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Frequency of Viable Seeds Obtained from Several *Lilium* spp. Cross－Pollinated at Different Floral Stages

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Summary

To study the influences of the floral stage at the time of pollination on the production of viable seeds in *Lilium* spp., flower buds and flowers were cross－pollinated 1 and 3 days before anthesis (D －1 and D －3), at anthesis (D 0) and 1, 3 and 5 days after anthesis (D +1, D +3, and D +5).

The optimum times of pollination for maximum seed production of each cross－combination were D －1, D 0 and D +1 in 'Enchantment' × *L. maculatum*, 'Enchantment' × 'Connecticut King' and 'Georgia' × 'Hinomoto', respectively. It was D 0, D +1 and D +3, respectively, for 'Connecticut King' × *L. maculatum* and 'Connecticut King' × 'Enchantment'. In *L. rubellum* × *L. speciosum* 'Uchida' a few seeds were produced only when they were pollinated at D －3 and D +5.

The viability of seeds obtained in four cross－combinations was estimated by a pattern of cumulative percentage germination of seeds for 30 days, cumulative percentage germination 30 days after sowing (PG), and mean days to germination (MDG). The results varied among cross－combinations and floral stages at pollination. Seeds, produced from pistils pollinated after anthesis, germinated more rapidly at the higher cumulative percentage than did those from pistils pollinated at and before anthesis.

On the basis of these results on the yield of viable seeds and seed germination, we conclude that a large number of high quality seeds can be produced consistently between compatible cross－combinations of *Lilium* spp. when pistils are pollinated within a few day after anthesis

Key Words: cross－pollination, *Lilium* spp., pistil, seed viability, timing of pollination.

Introduction

Obtaining many high quality seeds is important to yield good healthy plantlets in breeding program for *Lilium* spp. It has been reported that for *Lilium* spp., the success or failure of fertilization and the number of seeds produced are affected by temperature during cultivation, particularly at pollination (Van Tuyl et al., 1982) and pollen quality (Niimi and Shiokawa, 1992). Niimi et al. (1997) showed that flower age at pollination affects the number and germinability of seeds produced in cross－pollination of *L.* hybrid 'Enchantment'. The flowers of 'Enchantment', crossed at anthesis, produced fewer seeds than did those pollinated after anthesis, but the seeds, produced at anthesis, germinated most rapidly with the highest percentage germination as compared with those of other flower ages. This study was made to clarify whether the floral stage at the time of pollination which influences the number and viability of seeds, is limited to 'Enchantment' or common in *Lilium* spp.

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Materials and Methods

1. Plant materials and culture condition

Seven *Lilium* spp., *L. rubellum* Baker, *L. longiflorum* Thunb. 'Hinomoto' and 'Georgia', *L. speciosum* Thunb. 'Uchida', *L.* hybrid 'Enchantment', *L.* hybrid 'Connecticut King' and *L. maculatum* were raised as follows: three bulbs per 24－cm clay pot or six per plant box (54 × 34 × 20 cm) were planted in October 1996 and allowed to overwinter outdoors; these plants were transferred to an unheated plastic tunnel (4.5 × 20 × 2.4 m) before pollination, and afterward both sides of the tunnel were kept open during the experiments.

2. Pollination

After emasculation 1 or 2 days before pollination, pistils were covered with aluminum foil to avoid contamination and pollinated with fresh pollen which were collected at anthesis in a plastic tunnel, or with pollen stored at －10 ℃ as described previously (Niimi and Shiokawa, 1992). Germination of fresh or stored pollen was tested before pollination (Table 1) by culturing pollen in a 10－ml Erlenmeyer flask that contained 3 ml

Table 1. In vitro percentage germination of fresh and stored pollen tested at the time of pollination.

Lilium species	Full blooming time	Fresh pollen		Stored pollen	
		No. test	Germination (%)	No. test	Germination (%)
L. rubellun	27 May to 11 June	10	62.5	-[z]	-
L. logiflorum 'Georgia'	4 July to 12 July	6	63.6	-	-
L. longiflorum 'Hinomoto'	4 July to 12 July	5	47.1	-	-
L. hybrid 'Enchantment'	25 July to 2 July	6	87.5	-	-
L. hybrid 'Connecticut King'	5 July to 12 July	6	85.1	-	-
L. speciosum 'Uchida'	16 Aug. to 26 Aug.	6	80.4	-	-
L. maculatum	27 May to 6 June	-	-	5	53.8(1)[y]

[z]Not determined.

[y]Parenthesized figures indicate the storage term (month) of pollen used.

of liquid medium composed of 100 ppm H_3BO_3 and 10% (w/v) sucrose on a gyratory shaker (90 rpm) for 3 hr at 24 ± 1 ℃ under constant light. The pollen was fixed and stained with 0.08% lactophenol-cotton blue solution and 50 pollen grains per visual field in10 fields were observed under a light microscope. They were considered to be germinated when they produced a pollen tube that was at least equal to the pollen grain diameter.

Five to 20 pistils were pollinated 3 days before anthesis (D −3) to 5 days after anthesis (D +5).

3. *Seed germination*

Germination of seeds, obtained from pistils pollinated at different floral stages in 4 cross-combinations (Table 3), was tested by harvesting the enlarged capsules about 3 months after pollination. The number of seeds with well-developed embryo and endosperm was determined by the method described previously (Niimi et al., 1997).

Five seeds were sown per cell (5 × 5 × 5 cm) and filled with a potting medium (clay soil: peat moss = 1:1, v/v) in a plastic container. The experiment was arranged in a split-plot-design in 5 random blocks, each of which consisted of 2 cells for the seeds of each cross-combination. They were placed in a heated greenhouse kept at 10 ℃-20 ℃ (night) and 20 ℃-30 ℃ (day).

The number of germinated seeds was recorded every 3 days for 30 days. Mean days to germination (MDG) were calculated according to the formula, $\Sigma(fx)/\Sigma f$ where f is the number of germinated seeds on day x (Nichols and Heydecker, 1968).

4. *Statistical analysis*

Data were subjected to analysis of variance, and the least significant difference (LSD) procedure was used for mean separation when the F-test was significant at $p \leq 0.05$. However, standard errors were calculated in *L. rubellum* × *L. speciosum* 'Uchida' when data could not be subjected to analysis of variance.

Results

Experiment 1. Production of capsules and seeds from cross-pollinated flowers

All pollinated pistils in both 'Enchantment' × *L. maculatum* and 'Enchantment' × 'Connecticut King' developed capsules with seeds except that only 25% of the former and 39% of the latter crosses developed capsules when pollinated at D +5 (Table 2). More than 266 seeds developed in 'Enchantment' × *L. maculatum* and 150 in 'Enchantment' × 'Connecticut King' when flowers (D 0 and D +1) or flower buds at D -1 were crossed.

All pollinated pistils developed capsules with seeds in 'Connecticut King' × *L. maculatum* and 'Connecticut King' × 'Enchantment', although the floral stage at the time of pollination affected the number of seeds. More than 100 seeds were produced in pistils pollinated after anthesis in 'Connecticut King' × *L. maculatum* as in 'Connecticut King' × 'Enchantment'; in the latter, there were more than 150 seeds per capsule when pollinated at D +3.

In 'Georgia' × 'Hinomoto', all pistils which were pollinated at D 0 and D −1 developed capsules with more than 150 seeds. However, flowers buds pollinated at D -3 and flowers at D +3 and D +5 contracted diseases after pollination, wilted, and resulted in a few capsules with seeds.

Fifty to 100% of flowers or flower buds pollinated at each floral stage developed small capsules in *L. rubellum* × *L. speciosum* 'Uchida'; flower buds pollinated at D -3 and flowers at D +5 produced a few seeds per pistil.

Experiment 2. Seed germination

Seed germination of 'Georgia' × 'Hinomoto' was not tested because pistils pollinated at D +3 and D +5 contracted diseases as stated above. Seeds of four combinations began to germinate between 9 and 12 days after sowing (Fig. 1). Cumulative percentage germination of seeds, produced from pistils pollinated at each

Table 2. Numbers of capsules and seeds produced from flowers cross - pollinated at different floral stages (D - 3 to D+5) in *Lilium* spp.

Floral stages	'Enchantment' × *L. maculatum*			'Enchantment' × 'Connecticut King'			'Connecticut King' × *L. maculatum*		
	No. pistils pollinated	Capsule fomation (%)[z]	No. seeds per pistil pollinated	No. pistils pollinated	Capsule fomation (%)	No. seeds per pistil pollinated	No. pistils pollinated	Capsule fomation (%)	No. seeds per pistil pollinated
D−3	15	100	203b[w]	18	100	91c	15	100	58c
D−1	15	100	266a	15	100	160a	15	100	70c
D 0	15	100	283a	16	100	157a	14	100	77b
D+1	15	100	297a	15	100	150a	15	100	109a
D+3	14	100	179b	15	100	120b	13	100	116a
D+5	16	25	41c	18	39	35d	14	100	106ab

Floral stages	'Connecticut King' × 'Enchantment'			'Georgia' × 'Hinomoto'			*L. rubellun* × *L. speciosum* 'Uchida'		
	No. pistils pollinated	Capsule fomation (%)	No.seeds per pistil pollinated	No. pistils pollinated	Capsule fomation (%)	No.seeds per pistil pollinated	No. pistils pollinated	Capsule fomation (%)	No. seeds per pistil pollinated
D−3	15	100	104d	10	50	47b	6	83	0.2 ±[x]0.2
D−1	11	100	121cd	10	100	154a	10	80	0
D 0	15	100	141ab	10	100	150a	10	50	0
D+1	12	100	133abc	12	75	84b	8	88	0
D+3	8	100	150a	10	−[y]	−	5	80	0
D+5	13	100	129bc	10	−	−	7	71	2.4 ± 1.5

[z]Parenthesized figures indicate % capsule formation.

[y]Flowers pollinated at D+3 and D+5 contracted diseases after pollination and wilted.

[x] ± indicates standard error.

[w]Values followed by the same letter are not significantly different according to the LSD test at $P \leq 0.01$.

floral stage, differed among 4 combinations; the rate was better in seeds from pistils pollinated after anthesis than those pollinated at or before anthesis in each combination. All seeds of 'Enchantment' × 'Connecticut King' germinated rapidly, exceeding 80% within 21 days of sowing (Fig. 1A). The seeds, produced from pistil pollinated after anthesis, germinated more rapidly than did those before and at anthesis (Fig. 1B, C, D). Seeds of 'Connecticut King' × *L. maculatum* had a low cumulative percentage, compared to the other three combinations; the percentages of seeds from pistils pollinated at D 0 and D - 1 were less than 50%, 30 days after sowing (Fig. 1D).

The cumulative percentage germination of seeds 30 days after sowing (PG) was not significantly different in 'Enchantment' × 'Connecticut King' and 'Connecticut King' × *L. maculatum* (Table 3); it was statistically lower 'Connecticut King' × 'Enchantment' and 'Enchantment' × *L. maculatum*, in seeds from pistils pollinated at D - 1 in the former and those at D - 3 in the latter. MDG which ranged from 15 to 20 days was not significantly different in 'Enchantment' × 'Connecticut King' and 'Connecticut King' × 'Enchantment', whereas it was significantly different in 'Enchantment' × *L. maculatum* ($p \leq 0.05$) and in 'Connecticut King' × *L. maculatum* ($p \leq 0.01$). MDG was faster in seeds pollinated at D +3 and D +5 in the former and from at D - 1 and +5 in the latter.

Discussion

That a large number of seeds was produced in pistils pollinated from D - 1 to D +3 in all combinations except *L. rubellum* × *L. speciosum* 'Uchida' in which pistils pollinated at D - 3 and D +5 produced a few seeds (Table 2) indicates that more seeds can be successfully produced in pistils pollinated in a few days after anthesis rather than at or before anthesis in the compatible cross-combinations of *Lilium* spp., particularly in the Asiatic hybrids. Unlike the above five compatible cross-combinations, only pistils pollinated on D −3 and D +5 produced seeds in the cross-combination of *L. rubellum* × *L. speciosum* 'Uchida', indicating that this combination is somewhat incompatible. A similar combination, *L. rubellum* × *L. regale*, yielded similar results when pistils were pollinated at D +4 and D +5 (Niimi et al., 1996). These results indicate that pollination of younger flower buds or older flowers as well as cut-style pollination (Cheng and Mattson, 1972; Asano and Myodo, 1977; Van Tuyl et al., 1988) is an effective way to obtain seeds in such lowly cross-compatible combination of *Lilium* spp.

The viability of seeds is estimated by the speed, uniformity, and final percentage of germination (Forbes and Wattson, 1992). Few studies have actually demonstrated that the floral stage at pollination affects the viability of seeds in *Lilium* spp. In this study, the floral

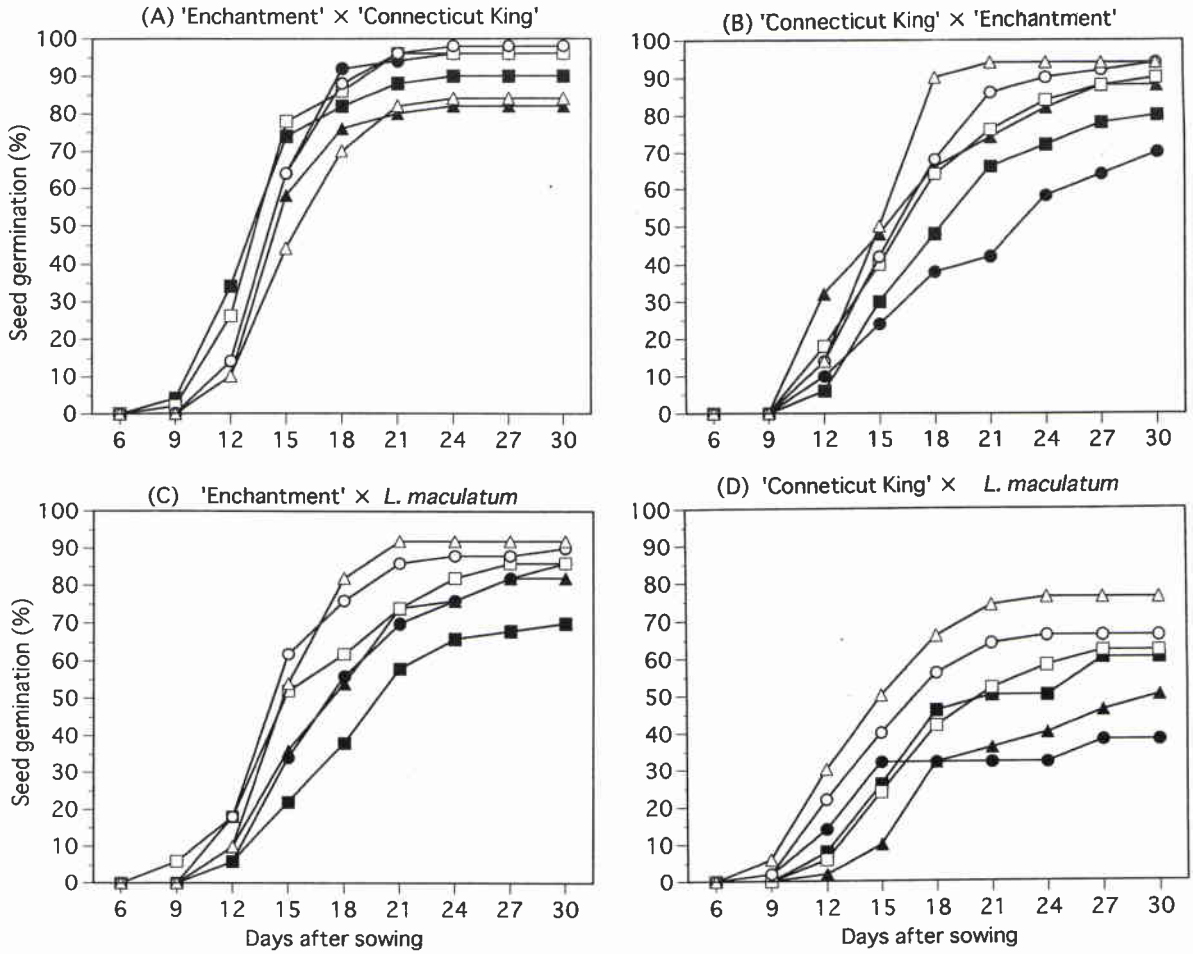

Fig. 1. Cumulative percentage germination curves of seeds produced from flowers cross-pollinated at D −3 (■), D −1 (●), D 0 (▲), D +1 (□), D +3 (○) and D +5 (△).

Table 3. Cumulative percentage germination of seeds on 30 days after sowing (PG) and mean days to germination of seeds (MDG[z]) produced from flowers cross-pollinated at different floral stages in *Lilium* spp.

Floral stages	'Enchantment' × 'Connecticut King'		'Connecticut King' × 'Enchantment'		'Enchantment' × *L. maculatum*		'Connecticut King' × *L. maculatum*	
	PG (%)	MDG (days)	PG (%)	MDG (days)	PG (%)	MDG (days)	PG (%)	MDG (days)
D−3	90	15	80ab	19	70b	19a	60	17abc
D−1	96	16	70b	20	86a	19a	38	15c
D 0	82	16	88a	19	82ab	18ab	50	20a
D+1	96	15	90a	18	86a	17ab	62	18ab
D+3	98	16	94a	17	90a	16b	66	16bc
D+5	84	17	94a	16	92a	16b	76	15c
Significance Floral stages[y]	NS	NS	*	NS	*	*	NS	**

[z]MDG= $\Sigma (fx)/ \Sigma f$ f: number of germinated seeds, x: germinated days.
[y]NS, *, ** indicate non-significant, significant at $P \leq 0.05$ and 0.01, respectively. Values followed by the same letter are not significantly different according to the LSD test at 1% or 5% level.

stage at pollination greatly influenced the viability of seeds produced in 4 cross-combinations, based on PG for 30 days (Fig. 1), the final percentage 30 days after sowing, and MDG (Table 3). There was very little difference in the viability of seeds produced from pistils pollinated at each floral stage in 'Enchantment' × 'Connecticut King' (Table 3, Fig. 1A), whereas there were some differences in viability among seeds from

pistils pollinated at each floral stage in the other three cross-combinations; seeds from flowers, pollinated after anthesis, had a similar viability with few exceptions (Table 3; Fig. 1 B, C, D). These results indicate that the quality of the F_1 generation can be influenced by the floral stage at the time of pollination and pistils pollinated after anthesis produce seeds with better quality than those pollinated at or before anthesis.

Based on the seed yield and germination, we conclude that more seeds with better quality can be produced consistently in compatible cross-combinations of *Lilium* spp. when pistils are pollinated within a few day after anthesis.

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様々な花齢で交雑受粉した数種類のユリの種子形成とその発芽力

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摘　要

交雑受粉時の花齢が種子数とその発芽力に及ぼす影響を調査した．開花3日および1日前のつぼみと開花当時，開花1日，3日と5日後の花の各交雑で得られたさく果および種子数は受粉時の花齢によって異なった．'エンチャントメント'×イワユリ，'エンチャントメント'×'コネチカットキング'，'ジョージア'×'ひのもと'では，開花当日，開花1日前，開花1日後の受粉，'コネチカットキング'×イワユリと'コネチカットキング'×'エンチャントメント'では，開花時または開花後の受粉でさく果および種子がよく形成された．一方，交雑不和合性を示したヒメサユリ×カノコユリ'内田'では，開花3日前と開花5日後に受粉した時のみに種子が得られた．

'エンチャントメント'×'コネチカットキング'，'コネチカットキング'×'エンチャントメント'，'エンチャントメント'×イワユリ，および'コネチカットキング'×イワユリの4交雑組合せから得られた種子の発芽力を，播種後の30日間の種子発芽率の推移，播種30日後の発芽測定終了時の累積発芽率(PG)および平均発芽日(MDG)に基づき測定した．受粉時の花齢は種子の発芽力に影響したが，その結果は各交雑で異なった．しかし，いずれの組合せでも開花後に受粉した雌ずいから得られた種子は，開花時あるいは開花前に受粉した雌ずいから得られた種子より発芽が速く，播種30日後の発芽率もよかった．

さく果およびさく果形成数，および種子の発芽力の結果から，ユリの交雑受粉は開花後数日以内に行うのが適当であることが明らかになった．