb production. In order to establish lily bulb production system, bulblets propagation was very important. As it is known that different species, different explants and different cultural methods led to different cultural results and the bulb scales were very successful explants. *In vitro* and more lily bulb scales were used as explants.

When buried in the ground for a long time, the bulbs infection rate with bacterial was very high and the effect of sterilization was not ideal which led to higher contamination rate of tissue culture. So, selecting the optimum concentration of sterilizing agent and treatment time was a key problem (Lu et al., 2005). The sensitivity of different genotypes to sterilizing agents and sterilization time was different, therefore, choosing different disinfection methods for different cultivars was quite necessary. Sucrose was the energy source of the medium; it could provide a good penetration relationship. Suitable sucrose concentration played an important role in the organogenesis and was essential for the formation and enlargement of the bulblets. Different genotypes of explants and different culture stages led to different optimum concentration of sucrose. Higher concentration of sucrose made explants absorb, transform and store more carbon and promoted bulblets' enlargement. However, too high concentration of sucrose made the osmotic pressure of medium too high, and damaged the induced bulblets. Therefore, an appropriate increase in sucrose concentration was good for the formation and enlargement of bulblets.

There were a lot of researches on the type, concentration, and ratio of plant hormones for lily bulblets' induction (Chen et al., 2001; Jiang et al., 2004; Novak, 1981; Rybcynski and Gomolinska, 1989; Takayama, 1979; Wang et al., 2004) and it was generally considered the growth factors. Naphthyl acetic acid (NAA) and cytokinin 6-benzyl aminopurine (6-BA) were the most suitable for lily scales tissue culture. Commonly used concentration of 6-BA was 0.4 ~2.0 mg·L^{-1}, and NAA was

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Table 1. The combination factors and levels of the treatment and disinfection of explants.

S/N	Factor	Level		
		1	2	3
1	1:500 carbendazim solution (min)	0	15	30
2	75% alcohol(s)	10	30	60
3	2% NaClO (min)	10	15	20

0.1 ~0.5 mg·L^{-1} (Ding et al., 2001; Liu et al., 1997; Wang et al., 1998).

The bud induction and proliferation were affected by the concentration of 6-BA and NAA and the ratio of them. So, choosing the suitable concentration of 6-BA and NAA was key factors. Paclobutrazol (PP_{333}) was a broad spectrum growth retardant which promoted the formation and enlargement of bulblets, root growth and dry matter accumulation. So, choosing the suitable concentration of PP_{333} was another key factor.

Temperature was the most important environmental condition for tissue culture. Generally, low temperature stopped the growth of plants; high temperature was bad for the growth (Wang, 2006). For example, the optimum temperature for *Lilium aurarum* Lindl. and *Lilium speciosum* Thunb. was 20℃ (Takayama, 1979). Light was another factor, it had a great impact on the growth and differentiation of cells, tissues and organs which could promote the absorption of nitrogen, potassium and sugar. Generally, it is believed that the higher concentration of mineral elements was good for the growth of stems and leaves, and lower concentration was good for root induction and rooting culture. Appropriate amount of NAA (or IBA) added to the medium could induce roots, and different concentrations of activated charcoal had a certain effect on the roots induction of the tube bulbs.

Longiflorum-Asiatic (LA) hybrids lily cultivar ‘eyeliner’ was a very beautiful lily cut flower. In order to produce cut flower, mother bulbs were the basic. So, *in vitro* micropropagation of bulblets of ‘eyeliner’ was studied in this paper.

MATERIALS AND METHODS

Experimental material and method

Lily bulbs used in the experiment were imported from the Netherlands, ‘eyeliner’ (LA Lily Hybrids) (size 12 to 14 cm). Multifactor orthogonal experimental design was used and the results of analysis of variance and multiple comparisons were analysed by SPSS software.

Treatment and disinfection of explants

Three factors and three levels were used: 1:500 carbendazim solution (min) (0/15/30), 75% alcohol(s) (10/30/60), and 2% NaClO (min) (10/15/20) as shown in Table 1. The L9 (3^4) orthogonal experimental design was used. Strong, watery, no scab, bright color bulbs were selected. The scales of lily bulbs were peeled before cleaning, and then were cultivated on the medium MS + 6 - BA 0.5 mg·L^{-1} + NAA 0.5 mg·L^{-1}.

Influence of different factors on the induction of lily buds, bulblets and the roots of bulblets

Five factors and three levels were used: 6-BA (mg·L^{-1}) (0.5/1.0/2.0), NAA (mg·L^{-1}) (0.1/0.3/0.5), sucrose concentration (g·L^{-1}) (30/60/90), temperature (℃) (20/25/28), light (darkness/10 h lightness, 14 h darkness/14 h lightness, 10 h darkness) as shown in Table 2. The L27 (3^{13}) orthogonal experimental design was applied. Finally, disinfected lily scales were cultured on the medium of orthogonal design of 27 groups.

Seven factors and three levels were used: 6-BA (mg·L^{-1}) (0.5/1.0/2.0), NAA(mg·L^{-1}) (0.1/0.3/0.5), sucrose concentration (g·L^{-1}) (30/60/90), the concentration of a large number of elements (MS/2MS/3MS), PP_{333} concentration (mg·L^{-1}) (1/2/3), temperature (℃) (20/25/28), and light (darkness/10 h lightness 14 h darkness/14 h lightness 10 h darkness). The L27 (3^{13}) orthogonal experimental design was applied (Table 3). The buds were divided into single bud, and then cultured on the medium of orthogonal design of 27 groups. 45 days later, lily bulblets were transferred to the same medium and continued to be cultured.

Five factors and three levels were used: the concentration of a large number of elements (1/4MS/1/2MS/MS), NAA (mg·L^{-1}) (0.3/0.5/0.8), the concentration of activated charcoal (g·L^{-1}) (0/1/3), temperature (℃) (20/25/28), light (darkness/10 h lightness 14 h darkness/14 h lightness 10 h darkness). The L18 (3^7) orthogonal experimental design was applied (Table 4). Lily bulblets were cultured on the rooting medium of the orthogonal design of 18 groups.

Statistics of the results

Each treatment was 50 bottles, and each bottle contained two explants. 10 to 15 days later, the numbers of contaminated explants were observed and recorded, 45 days later, the number of explants induced bud and the number of final calculated contamination rate and induction rate were observed and recorded.

30 days laters in bud induction stage, the induction conditions (including the survival number, the number of explants which induced buds, the total number of induced buds, bud size and bud growth conditions, etc.) were measured and recorded, then the bud induction rate and average number of induction buds were calculated .

45 days later in bulblets induction stage, the number of bulblets were observed and recorded, then the bulblets rate was calculated. 45 days after transfer, the fresh weight, diameter, average proliferation number and height of bulblets were measured and recorded.

30 days later in rooting stage, the total numbers of roots of bulblets were observed .The length of roots of bulblets, and the number of bulblets which induced roots were measured and

Table 2. The combination factors and levels of different factors on the induction of lily buds.

S/N	Factor	Level		
		1	2	3
a	6-BA (mg·L^{-1})	0.5	1	2
b	NAA (mg·L^{-1})	0.1	0.3	0.5
c	Sucrose concentration (g·L^{-1})	30	60	90
d	Temperature (℃)	20	25	28
f	Light	darkness	10 h lightness 14h darkness	14h lightness 10h darkness

Table 3. The combination factors and levels of the bulblets induction experiment.

S/N	Factor	Level		
		1	2	3
A	6-BA (mg·L^{-1})	0.5	1	2
B	NAA (mg·L^{-1})	0.1	0.3	0.5
C	Sucrose concentration (g·L^{-1})	30	60	90
D	The concentration of a large number of elements	MS	2MS	3MS
E	PP_{333} concentration (mg·L^{-1})	1	2	3
F	Temperature (℃)	20	25	28
G	Light	darkness	10 h lightness 14 h darkness	14 h lightness 10 h darkness

Table 4. The combination factors and levels of the rooting culture experiment.

S/N	Factor	Level		
		1	2	3
A	The concentration of a large number of elements	1/4MS	1/2MS	MS
B	NAA (mg/L)	0.3	0.5	0.8
C	The concentration of activated charcoal (g/L)	0	1	3
D	Temperature (℃)	20	25	28
E	Light	darkness	10 h lightness 14 h darkness	14 h lightness 10 h darkness

recorded. Finally, the average root number, average root length and rooting rate were calculated.

RESULTS

Influence of different factors on the induction of 'eyeliner' buds

The results of treatment and disinfection of the explants of 'eyeliner' are shown in Table 5. The analysis of variance for the results of treatment and disinfection of 'eyeliner' explants is shown in Table 6. It was indicated that the optimum treatment and disinfection methods of 'eyeliner' bulb scales were soaked in 1:500 carbendazim solution for 30 min, disinfected in 75% alcohol for 10 to 60 s, and disinfected in 2% NaClO solution for 15 min (Figures 1, 2 and 3).

Table 7 shows that the influence of 6-BA concentration on the induction rate of 'eyeliner' buds reached a highly significant level. The influence of NAA concentration on the size of buds also reached a significant level; the influence of interaction effect of 6-BA and NAA on the bud induction rate and the average number of induced buds reached a highly significant level. Therefore, the optimum concentration of 6-BA and NAA was determined by the influence of interaction effect. The interaction are shown in Tables 8 and 9; A1B1 (0.5 mg·L^{-1}6-BA, 0.1 mg·L^{-1} NAA) had the highest induction rate of buds, but A3B2 (2.0 mg·L^{-1}6-BA, 0.3 mg·L^{-1} NAA) was the most induced buds.

Table 9 shows that the averages of A1B1 and A3B2 were very close (A1B1 was 3.83, A3B2 3.87). Therefore, from comprehensive point of view, A1B1 (0.5 mg·L^{-1}6-BA,

Table 5. The results of treatment and disinfection of 'eyeliner' explants.

Test number	A	B	C	D	Contamination rate (%)	Induction rate (%)
1	1	1	1	1	86	57.1
2	1	2	2	2	63	81.1
3	1	3	3	3	42	37.9
4	2	1	2	3	45	76.4
5	2	2	3	1	30	48.6
6	2	3	1	2	57	51.2
7	3	1	3	2	18	46.3
8	3	2	1	3	41	66.1
9	3	3	2	1	22	87.2

Table 6. The analysis of variance for the experimental results of treatment and disinfection of 'eyeliner' explants.

Source of variation	Sum of squares of deviation	df	Mean square	*F*	P	Sig.
Contamination rate						
A	773.65	2	386.825	96.265	0.01	*
B	53.348	2	26.674	6.638	0.131	
C	569.556	2	284.778	70.869	0.014	*
Error	8.037	2	4.018			
Total	1404.591	8				
Induction rate						
A	51.66	2	25.83	2.707	0.27	
B	22.282	2	11.141	1.168	0.461	
C	820.869	2	410.434	43.018	0.023	*
Error	19.082	2	9.541			
Total	913.893	8				

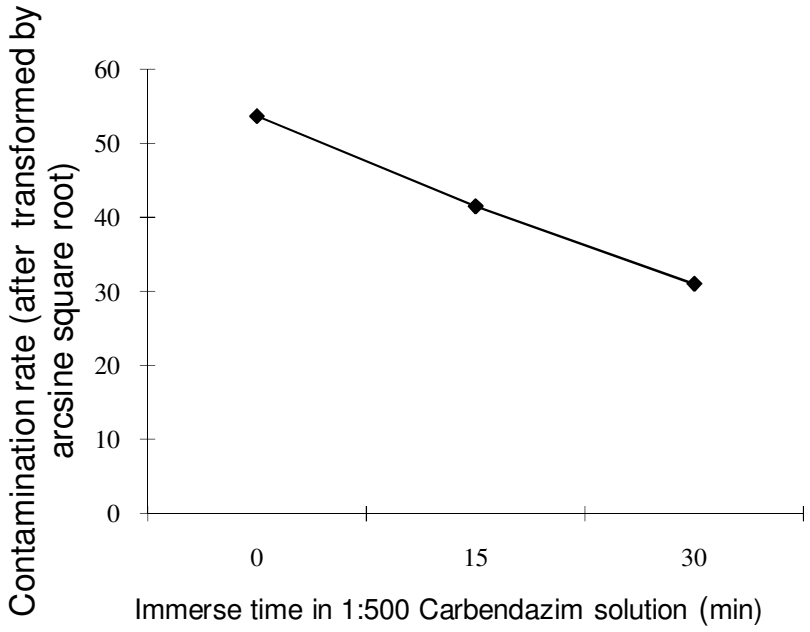

Figure 1. The influence of different treatment time of carbendazim on the contamination rate of 'eyeliner' scales.

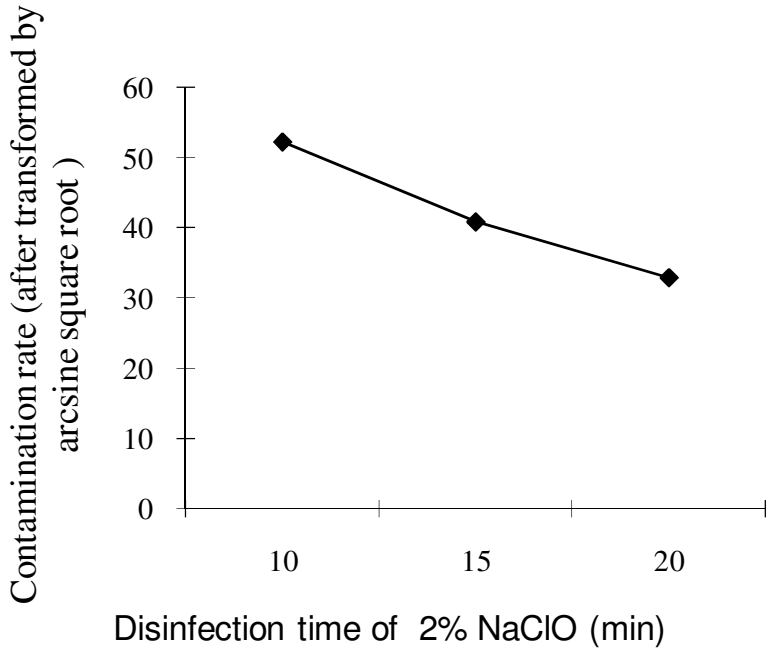

Figure 2. The influence of the disinfection time of 2% NaClO on the contamination rate of 'eyeliner' scales.

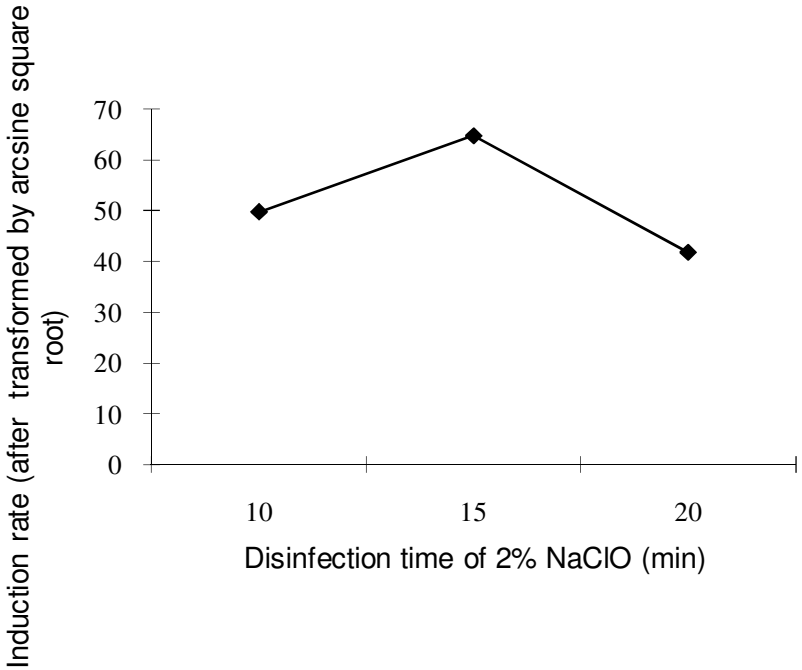

Figure 3. The influence of the disinfection time of 2% NaClO on the induction rate of 'eyeliner' scales.

0.1 mg·L^{-1} NAA) had better effect on the induction of 'eyeliner' buds.

As it could be seen from Table 7, the sucrose concentration had the greatest effect on the size of buds, and reached a significant level, however, the influence of sucrose concentration on the induction rate and the average number of proliferation buds did not reach a significant level. The decreasing order of different

Table 7. The analysis of variance for the experimental results of different factors on ‘eyeliner’ buds induction.

Source of variation	Sum of squares of deviation	df	Mean square	*F*	P	Sig.
Induction rate						
A	82.559	2	41.28	31.616	0	**
B	4.342	2	2.171	1.663	0.23	
C	0.695	2	0.348	0.266	0.771	
D	907.388	2	453.694	347.485	0	**
E	4.555	2	2.277	1.744	0.216	
A*B	89.822	4	22.456	17.199	0	**
Error	15.668	12	1.306			
Total	1105.029	26				
Average buds						
A	0.202	2	0.101	2.084	0.167	
B	0.009	2	0.004	0.092	0.913	
C	0.327	2	0.163	3.366	0.069	
D	1.949	2	0.974	20.084	0	**
E	1.849	2	0.924	19.053	0	**
A*B	1.129	4	0.282	5.817	0.008	**
Error	0.582	12	0.049			
Total	6.047	26				
Bud size						
A	0.032	2	0.016	0.204	0.818	
B	1.05	2	0.525	6.732	0.011	*
C	0.765	2	0.383	4.907	0.028	*
D	0.716	2	0.358	4.594	0.033	*
E	1.39	2	0.695	8.912	0.004	**
A*B	0.261	4	0.065	0.838	0.527	
Error	0.936	12	0.078			
Total	5.15	26				

concentrations of sucrose inducing different buds was: 90 $g \cdot L^{-1}$>60 $g \cdot L^{-1}$>30 $g \cdot L^{-1}$. This result show that the suitable concentration of sucrose for the induction of ‘eyeliner’ buds was 90 $g \cdot L^{-1}$.

Table 7 shows the influence of different temperature on bud induction rate; the average number of buds and bud size were different in the order: 25>20>28℃, and the influence of different temperature on bud induction rate and the average number of proliferation buds reached a highly significant level (Table 7). It was indicated that 25℃ was the optimum temperature for the induction of ‘eyeliner’ buds.

As can be seen from Table 7, the different light conditions had significant effect on the average number of buds and bud size, but had no significant effect on the induction rate; it induced the most robust and the largest number of buds in the dark. Therefore, darkness is the optimum light condition for the induction of ‘eyeliner’ buds.

Influence of different factors on the induction of ‘eyeliner’ bulblets

The buds were separated into single bud. 20 days later, lily bulblets came into being. The results are shown in Table 10, the analysis of variance is shown in Table 11. By comprehensive analysis of all aspects, it could be concluded that 1.0 $mg \cdot L^{-1}$6-BA, and 0.5 $mg \cdot L^{-1}$ NAA were the optimum conditions for the formation and enlargement of the ‘eyeliner’ bulblets; 90 $g \cdot L^{-1}$ sucrose was the optimum condition of the induction of ‘eyeliner’ bulblets; the optimum concentration of a large number of elements for the induction of ‘eyeliner’ bulblets was 2 MS; the optimum concentration of PP_{333} for the induction of ‘Eyeliner’ bulblets was 2 $mg \cdot L^{-1}$; 20℃ was the best condition for ‘eyeliner’ bulblets induction and 14 h light + 10 h darkness was optimum for the induction of ‘eyeliner’ bulblets.

Table 11 shows that 6-BA had a great effect on bulblets

Table 8. The interaction between factor A and B (buds induction rate).

A level	B Level		
	B_1	B_2	B_3
A_1	63.2	58.3	54.7
A_2	62.3	57.6	62.3
A_3	49.5	58.2	53.4

A_1 means the first level of factor A and so on.

Table 9. The interaction between factors A and B (the average number of induced buds)

A level	B Level		
	B_1	B_2	B_3
A_1	3.83	3.6	3.33
A_2	3.6	3.2	3.6
A_3	3.37	3.87	3.8

rate, the average number of bulblets and the height. When its concentration was 1.0 mg·L^{-1}, the average number of bulblets and height were up to the highest level. The concentration of NAA had a significant effect only on height, and the height showed increasing trend with increase in NAA concentration. When its concentration was 0.5 mg·L^{-1}, the height was the highest. The interaction effect of 6-BA and NAA had no significant effect on the five indicators. So, by comprehensive analysis of all aspects, it was found that 1.0 mg·L^{-1}6-BA, 0.5 mg·L^{-1} NAA was the optimum conditions for the formation and enlargement of the 'eyeliner' bulblets.

Table 11 shows that the sucrose concentration had a significant effect on the fresh weight of bulblets, bulblets rate, average number of bulblets and height, and had a significant effect on the bulblets diameter. The five indicators showed increasing trend with increase in the sucrose concentration; when the sucrose concentration was 90 g·L^{-1}, the above five indicators reached the optimum level. Comprehensive analysis could be concluded: 90 g·L^{-1} sucrose was the optimum condition for the induction of 'eyeliner' bulblets.

When the concentration of a large number of elements was 2 MS, the bulblets rate, average number of bulblets and height reached the optimum level. Table 10 shows that the influence of the concentration of a large number of elements on the bulblets rate, average number of bulblets and height reached a significant level. Therefore, the optimum concentration of a large number of elements for the induction of 'Eyeliner' bulblets was 2 MS.

Table 11 shows that the influence of the concentration of PP_{333} on bulblets rate and height reached a significant level, but different concentrations of PP_{333} affected two indicators differently. When the concentration of PP_{333} was 2 mg·L^{-1}, the bulblets rate was the highest (Figure 4). The height decreased with the concentration of PP_{333} increase. When the concentration of PP_{333} was 1 mg·L^{-1}, the height was the highest (Figure 5). It was indicated that PP_{333} inhibited the growth induction of 'eyeliner' bulblets, and promoted their formation. Also, in the induction of 'eyeliner' bulblets stage, the importance of indicators bulblets rate, average number of bulblets, fresh weight of bulblets and bulblets diameter height was much higher than the height. Therefore, the optimum concentration of PP_{333} for the induction of 'eyeliner' bulblets was 2 mg·L^{-1}.

Table 11 shows that the influence of temperature on bulblets weight and bulblets diameter reached a significant level, and influence of temperature on bulblets rate and average number of bulblets reached a significant level. The four indicators were all increased with temperature decrease; when the temperature was 20℃, 'eyeliner' bulbles induction was the best.

The analysis is shown in Table 11: light had an effect only on the enlargement of bulblets. With the number of illumination increased, the bulblets enlargement was more obvious. When the number of illumination was 14 h, the bulblets fresh weight and diameter achieved the best level. Therefore, 14 h light +10 h darkness was optimum for the induction of 'eyeliner' bulblets.

Influence of different factors for the induction of roots of 'eyeliner' bulblets

'Eyeliner' bulblets began to have roots after 17 days induction of roots, and the induction of roots were almost complete around 30 days. The results are shown in Table 12; the analysis of variance is shown in Table 13. Therefore, 1/2 MS was the optimum concentration of a large number of elements for the induction of roots of 'eyeliner' bulblets; 0.8 mg·L^{-1} was the optimum concentration of NAA for the induction of roots of 'eyeliner' bulblets; 3.0 g·L^{-1} was the optimum concentration of activated charcoal; the optimum temperature for the induction of roots of 'eyeliner' bulblets was 25℃ and 14 h was the optimum light hours for the induction of roots of 'eyeliner' bulblets.

It can seen from Table 13 that when the concentration of a large number of elements was 1/2 MS, the average number of roots, root length and rooting rate all reached the highest value and the concentration of a large number of elements had a significant effect on the average number of roots and root length, and had significant effect on rooting rate. So, 1/2 MS was the optimum concentration of a large number of elements for the induction of roots of 'eyeliner' bulblets.

It can be seen from Table 13 that the concentration of NAA had a significant effect on the average number of roots and rooting rate, and that the concentration of NAA had a significant effect on the average root length. With

Table 10. The experimental results of the influence of different factors on 'eyeliner' bulblets induction.

S/N	A	B	A* B	A*B	C	D	E	F	G	H	I	J	K	Fresh weight of bulblet (g)	Bulblets diameter (mm)	Bulblets rate (%)	Average proliferation bulblet	Height (cm)
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.73	11.26	94	3.3	10.9
2	1	1	1	1	2	2	2	2	2	2	2	2	2	2.25	12.58	100	3.7	11.1
3	1	1	1	1	3	3	3	3	3	3	3	3	3	2.18	12.17	87	3.1	9.6
4	1	2	2	2	1	1	1	2	2	2	3	3	3	1.69	11.05	92	2.8	10.5
5	1	2	2	2	2	2	2	3	3	3	1	1	1	1.98	11.59	100	3.1	11.3
6	1	2	2	2	3	3	3	1	1	1	2	2	2	2.31	12.97	91	3.6	10.8
7	1	3	3	3	1	1	1	3	3	3	2	2	2	1.52	10.63	89	3	11.6
8	1	3	3	3	2	2	2	1	1	1	3	3	3	2.05	11.88	100	3.9	11.9
9	1	3	3	3	3	3	3	2	2	2	1	1	1	2.2	12.12	94	3	10.8
10	2	1	2	3	1	2	3	1	2	3	1	2	3	1.94	11.33	91	4.2	11.9
11	2	1	2	3	2	3	1	2	3	1	2	3	1	2.37	13.05	90	3.5	11.4
12	2	1	2	3	3	1	2	3	1	2	3	1	2	2.28	12.81	95	4.1	11.2
13	2	2	3	1	1	2	3	2	3	1	3	1	2	1.88	11.6	93	3.7	10.2
14	2	2	3	1	2	3	1	3	1	2	1	2	3	1.21	10.34	85	2.9	11
15	2	2	3	1	3	1	2	1	2	3	2	3	1	2.98	13.49	98	4.4	11.6
16	2	3	1	2	1	2	3	3	1	2	2	3	1	0.92	9.65	83	3.6	10.7
17	2	3	1	2	2	3	1	1	2	3	3	1	2	1.85	11.53	88	3.8	12
18	2	3	1	2	3	1	2	2	3	1	1	2	3	2.11	11.96	95	4.2	12.1
19	3	1	3	2	1	3	2	1	3	2	1	3	2	2.3	12.62	90	2.9	8.6
20	3	1	3	2	2	1	3	2	1	3	2	1	3	1.47	10.46	82	3.1	9.7
21	3	1	3	2	3	2	1	3	2	1	3	2	1	1.93	11.2	92	3.6	11.7
22	3	2	1	3	1	3	2	2	1	3	3	2	1	1.79	11.54	84	2.8	9.3
23	3	2	1	3	2	1	3	3	2	1	1	3	2	1.6	10.82	87	3	9.8
24	3	2	1	3	3	2	1	1	3	2	2	1	3	3.26	14.28	97	3.7	12.9
25	3	3	2	1	1	3	2	3	2	1	2	1	3	1.15	9.91	81	2.8	10
26	3	3	2	1	2	1	3	1	3	2	3	2	1	3.11	14.17	88	2.9	11.3
27	3	3	2	1	3	2	1	2	1	3	1	3	2	2.08	11.56	96	3.4	12.6

increase in the concentration of NAA, three indicators were gradually enhanced. When the concentration of NAA was 0.8 $mg \cdot L^{-1}$, the measured indicators reached the best level. Therefore, 0.8 $mg \cdot L^{-1}$ was the optimum concentration of NAA for the induction of roots of 'eyeliner' bulblets.

Analysis of variance (Table 13) shows that the concentration of activated charcoal had a significant effect on roots length and had a significant effect on the average number of roots and rooting rate. When activated charcoal was not added, three indicators reached the worst level. Therefore, 3.0 $g \cdot L^{-1}$ was the optimum concentration of activated charcoal for theinduction of roots of 'eyeliner' bulblets.

It can be seen from Table 13 that temperature had a great effect on the rooting, rooting rate and the number of roots; reached a significant level. But the same conclusion came into being: when the temperature was 25℃, the three indicators

Table 11. The analysis of variance for the experimental results of different factors on 'eyeliner' bulblets induction.

Source of variation	Sum of squares of deviations	df	Mean square	F	P	Sig.
Fresh weight of bulblets						
A	0.077	2	0.038	0.49	0.63	
B	0.19	2	0.095	1.214	0.346	
C	2.287	2	1.143	14.641	0.002	**
D	0.081	2	0.04	0.517	0.615	
E	0.119	2	0.059	0.759	0.499	
F	2.546	2	1.273	16.3	0.002	**
G	1.352	2	0.676	8.659	0.01	*
A*B	0.505	4	0.126	1.615	0.261	
Error	0.625	8	0.078			
Total	7.78	26				
Bulblets diameter						
A	0.036	2	0.018	0.032	0.969	
B	1.29	2	0.645	1.128	0.37	
C	9.354	2	4.677	8.176	0.012	*
D	0.054	2	0.027	0.047	0.954	
E	0.808	2	0.404	0.706	0.522	
F	11.548	2	5.774	10.094	0.006	**
G	5.898	2	2.949	5.155	0.036	*
A*B	2.731	4	0.683	1.193	0.384	
Error	4.576	8	0.572			
Total	36.295	26				
Bulblets rate						
A	285.613	2	142.807	25.883	0	**
B	11.133	2	5.566	1.009	0.407	
C	175.879	2	87.94	15.939	0.002	**
D	431.145	2	215.572	39.072	0	**
E	316.999	2	158.5	28.727	0	**
F	83.588	2	41.794	7.575	0.014	*
G	15.484	2	7.742	1.403	0.3	
A*B	17.449	4	4.362	0.791	0.563	
Error	44.139	8	5.517			
Total	1381.429	26				
Average proliferation bulblets						
A	2.376	2	1.188	23.756	0	**
B	0.127	2	0.063	1.267	0.333	
C	0.996	2	0.498	9.956	0.007	**
D	1.127	2	0.563	11.267	0.005	**
E	0.242	2	0.121	2.422	0.15	
F	0.722	2	0.361	7.222	0.016	*
G	0.08	2	0.04	0.8	0.482	
A*B	0.098	4	0.024	0.489	0.745	
Error	0.4	8	0.05			
Total	6.167	26				
Height						
A	2.154	2	1.077	5.828	0.027	*
B	2.987	2	1.494	8.082	0.012	*

Table 11. Contd.

C	5.194	2	2.597	14.052	0.002	**
D	6.483	2	3.241	17.539	0.001	**
E	5.836	2	2.918	15.79	0.002	**
F	1.603	2	0.801	4.337	0.053	
G	0.099	2	0.049	0.267	0.773	
A*B	1.566	4	0.391	2.118	0.17	
Error	1.479	8	0.185			
Total	27.401	26				

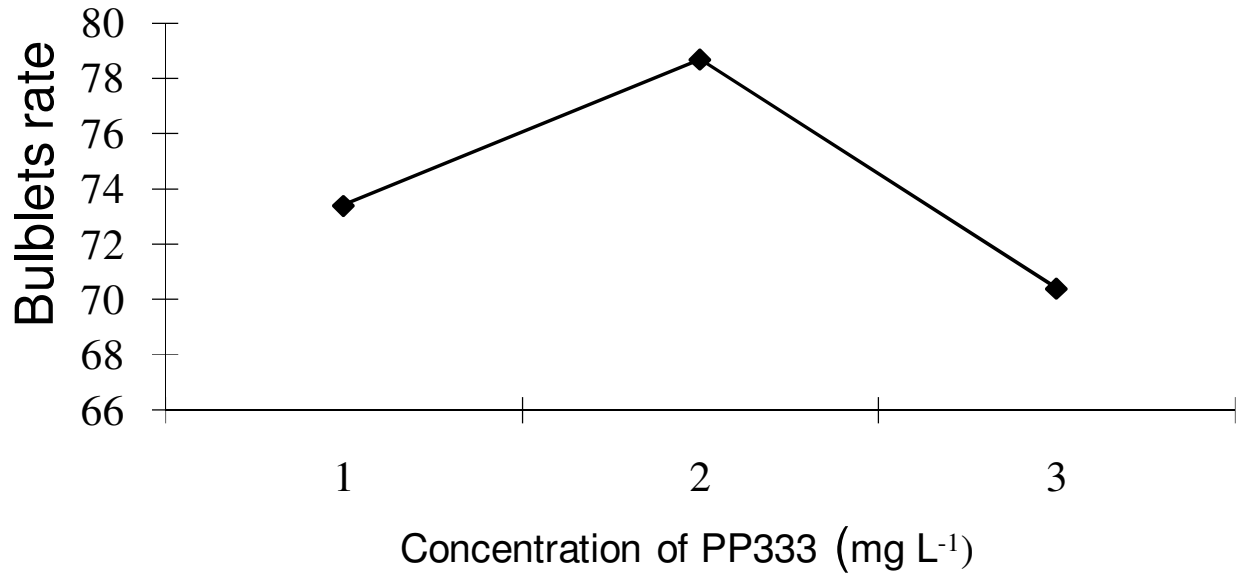

Figure 4. The influence of different PP_{333} concentration on the bulb formation rate of 'eyeliner'.

reached the optimum level. Therefore, the optimum temperature for the induction of roots of 'eyeliner' bulblets was 25°C.

Analysis of variance (Table 13) shows that the influence of light on rooting rate did not reach a significant level, but reached a significant level on the average number of roots and average root length and different hours of light induced different number of roots and average root length, in the order: 14>10>0 h. Thus, 14 h was the optimum light hours for the induction of roots of 'Eyeliner' bulblets.

DISCUSSION

The experimental materials were imported from Netherlands, and the bulbs had been planted once. Due to contact with the substrate, the bulbs were infected with part of the fungal, so, lily bulbs pretreatment must be completed. The pretreatment was necessary for the induction of scales. It was indicated that the optimum treatment and disinfection methods of 'eyeliner' bulb scales were soaked in 1:500 carbendazim solution for 30 min, disinfected in 75% alcohol for 10 to 60 s, and disinfected in 2% NaClO solution for 15 min.

'Eyeliner' was the hybrids of Asiatic and the hybrids between L/A, the size of bulbs were 12 to 14 cm, the scales were relatively thin, and the materials used in this experiment were middle scales, so scales contained less nutrients, and smaller incision size of scales. To induce the induction of 'eyeliner' buds, the optimum condition of culture was 0.5 mg·L^{-1}6-BA, and 0.1 mg·L^{-1}NAA. The suitable concentration of sucrose for the induction of'eyeliner' buds was 90 g·L^{-1}. 25°C was the optimum temperature for the induction of 'eyeliner' buds and darkness is the optimum light condition for the induction of 'eyeliner' buds. The optimum conditions for the formation and enlargement of the 'eyeliner' bulblets was 1.0 mg·L^{-1} 6-BA, 0.5 mg·L^{-1} NAA, 90 g·L^{-1} sucrose 2MS, 2 mg·L^{-1} of PP_{333}, 20°C,14 h light and 10 h darkness. The

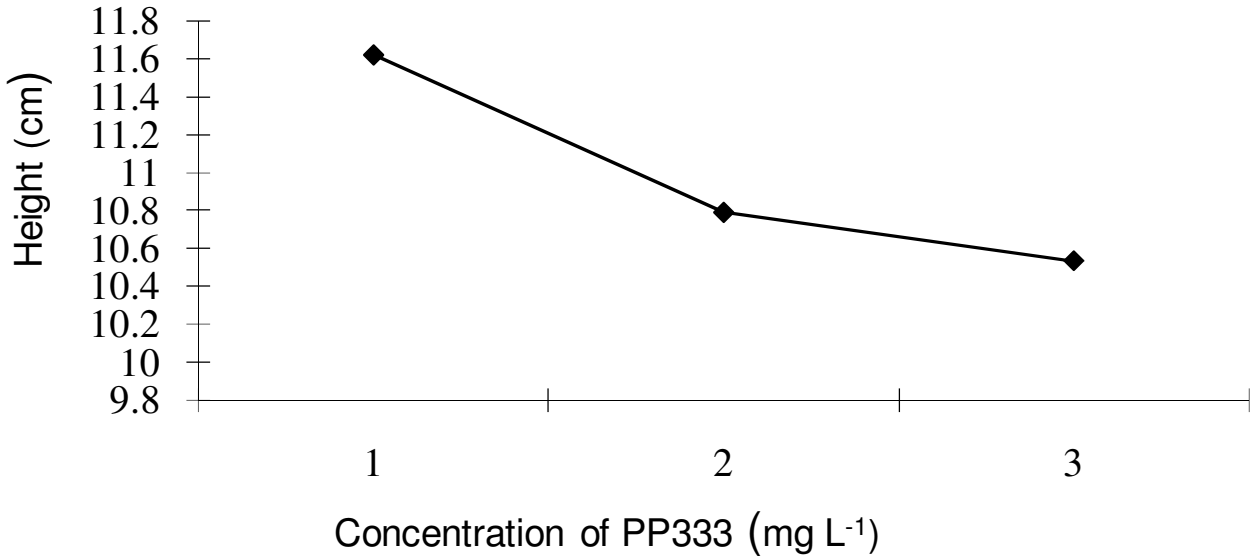

Figure 5. The influence of different PP_{333} concentration on the height of 'eyeliner'.

Table 12. The experimental results of the influence of different factors on 'eyeliner' rooting culture.

S/N	A	B	C	D	E	F	G	Average number of roots	Average roots length (cm)	Roots rate (%)
1	1	1	1	1	1	1	1	3.7	2.4	90
2	1	2	2	2	2	2	2	5.2	3.9	96
3	1	3	3	3	3	3	3	6	4.9	95
4	2	1	1	2	2	3	3	5.8	4.4	95
5	2	2	2	3	3	1	1	6.3	5.8	93
6	2	3	3	1	1	2	2	7	6.1	100
7	3	1	2	1	3	2	3	5.5	4.8	97
8	3	2	3	2	1	3	1	5.9	5.2	99
9	3	3	1	3	2	1	2	4.9	3.5	94
10	1	1	3	3	2	2	1	4.5	3.4	89
11	1	2	1	1	3	3	2	5.2	3.9	84
12	1	3	2	2	1	1	3	5.5	4.1	97
13	2	1	2	3	1	3	2	4.7	3	92
14	2	2	3	1	2	1	3	7.1	5.7	98
15	2	3	1	2	3	2	1	7.6	5.9	100
16	3	1	3	2	3	1	2	7.5	5.4	98
17	3	2	1	3	1	2	3	4.4	3.1	87
18	3	3	2	1	2	3	1	7.1	5.5	97

Table 13. The analysis of variance for the experimental results of different factors on rooting culture of 'eyeliner'.

Source of variation	Sum of squares of deviations	df	Mean square	F	P	Sig.
Average number of roots						
A	5.991	2	2.996	15.45	0.003	**
B	3.484	2	1.742	8.986	0.012	*
C	3.441	2	1.721	8.874	0.012	*
D	3.974	2	1.987	10.249	0.008	**

Table 13. Contd.

E	3.968	2	1.984	10.232	0.008	**
Error	1.357	7	0.194			
Total	22.216	17				
Average roots length						
A	5.803	2	2.902	19.913	0.001	**
B	3.72	2	1.86	12.765	0.005	**
C	4.69	2	2.345	16.093	0.002	**
D	2.743	2	1.372	9.413	0.01	*
E	3.943	2	1.972	13.531	0.004	**
Error	1.02	7	0.146			
Total	21.92	17				
Roots rate						
A	157.018	2	78.509	7.886	0.016	*
B	163.092	2	81.546	8.191	0.015	*
C	107.846	2	53.923	5.416	0.038	*
D	218.938	2	109.469	10.995	0.007	**
E	3.36	2	1.68	0.169	0.848	
Error	69.691	7	9.956			
Total	719.945	17				

optimum conditions for the formation and enlargement of the induction of roots of ‘eyeliner’ bulblets were 1/2 MS, 0.8 mg·L^{-1} of NAA, 3.0 g·L^{-1} of activated charcoal, 25℃, 14 h.

In conclusion, in this work, the optimum conditions for the LA hybrids lily cultivar ‘eyeliner’ micro-propagation *in vitro* were obtained, and it contributed to the success of the propagation of lily. In the further work, other optimum conditions for other cultivar of lily will be studied, and the propagation of lily in our country will develop more and more speedily.

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