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Temperature Sensitivity for Flowering of Bulblets in *Lilium formolongi*

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Abstract. *Lilium formolongi* 'F1 August' plantlets with scale-leaves and scale-bulb were treated at 10, 15, 20, 25°C for 15, 30, or 45 days and planted in February, March, April and May. In the April planting, flowering percentage was below 10% and in the May planting no flowering occurred. Sprouting and flowering percentages were lower at the late planting times. Days to flowering of the April planting was 110.8 days compared to 128 days for the February planting. Plant height and numbers of leaves were reduced to 7.2 in May planting, compared to 40.5 leaves in February planting, and almost no flowers emerged in either the April or May plantings. Plantlets exposed to 10 or 15°C flowered at 80 percent or higher at all treatment durations, but at 20 or 25°C flowering percentages were lower, with 30% or less in the 25°C treatment. In the 15°C treatment days to flowering were less than 100 days, while the number of flowers and flower bud differentiation were greatest in the 15°C treatment.

Cytokinin and auxin were analyzed in bulblets grown at 15, 20 and 25°C. T-zeatin content was three times greater in the 15°C treatment than at 25°C, but the content of indoleacetic acid (IAA) was less at 15°C than at 20 and 25°C. In the 15°C treatment, t-zeatin content was about twice the IAA content in the scale- bulblet. The auxin and cytokinin balance may affect flower bud differentiation. 15°C with 30 days was most effective for flowering in scale-bulblet and planting of February and March were effective for flowering too.

Key words : duration, growth temperature, indoleacetic acid, trans-zeatin riboside

Introduction

Lily is famous for cut-flower (Suh, 2001). Especially *Lilium formolongi* was high price in flower market than other lilies. *Lilium formolongi* was the product of the crossing between *L. formosanum* and *L. longiflorum* (Chou, 1983; Comb, 1949; Hiramatsu et al., 2002; Wilson 1925). *Lilium formolongi* is propagated mainly by seeds. Seeds sown during December to February flowered in August (Goo et al., 1995, 2003). The price of cut flower is very low due to all plants flowering and being harvested at the same time in August; thus, the income of farmers is very low. Hence, another culture system for cut flower production during September until October is required to be established in *Lilium formolongi* with the use of bulbs and bulblets (Okubo et al., 1988). The objectives of this research were to establish a culture system with the use of bulblets and plantlets from scale bulblets in order to determine the temperature sensitivity for shoot development and elongation of *Lilium formolongi* bulblets and plantlets.

Materials and Methods

Lilium formolongi 'F1 August' bulblets formed from scale about 1cm diameter were planted during February, March, April and May for flowering. Five hundred bulblets were used per treatment. A randomized complete block design and Duncan's multiple range test were used for this experiment. 'F1 August' plantlets with scale-leaf and scale-bulb were treated at 10, 15, 20, or 25°C for 15, 30, or 45 days in growth chambers in order to increase the flowering percentage. Light or dark condition during temperature treatments were also tested for an influence on flowering.

In t-zeatin and indole acetic acid (IAA) determination, t-zeatin and IAA were extracted with 30% methanol/ 0.1 M acetic acid 4 ml each. The extracted solution was adjusted to pH 3.0. The solution was concentrated for one night under vacuum. 500 μl to 500 μl concentrated solution another 500 μl of TBS buffer (pH 7.5) was added and mixed with vortex. 100 μl test solution was

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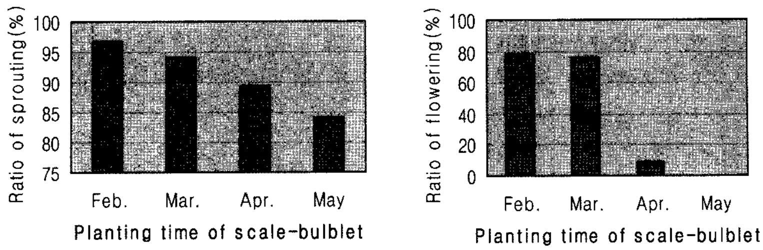

Fig. 1. Ratio of sprouting and flowering according to planting time of scale-bulblet in *L. formolongi* 'F1 August'.

pipetted into plate wells coated with antibody in the wall and incubated for three hours at 4°C. The plate well was washed two times with washing solution. After adding 200 μl substrate solution in the well and incubating for one hour at 37°C, the responded wells were tested at 405 nm optical density with ELISA reader (Ito et al., 1999). Cytokinin standard solution was used with trans-zeatin riboside and antibody was used with t-ZR (PDK 09348/0096) and IAA (PDK 09346/0096).

Results and Discussion

Sprouting percentages were above 80% for all planting times (Fig. 1) but in April flowering percentages were below 10% and in the May planting there was no flowering. Sprouting decreased with the later planting dates. Days to flower for the April planting were 110.8 days, which was less than for the earlier planting times (Table 1). Plant height and numbers of leaf were reduced in May planting, with leaf number 7.2 compared to 40.5 leaves for the February planting (Table 2).

For bulblets subjected to 5 or 8°C temperatures for 2 or 4 weeks, the flowering ratio was below 50%. Flowering ratio for the 5°C treatment was about 10% higher than for the 8°C treatment (Table 3). In the combinations of temperature and duration (Table 4), the flowering ratio was above 80% in the 10°C treatment, above 90% in the 15°C treatment, but at higher temperatures, the flowering ratio was lower below 30% in the 25°C treatment. This result was similar to the study of paprika (Choi et al, 2004). In this result, 15°C treatment was more effective to initiation of flower bud and development of flower. Days to flowering were fewer than 100 days in the 15°C treatment. Bolting ratio was higher in the 15°C treatment than others. More flower bud differentiation occurred in temperatures below 15°C. Flower number was highest in the 15°C treatment. Plants were taller at 15°C than for other

Table 1. Days to sprouting and flowering on planting time of scale-bulblet in *L. formolongi* 'F1 August'.

Planting time of bulblet	Days to sprouting	Days to flowering
February	30.5a^z	121.3a
March	20.8ab	118.1ab
April	18.5b	110.8b
May	17.9b	-

^{z}Mean separation within columns by Duncan's multiple range test at 5% level.

Table 3. Characteristics of flowering by the treatment temperature and duration time of scale-bulblet in *L. formolongi* 'F1 August'.

Treatment	Flowering ratio (%)	No. of flower
Control	34.2b^z	1.0a
8°C 2 weeks	32.5b	1.0a
4 weeks	38.7ab	1.0a
5°C 2 weeks	48.3a	1.1a
4 weeks	42.6ab	1.0a

^{z}Mean separation within columns by Duncan's multiple range test at 5% level.

Table 2. Plant growth and flower characteristics on planting time of scale-bulblet in *L. formolongi* 'F1 August'.

Planting time of bulblet	Plant height (cm)	No. of leaf	No. of flower	Width of flower (cm)	Height of flower (cm)
February	104.0a^z	40.5a	1.3	13.1	16.3
March	111.7a	38.4a	1.1	12.7	16.6
April	58.2b	25.3b	-	-	-
May	26.3c	7.2c	-	-	-

^{z}Mean separation within columns by Duncan's multiple range test at 5% level.

Table 4. Ratio of bolting, flowering and days to bolting and flowering by the treatment of temperature and duration on plantlet from scale-bulblet in *L. formolongi* 'F1 August'.

Treatment	Bolting ratio (%)	Days to bolting	Flowering ratio (%)	Days to flowering
10°C 15 days	88.9ab[z]	43.3b	88.9ab	102.3ab
30 days	83.3ab	45.6b	83.3ab	101.4ab
45 days	88.9ab	52.3ab	88.9ab	112.6ab
15°C 15 days	94.4a	37.2b	94.4a	98.1b
30 days	88.9ab	40.2b	88.9ab	94.5b
45 days	94.4a	42.6b	94.4a	92.6b
20°C 15 days	35.3c	61.0a	35.3c	122.7a
30 days	75.0b	56.7ab	64.0b	120.4a
45 days	55.0bc	61.3a	55.0bc	123.2a
25°C 15 days	27.8c	62.2a	27.8c	124.8a
30 days	23.8c	65.4a	23.8c	130.2a
45 days	30.0c	63.5a	30.0c	127.6a

[z]Mean separation within columns by Duncan's multiple range test at 5% level.

Table 5. Plant growth and flower characteristics on planting time of plantlet from scale-bulblet in *L. formolongi* 'F1 August'.

Treatment	Plant height (cm)	No. of leaf	No. of flower	Width of flower (cm)	Diameter of stalk (mm)
10°C 15 days	118.0ab[z]	32.3b	1.6ab	14.3b	4.3b
30 days	120.3ab	33.6b	1.3b	13.8b	5.1ab
45 days	123.8ab	33.5b	1.2b	14.1b	5.2ab
15°C 15 days	123.0ab	35.1ab	1.8a	14.2b	4.2b
30 days	128.0ab	33.0b	2.0a	14.3b	6.8a
45 days	132.4a	36.7ab	1.9a	14.6b	5.3ab
20°C 15 days	143.5a	32.5b	1.0b	14.3b	3.8b
30 days	138.2a	32.1b	1.1b	14.1b	4.1b
45 days	140.3a	34.0ab	1.2b	14.2a	3.6b
25°C 15 days	105.0b	33.0b	1.0b	16.5a	5.0ab
30 days	145.0a	42.0a	2.0a	17.5a	5.0ab
45 days	127.5ab	35.4ab	1.2b	14.6a	3.8b

[z]Mean separation within columns by Duncan's multiple range test at 5% level.

Table 6. Bolting and flowering by the treatment of temperature and duration with light condition on plantlet from scale-bulblet in *L. formolongi* 'F1 August'.

Treatment	Bolting ratio (%)	Days to flowering	Flowering ratio (%)
Control	-	-	-
15°C 2 weeks	39.7b[z]	97.1b	83.7a
4 weeks	42.5b	98.5b	82.3a
6 weeks	41.3b	95.8b	87.6a
20°C 2 weeks	62.5a	123.2a	53.2b
4 weeks	64.2a	113.6ab	61.7b
6 weeks	58.4a	125.4a	53.4b

[z]Mean separation within columns by Duncan's multiple range test at 5% level.

temperature treatments (Table 5).

The effect of light and dark conditions at 15 or 20°C on plantlet, bolting ratio was higher at 20°C, and days to flowering were fewer at 15°C in both light and dark condition. Flowering ratio was affected more by the temperature than by light or dark and it was higher at 15°C (Tables 6, 7). The low temperature of night is effective for compact plant in the result of pepper (Seo et al,

Table 7. Bolting and flowering by the treatment of temperature and duration with dark condition on plantlet from scale-bulblet in *L. formolongi* 'F1 August'.

Treatment	Bolting ratio (%)	Days to flowering	Flowering ratio (%)
Control	-	-	-
15°C 2 weeks	43.5b[z]	107.1ab	81.0a
4 weeks	46.3b	101.3ab	85.8a
6 weeks	45.6b	95.7b	52.6ab
20°C 2 weeks	66.8a	124.3a	63.2ab
4 weeks	68.3a	118.5a	51.8ab
6 weeks	60.2a	127.2a	43.6b

[z]Mean separation within columns by Duncan's multiple range test at 5% level.

Table 8. Effects of temperature on t-zeatin and IAA content of bulblets and leaves in *L. formolongi*'F1 August'.

Hormone ($ng\cdot g^{-1}$ FW)	Temperature (°C)					
	15		20		25	
	Bulblet	Leaf	Bulblet	Leaf	Bulblet	Leaf
T-zeatin	239.0±10.2[z]	173.0±8.5	170.3±8.6	87.3±4.5	73.9±4.7	145.0±7.4
IAA	124.6±9.3	159.2±10.1	147.4±9.8	172.3±12.1	141.2±10.2	155.7±14.2

[Z]Mean±Standard error (n=30)

2006).

In the analysis of hormones in bulblets (Table 8), t-zeatin content was greater in the 15°C treatment than at 20 or 25°C, at 239 $ng\cdot g^{-1}$, it was about three times greater than in the 25°C treatment. T-zeatin content was much in the bulblet than leaf with low temperature treatment and opposite with high temperature treatment. The content of indoleacetic acid (IAA) in the bulblets (124.6 $ng\cdot g^{-1}$) given the 15°C treatment was less than for bulblets given 20 and 25°C treatments. In the 15°C treatment, t-zeatin content was about two times greater than the IAA content in the scale- bulblet. If leaves perceive the temperature, flower bud differentiation within the plant body might be caused by an optimum balance between cytokinin (T-zeatin) and auxin (IAA) (Kim et al., 1968). The result of IAA was similar to the result of Xu et al. (2008), IAA was produced much in the low temperature treatment and the position was internodes and anthers.

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신나팔나리(*Lilium formolongi*) 인편자구의 개화를 위한 온도 감응

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적 요. 본 실험은 신나팔나리 인편자구 유묘 및 인편자구를 이용한 새로운 재배작형개발을 위해 온도처리 및 정식시기별 생육 및 개화 경향을 조사하고자 신나팔나리 'F1 August'의 인편자구 유묘와 인편자구는 10, 15, 20 및 25°C에 15, 30 및 45일 동안 처리하였고, 인편자구는 2, 3, 4 및 5월에 각각 정식하였다. 정식시기별 인편자구의 개화율에 있어 4월 정식구는 개화율이 10% 미만이었으며 5월 정식구에서는 전혀 개화하지 않았다. 맹아 및 개화율은 정식시기가 늦을수록 낮았다. 인편자구 4월 정식구의 개화소요일수는 2월 정식구 128일에 비해 110.8일로 다소 짧았다. 5월 정식구의 엽수는 2월 정식구의 엽수 40.5매보다 훨씬 적은 7.2매 였으며 초장도 짧았고 4월 및 5월 정식구는 초기 생육기에 저온을 받지 않아 생육도 부진했을 뿐만 아니라 개화도 거의 되지 않았다. 온도처리에 따른 인편자구 유묘는 10 또는 15°C에 처리한 유묘는 처리기간에 관계없이 80% 이상 개화하였으나, 20°C 또는 25°C에 처리한 유묘의 개화율은 30% 이하로 낮았으며 25°C 처리구는 가장 낮았다. 15°C 처리구는 개화소요일수가 100일 이하로 가장 짧았고, 꽃수 및 추대율도 가장 높았다.
사이토카이닌 및 옥신은 15, 20 및 25°C 온도 감응한 인편자구 유묘를 분석하였다. T-zeatin 함량은 15°C 처리구에서 25°C 처리구보다 3배나 많았고, IAA 함량은 20 및 25°C 처리구보다 낮았다. 15°C 처리구의 t-zeatin 함량은 IAA 함량의 약 2배에 달했다. 이처럼 옥신과 사이토카이닌의 균형이 화아분화에 영향을 미치는 것으로 나타났다. 인편자구 유묘의 온도처리 시 광을 동시에 처리하는 것이 개화소요일수에 다소 효과적이었다.
따라서 인편자구 유묘는 15°C에 30일 처리가 개화에 효과적이었고 인편자구는 2월 및 3월 정식이 생육시 저온을 받아 개화에 효과적이었다.

주제어 : 기간, 생육온도, 인돌초산, trans-zeatin riboside