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Translocation of maturity factors in the double-nose bulbs of *Lilium longiflorum* and *L. ×elegans*

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ABSTRACT

Translocation of maturity factors through the basal plate of double-nose *L. longiflorum* Thunb. 'Nellie White' and *L. ×elegans* Thunb. was investigated. Both mother (M) and daughter (D) scales were attached (+M+D) or the scales were removed (-M-D) from one or both sides of the double-nose bulb. The double-nose 'Nellie White' bulbs were grouped into +M+D/+M+D, +M+D/-M-D, and -M-D/-M-D. The basal plate of the +M+D/-M-D 'Nellie White' bulb was separated 0, 10, 20, 30, and 40 days after potting. The basal plate of the +M+D/-M-D 'Inferno' bulb was separated at 0, 4, 8, and 16 days after potting. The maturity factor evaluated by the speed of shoot emergence and the number of flowers and leaves was translocated from the +M+D bulb to the -M-D bulb of the non-separated +M+D/-M-D bulb. The translocation of maturity factors in 'Nellie White' bulbs was completed before bulb separation in 40 days after potting. In 'Inferno' no differences in the number of flowers and leaves were observed between shoots that emerged from the +M+D bulb and the -M-D bulb of the +M+D/-M-D bulb, indicating that the maturity factors in 'Inferno' were not translocated. The differences in translocation responses between 'Nellie White' and 'Inferno' are that the shoot apex in 'Nellie White' is under the vegetative growth stage and the shoot apex in 'Inferno' is under the reproductive development stage when the vernalized bulbs are potted following scale removal treatment.

Keywords: basal plate, flower bud formation, vernalization.

INTRODUCTION

Bulb for forcing the Easter lily (*Lilium longiflorum* Thunb.) and the Asiatic hybrid lily (*Lilium ×elegans* Thunb.) is composed of mother and daughter scales, basal plate, and basal roots (De Hertogh *et al*., 1971; Roh 1990; Roh and Wilkins, 1977a, b). Bulb vernalization of the Easter lily (Roh and Wilkins, 1977c) and Asiatic hybrid lilies (Lee *et al*., 2008; Roh, 1985) induced a uniform shoot emergence and early flowering. However, bulb vernalization of the

Easter lily reduced the number of flower buds (Roh and Wilkins, 1977c), while increasing the number in the Asiatic hybrid lily (Lee *et al*., 2008, 2010). Roh and Wilkins (1977a) reported that when the bulb harvest dates were delayed, the maturity factors were primarily concentrated in the daughter scales, which had shifted away from the mother scales. Maturity in *L. longiflorum* is defined as shoot emergence without delay and acceleration of flowering responding to bulb vernalization (De Hertogh *et al*., 1971).

Once maturity is induced by bulb vernalization (De Hertogh *et al*., 1971) in both the Easter lily (Roh and Wilkins, 1977c) and Asiatic hybrid lily (Lee *et al*., 2008; Roh, 1985; 1990), maturity factors in the bulb is considered as the main physiological response and the translocation must be completed before the basal plate of a double-nose bulb is separated into two single-nose bulbs. The basal plate of double-nose bulbs should be connected to observe the translocation of the maturity factors.

There was no report on the translocation of maturity factors influenced by the timing of separation of a double-nose bulb into two single-nose bulbs in *L. longiflorum* and further in *L.* ×*elegans* Asiatic hybrid lily. Therefore, translocation of maturity factors from the bulb with all scales that were not removed (+M+D bulb) to the bulb after removing mother scales (M) and daughter scales (D) (-M-D bulb) in the +M+D/-M-D double-nose bulb was investigated. These experiments were initiated using double-nose bulbs to (a) evaluate the differences in the translocation of maturity factors between 'Nellie White' and 'Inferno' and (b) investigate the effect of separation of double-nose (DN) bulbs into two single-nose bulbs in 'Nellie White' and 'Inferno'.

MATERIALS AND METHODS

Plant materials and general culture

Double-nose *L. longiflorum* 'Nellie White' and *L.* ×*elegans* 'Inferno' lily bulbs were used in the experiments. All scales were intact on one side of a double-nose bulb [(+M+D) bulb] and all scales, except the 5 innermost small scales were removed on the other side [(-M-D) bulb] which was not separated through the basal plate of the double-nose bulb (+M+D/-M-D bulb). The scales were not removed (+M+D/+M+D bulb) or removed from both sides (-M-D/-M-D bulb). The shoot in 'Nellie White' did not elongate and did show an evident stem surrounded by the unfolded leaves (Fig. 1). However, in 'Inferno' the shoots were elongated by approximately 1 – 2 cm with folded leaves.

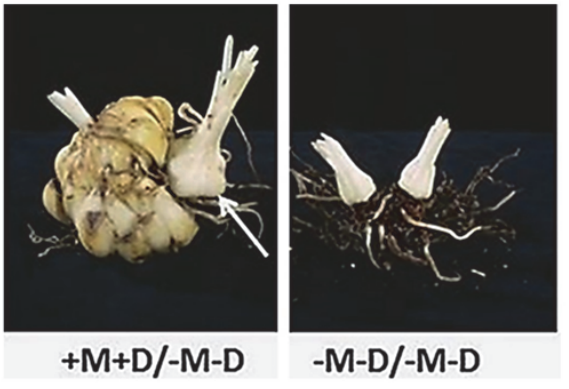

Figure 1. Scale removal treatments of double-nose *L. longiflorum* 'Nellie White'.
All scales were not removed (+M+D bulb) or removed leaving 5 inner scales (arrow) (-M-D bulb). Scales in the -M-D bulb connected to the +M+D bulb in +M+D/-M-D bulb through the basal plate were enlarged as compared to scales attached to the -M-D bulb in -M-D/-M-D bulb. Bulbs were lifted 20 days after potting at the sign of shoot emergence from the -M-D axis for photographing

After the scales were removed, the bulbs were potted singly about 5 cm deep from the surface of the growing medium to the nose of the +M+D bulb or 10 cm deep from the tip of the shoot in 15 cm pots filled with a growing medium as described (Lee *et al*., 2008) and grown in a greenhouse maintained at 20/16^{o}C (day: 08:00-16:00hr/night: 16:00 – 08:00hr). There were 10 bulbs per treatment and each plant was considered an experiment unit. All pots were completely randomized following bulb separation during forcing in the greenhouse.

Translocation in* L. longiflorum *'Nellie White' as influenced by removal of the scales and separation of the basal plate of the double-nose bulb

Bulbs (18 – 21 cm in circumference) were treated for 8 weeks at 2.5^{o}C, and after scale removal treatments in the double-nose bulbs (+M+D/+M+D, +M+D/-M-D, and -M-D/-M-D bulb) bulbs were potted on Jan. 4 (Table 1). The basal plate of the +M+D/-M-D bulb was separated into two single-nose bulbs using a scalpel and forceps 10, 20, 30, or 40 days after potting (Table 2). The growing medium was carefully separated without disturbing the basal roots and stem roots when the double-nose bulbs were separated 40 days after potting.

Translocation in* L. ×elegans *'Inferno' - separation of the double-nose bulb after potting

Double-nose bulbs (14 – 17 cm in circumference) were packed in vermiculite and Metro Mix 250 growing medium (Sun Grow Horticulture, Bellevue, WA, USA) (1:1, by volume, 50% moisture; packing medium) after 8 weeks at 2.5^{o}C. Scales were removed as described in *L*. *longiflorum*, and bulbs were first placed on top of 5 cm deep of the packing medium and covered with another layer of 5 cm packing medium. Bulbs were stored at 21/12.5^{o}C for 3 days in the packing medium, and then the presence of flower buds was confirmed by dissecting the shoot apices from 5 bulbs under a microscope (AO - American Optical Microscope, Buffalo, NY, USA). Bulbs were lifted from the packing medium 0, 4, 8, 12, and 16 days after storing bulbs, and then double-nose bulbs were separated, and then potted on Jan. 14 as described above.

Data analysis

The number of days to shoot emergence and flowering was counted from the date of potting. At anthesis, the number of flowers and leaves, and plant height were recorded as described previously (Roh and Wilkins, 1977b) for both cultivars. Data were subjected to analysis of variance using general linear model procedures (Lee *et al*., 2008). The variables were scale removal treatment (Table 1) separation of the double-nose 'Nellie White' bulbs (Table 2), and scale removal and bulb separation for 'Inferno'. The number of flower buds (x) was transformed using the formula $[(x + 0.5)^{1/2}]$ and analyzed. Regression analysis was performed for the variables of bulb separation in time.

RESULTS AND DISCUSSIONS

The biochemical nature of maturity factors in *Lilium* is not fully understood yet. Therefore, physiological characteristics such as shoot emergence, flowering, plant height, and the number of flower buds are used as indicators of maturity. Bulb maturity in terms of shoot emergence and flowering may also depend on the fulfilment of cold temperature requirements for the daughter axis (Roberts and Moller, 1971; Roberts e*t al*., 1978).

Some factors associated with the speed of shoot emergence and flowering, and the number of flower buds in 'Nellie White' bulbs are partially translocated from the +M+D side to the -M-D side through the basal plate of the +M+D/-M-D double-nose bulb when double-nose

bulbs are not separated (Roh and Wilkins, 1977a, b). However, the scales present attached to the +M+D axis may not have sufficient reserves to fully support the flowering and increase the number of flower buds in the -M-D side to the same level as the +M+D side of the +M+D/-M-D double-nose bulb. The exact nature of the reserves that are translocated from the +M+D side to the -M-D side in double-nose bulbs, whether they are carbohydrates or glycerol, a primary metabolite primarily synthesized in the outer scales (Lazare *et al.,* 2019), is not yet known.

Translocation in* L. longiflorum *'Nellie White' as influenced by removal of the scales from the non-separated double-nose bulb

When the basal plate of the double-nose bulb was not separated and all scales were not removed from both sides of the double-nose bulbs (+M+D/+M+D bulb), shoots emerged in 15 days with a difference of 0.5 days between the two bulbs. However, shoots emerged in 8 or 9 days when the scales of both single bulbs in the double-nose bulb were removed and non-separated (-M-D/-M-D bulb), which was significantly earlier as compared to shoot emergence from the +M+D/+M+D bulb. Shoots from the -M-D bulb emerged significantly later in 11 days in the +M+D/-M-D bulb than in -M-D/-M-D bulb, but earlier than the +M+D/+M+D bulb which emerged in 15 days from the +M+D bulb (Table 1, Fig. 1).

Shoot from the -M-D bulb in the +M+D/-M-D bulb emerged 4.1 days earlier than that from the +M+D bulb; however, 3 days later from the -M-D bulb in the -M-D/-M-D bulb (Table 1). Flowering was significantly delayed from 110 – 112 days when scales were attached to both bulbs (+M+D/+M+D bulb) to 126 days when scales were removed from both shoots (-M-D/-M-D bulb) (Table 1; Fig. 2). Flowering was significantly accelerated to 119 days from the -M-D bulb in the +M+D/-M-D bulb, as compared to bulbs which took 110 – 112 days in the +M+D/+M+D bulbs. The number of flowers was 3.5/3.7 in the +M+D/+M+D bulb, and significantly fewer than 0.9 flowers (-M-D/-M-D bulb) (Table 1). However, the number of flowers from the -M-D bulb was 2.0 when connected to the +M+D bulb which produced 3.4 flowers. The difference in the number of flowers was the greatest (1.4) in the +M+D/-M-D bulb as compared to the +M+D/+M+D bulbs (0.2) and the -M-D/-M-D bulbs (0.0).

Plant height in 'Nellie White' increased from 14 - 16 cm in the -M-D/-M-D bulb to 17 cm from the -M-D bulb in the -M-D/+M+D bulb (data not presented). The number of leaves was significantly increased from the -M-D bulb (77 leaves) in the -M-D/+M+D bulb as compared to 40 - 44 leaves from the -M-D/-M-D bulb. The reduction of plant height from both +M+D and -M-D bulbs as compared to the heights in both shoots (24 cm) of the +M+D/+M+D bulb showed a similar trend in the number of flower buds.

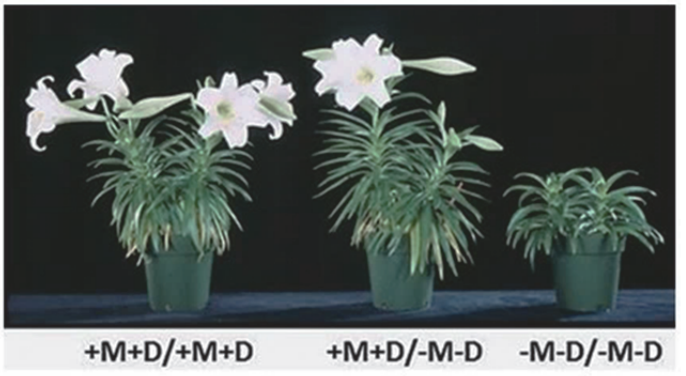

Figure 2. Flowering of double-nose *L. longiflorum* 'Nellie White' bulb as affected by scale removal treatments. From left to right; non-separated +M+D/+M+D; +M+D/-M-D; M-D/-M-D double-nose bulb

Table 1. The effect of scale removal treatment on the growth and flowering of *L. longiflorum* 'Nellie White'

Treat-ment		Number of days to						No. of leaves			Number of flowers		
Scale treatment [z]		Shoot emergence			Flowering								
+M +D (A)	-M -D (B)	A	B	DI [y]	A	B	DI	A	B	DI	A	B	DI
+	+	15	15	0.5	110	112	-2.3	78	81	-3.0	3.7	3.5	0.2
-	-	9	8	0.9	126	126	0.9	40	44	-3.8	0.9	0.9	0.0
+	-	15	11	4.1	117	119	-2.0	75	77	-1.7	3.4	2.0	1.4
HSD [x] $p<0.01$		2.9	2.4	1.2	2.3	3.1	0.97	10.8	9.7	0.92	1.52	2.03	0.58

[z] Scale treatment; +, all scales were attached to one side (+M+D bulb; A); -, all scales were removed from the other bulbs (+M+D or -M-D bulb; B) of the double-nose bulb (+M+D/-M -D bulb).; [y] Difference (DI) in shoot emergence, flowering, number of flowers, and number of leaves between the +M+D bulb (A) and the -M-D bulb (B); [x] Tukey's HSD (honestly significant difference) test at p<0.01.

Translocation in* L. longiflorum *'Nellie White' as influenced by the removal of the scales and the separation of the basal plate of the double-nose bulb

When the basal plate of the M+D/-M-D double-nose bulb was separated 10, 20, 30, and 40 days after potting, a shoot emerged in 11 days from the -M-D bulb which was not significantly different from 11 – 13 days, depending on the separation dates in the +M+D/-M-D bulb (Table 2). Shoot emergence from the +M+D bulb took 18 days when the basal plates were separated 30 days after potting. The differences of shoot emergence between +M+D/-M-D bulbs was 2.6 days when double-nose bulbs were separated 10 days after potting, and differences were greater than 3.7 days when +M+D/-M-D bulbs were separated in 20 and 40 days. There were no significant differences in flower numbers from the +M-D bulbs when double-nose bulbs were separated 20 - 40 days after potting over bulbs when the bulbs were not separated. Plant heights from the +M+D and the -M-D bulbs did not differ significantly as affected by separation dates of the +M+D/-M-D bulb (data not presented). However, the number of leaves from the -M-D bulb was increased to 74 when bulbs were separated 40 days after potting, which was not significantly different from the bulbs that were not separated (data not presented). When bulbs were separated 10 and 20 days after potting, the number of leaves was the fewest (56 leaves), which was significantly different from bulbs that were not separated or were separated 30 and 40 days after potting.

Translocation in* L. ×elegans *'Inferno' – removal of scales and separation of the double-nose bulb

Only two -M-D 'Inferno' bulbs of the -M-D/+M+D bulbs emerged, and shoot emergence (16 days) was not different from that (17 days) of the +M+D/+M+D bulbs (Table 4). When bulbs were not separated or separated 4 and 8 days after potting, shoot emergence (16 days) was not different from that (17 days) of the +M+D/+M+D bulbs (Table 3).

When bulbs were not separated or separated 4 and 8 days after potting, shoot emergence from the -M-D bulb took 13 and 12 days, respectively, and shoot emergence was delayed to 15 or 17 days when bulbs were separated 12 and 16 days after potting, respectively.

Shoot emergence of 'Inferno' bulbs that were separated 16 days after potting of the +M+D/+M+D bulb took 18/17 days, which was not different from bulbs that were not separated or the -M-D bulb of the +M+D/-M-D bulb that were separated 16 days after

potting. However, both shoots from the -M-D/-M-D bulb failed to flower even when both bulbs were not separated (Table 3).

Table 2. The effect of scale removal treatment and bulb separation on the growth and flowering of *L. longiflorum* 'Nellie White'

Treatment			Number of days to						Number of flowers		
SC [z]		Sepa-ration [y]	Shoot emergence			Flowering					
A	B		A	B	DI [x]	A	B	DI	A	B	DI
+	-	0 [w]	15	11	4.1	117	119	-2.0	3.4	2.0	1.4
+	-	10	15	12	2.6	115	123	-7.1	3.4	0.9	2.5
+	-	20	17	11	5.4	114	124	-9.7	3.1	1.1	2.0
+	-	30	18	13	5.0	117	126	-8.8	3.5	1.7	1.8
+	-	40	15	11	3.7	117	128	-9.9	3.0	1.9	1.1
HSD [v] $p<0.01$			2.2	2.1	1.9	5.3	6.4	3.8	0.87	0.92	0.94

[z] Scale treatment (SC); +, all scales were attached to one side (+M+D bulb; A); -, scales were removed from the other side (-M-D bulb; B) of the double-nose bulb (+M+D/-M-D bulb); [y] The basal plate of the double-nose bulb was not separated (-) or separated 10, 20, 30, and 40 days after potting on Jan. 4; [x] Difference (DI) in shoot emergence, flowering, and the number of flowers between the +M+D bulb (A) and the -M-D bulb (B); [w] Number of days after potting when the basal plate of the double-nose bulb was separated; [v] Tukey's HSD (honestly significant difference) test at p<0.01.

The -M-D bulb flowered ranging from 54 to 58 days when the +M+D/-M-D bulb was not separated or separated between 4 and 16 days after potting, respectively, which were not different from the flowering of the +M+D bulbs. Plant height of the +M-D bulb did not differ significantly between the +M+D/+M+D bulbs that were not separated; however, plant height was significantly shorter when separated 4, 8, 12, and 16 days after potting, ranging from 23 to 29 cm. The number of flowers was not significantly different by scale removal treatments, which produced 4.1 and 4.4 flowers from the +M+D bulb, and 4.0 and 4.6 flowers from the -M-D bulb when double-nose bulbs were not separated and separated 4 to 16 days after potting, respectively (Table 3). This could be attributed to the fact that flower bud initiation is completed before shoot emergence when bulbs are fully vernalized (Lee *et al.,* 2008, 2010; Ohkawa *et al.,* 1990; Pergola and Roh, 1987; Roh, 1985; 1990).

CONCLUSIONS

In *L. longiflorum* 'Nellie White', maturity factors evaluated by the speed of shoot emergence, the number of flowers and leaves, and plant height are translocated from the +M+D bulb to the -M-D bulb through the basal plate of the vernalized and non-separated double-nose +M+D/to -M-D bulb. The translocation is successful before the bulb separation in 40 days after potting before the initiation of flower buds. In *L. ×elegans* 'Inferno', maturity factors were not translocated and were not affected by scale removal treatment in the -M-D bulb of the +M+D/-M-D double-nose bulb. The observed differences in translocation responses of maturity factors might be attributed to the contrasting differences in the time of flower bud initiation; shoot apex under a vegetative growth in 'Nellie White' and under a reproductive development in 'Inferno' at the time of potting of the vernalized bulbs after scale removal treatments.

Table 3. The effect of scale removal treatment and bulb separation on the growth and flowering of *L.* ×*elegans* 'Inferno'

Treatment			Number of days to				Plant height (cm)		Number of flowers	
Scale [z]		Separa-	Shoot emergence		Flowering		A	B	A	B
A	B	tion [y]	A	B	A	B				
+	+	0	17	17	54	54	33	33	4.6	4.7
+	-	0	17	16	54	54	34	34	4.1	4.0
-	-	0	16 (2)[x]	--[w]	--	--	--	-	--	--
+	-	4	17	13	57	57	26	29	4.2	4.6
-	-	4	--	--	--	--	--	--	--	
+	-	8	17	12	56	56	29	32	4.6	4.4
-	-	8	--	--	--	--	--	--	--	
+	-	12	17	15	55	56	25	27	4.7	4.3
-	-	12	17 (5)	17 (3)	--	--	--	--	--	
+	-	16	17	17	57	58	23	23	4.4	4.1
-	-	16	18 (7)	17 (5)	--	--	--	--	--	--
+	+	16	18	17	55	55	32	33	4.8	4.8
Level of significance										
HSD [v] $p<0.01$			1.9	3.2	2.8	4.1	3.4	2.5	0.54	0.65
Regression analysis [u]										
Separation - linear			ns	*	ns	ns	*	*	ns	ns
-quadratic			ns	ns	ns	ns	**	**	ns	ns

*[z] Scale treatment; +, all scales were attached to one side (+M+D bulb; A); -, all scales were not or removed from the other side (+M-D or -M-D bulb; B) of double-nose bulb (+M+D/-M-D bulb); [y] The basal plate of the double-nose bulb was not separated (0) or separated 10, 20, 30, and 40 days after potting; [x] Number of days to shoot emergence and the number of bulbs with emerged shoots when all scales were removed from both bulbs of the double-nose bulb (-M-D/-M-D bulb); [w] Failed to emerge and flower when all scales were removed from both axes; [v] Tukey's HSD (honestly significant difference) test; [u] ns, *, **: non-significant, significant at 5% and 1%.*

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