

EFFECT OF STYLE CONDITION ON SEED PRODUCTION IN *LILIUM* 'ASIATIC' HYBRIDS.

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Abstract

Effect of style condition on seed set in compatible combinations of *Lilium* 'Asiatic' hybrids was investigated. The number of seeds produced in cut style pollination decreased when the shorter style was treated in six compatible combinations. On the other hand, there was no clear decrease in the number of seeds produced with a shorter distance from pollen to ovary in longitudinal cut style pollination. Results suggest that the lower numbers of seed set in cut style pollination is caused by the pollination method itself.

Key word : cut-style pollination, *Lilium*, seed.

Introduction

The cut-style technique has been developed to overcome pre-fertilization barriers in interspecific crosses in *Lilium* ^(1,2). The cut-style method followed by embryo rescue, including ovary-ovule culture ⁽³⁾, has produced many interspecific hybrid lilies in various combinations of distant related species ^(4,5,6,7,8). The cut-style method followed by embryo rescue, however, produces only a low level of germination, suggesting lower fertilization frequency after cut-style pollination ⁽⁹⁾. To clarify the effects of the interaction of pollen tube growth and pistil conditions on seed set, compatible crossings with a manipulated style should be studied to exclude crossing barriers. The aim of this work is to investigate the effect of style condition on seed set in compatible combinations of *Lilium* 'Asiatic' hybrids.

Materials and Methods

Plant Materials : 'Asiatic' hybrid lily bulbs, 'Connecticut King' (CK), 'Adelina' (AD), 'Make up' (MU), 'Toscana' (TC) and 'Shiraze' (SH) were grown in a greenhouse without heating. Various combinations of compatible cross pollination and incompatible self pollination were done with some manipulations of the style. All experiments were done in May and June from 1995 to 1999.

Pollination method : Flower buds were emasculated 1 day before anthesis and the stigma was covered with aluminum foil. Three pollination methods : normal (stigmatic) pollination (SP), cut style pollination (CSP) and longitudinal

cut style pollination (LCSP) were compared.

CSP : When a stigmatic exudate appeared, styles were cut off at various lengths above the ovary or just below the stigma (Fig. 1), followed by a 5mm longitudinal slit made in the rest style. The styles were cover with aluminum foil after mounting pollen into the slit.

LCSP : Styles were cut longitudinally to various depths, equivalent to the distance to the ovary in CSP (Fig.2), thereafter pollen was mounted into the slit. In this method, the distance from pollen to ovary is the same as in CSP but the whole style is preserved. After pollen mounting the style was covered with aluminum foil.

After 90 days all pods were harvested and the number of seeds with a developed embryo was counted.

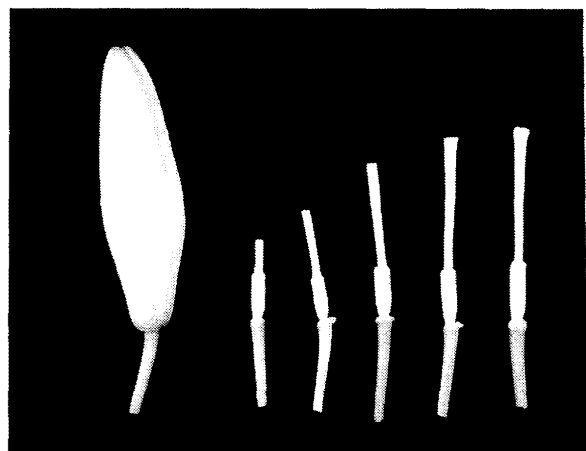


Fig. 1 Cut style pollination method. Styles were cut off at various lengths above the ovary. From right to left : intact style, style was cut off just below the stigma, 3, 2 and 1 cm above the ovary, respectively.

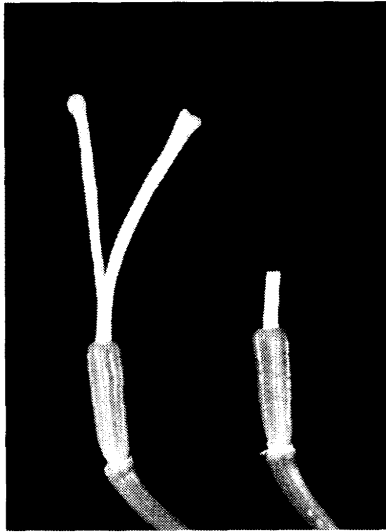


Fig. 2 Longitudinal cut style pollination method (left).

Morphological observation of the pollen tube : The styles and ovaries were harvested at 24 or 48 hrs after SP or CSP (1cm), and fixed immediately with FAA (formalin : acetic acid : 70% ethanol, 5 : 5 : 90). The fixed specimens were dehydrated in an ethanol-acetone series, critical point dried, coated with Pt, and observed under scanning electron microscope (SEM) (Hitachi, S-2150). Other specimens for optical microscope observation were stained with aniline blue.

Results

Effects of style condition on seed set.

In compatible combinations there was no difference between SP and manipulated style pollination methods (CSP and LCSP) in the development of capsule (Fig. 3). The style length in CSP did not affect capsule development. On the other hand, the self incompatible combination (CK x CK) produced a poor development of capsules regardless of style conditions (Fig. 3).

The number of seeds per capsule in SP varied depending on ovary parents and combinations. MU produced more than one hundred seeds per capsule; on the other hand SH had rather a low number of seeds. The number of seeds in CSP decreased when the shorter style was left in all compatible combinations (Fig. 4). Self-incompatible combinations (CK x CK, MU x MU) produced lower numbers of seeds regardless of style length in CSP.

In compatible combination with LCSP there was no clear decrease of seed number with a shortening of distance from

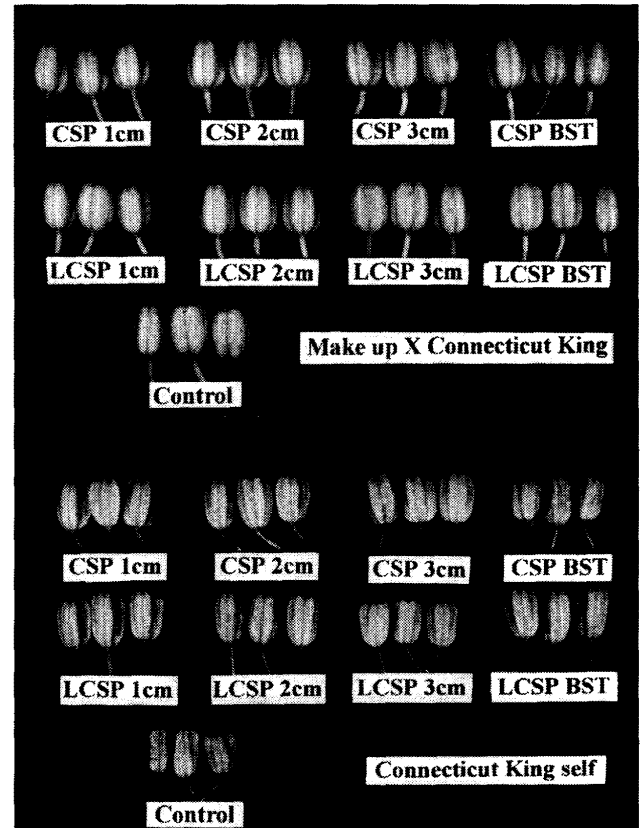


Fig. 3 Capsule development after 90 days pollination. Upper : compatible combination, MU x CK. Lower : incompatible self pollination, CK x CK.

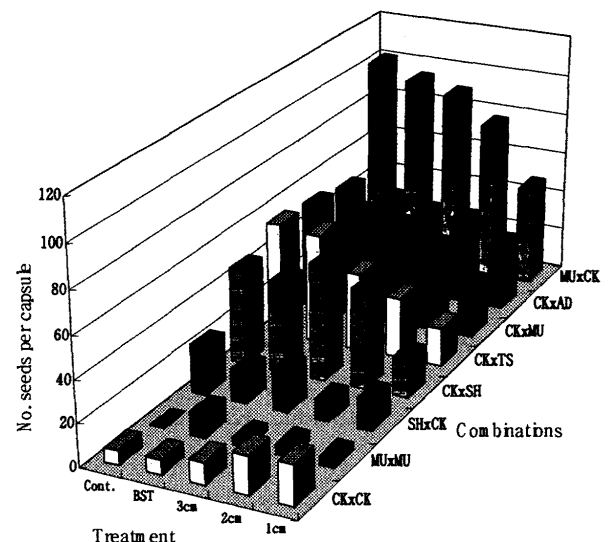


Fig. 4 Numbers of seeds produced in cut style pollination. Cont. : stigmatic pollination, BST : style was cut off just below the stigma, 1-3cm : style was cut off at 1-3cm above the ovary.

Table 1 Number of seeds produced in longitudinal cut style pollination.

Treatment*	Combinations				
	MuxCK	CKxMU	SHxCK	CKxSH	CKxCK
1cm*	65.0±1.9	33.0±1.9	23.1±2.5	47.8±6.0	9.1±1.1
2cm	79.1±3.0	33.1±1.2	27.2±4.1	32.5±5.6	5.4±0.3
3cm	78.8±2.4	33.4±2.2	34.6±4.1	50.3±7.3	6.9±0.3
Stigma	88.5±3.0	17.5±1.2	28.1±2.6	68.2±6.0	8.0±0.6
Control	101.1±3.2	28.1±2.9	23.3±2.5	46.2±8.3	6.6±0.8

*longitudinal slit was made until 1-3cm above the ovary, or just below the stigma (see Fig. 2).
Control : stigmatic pollination.

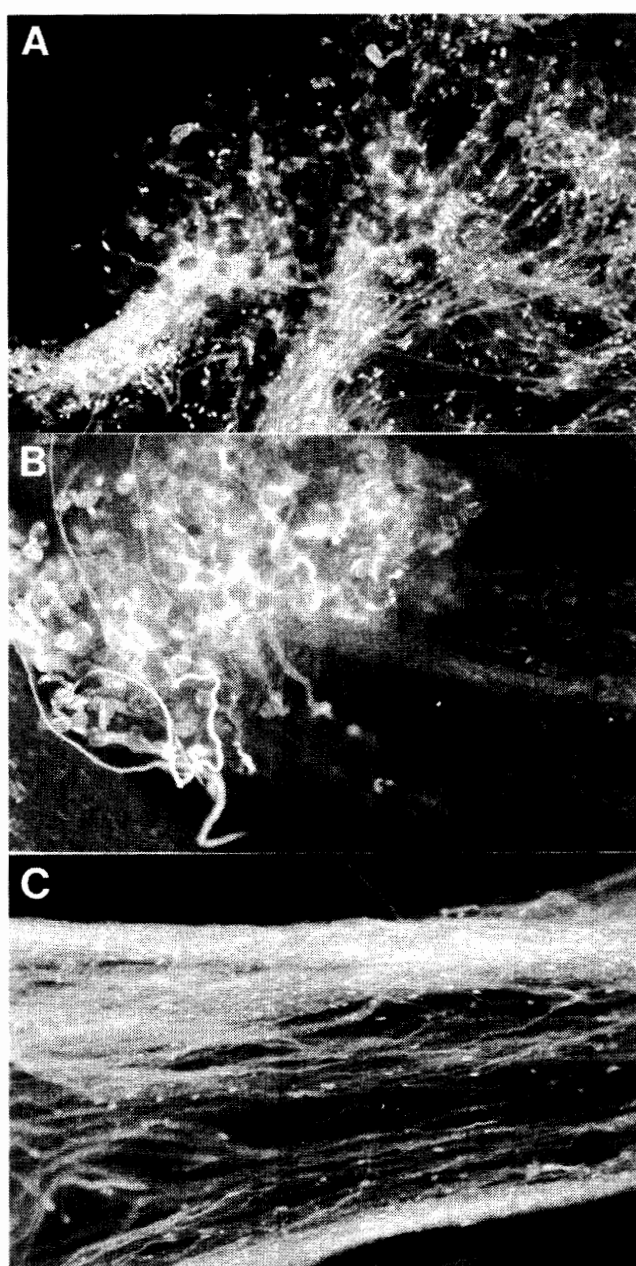


Fig. 5 Pollen tube growth in cut or intact style.
A : 24 hr after SP, B : 24 hr after CSP, C : 48 hr after CSP.

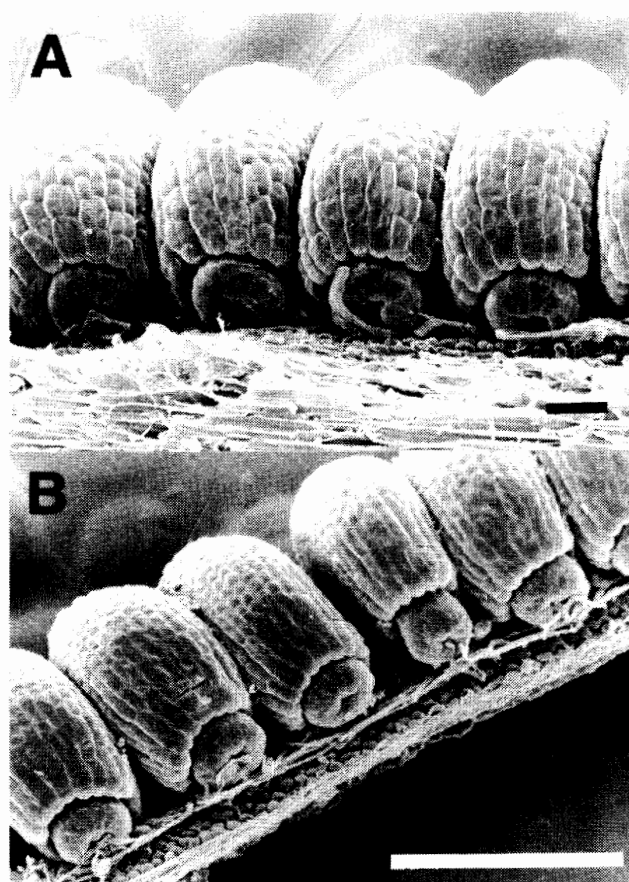


Fig. 6 Pollen tubes ignore the micropyles in CSP.
A : CSP, x 100, bar=100 μ m, B : SP, x 80, bar=500 μ m.

pollen to ovary, except for MU x CK (Table 1).

Pollen growth in both SP and CSP.

Pollen mounted on stigmas germinated and their pollen tubes penetrated the stigma and grew into the style within 24 hr (Fig. 5-A). On the other hand pollen mounted into slits of 1cm cut styles germinated but the pollen tubes did not grow into style after 24 hr (Fig. 5-B). After 48 hr of pollination vigorous pollen tube growth in styles was observed

in CSP (Fig.5-C). SEM observation showed that pollen tubes often ignored CSP micropyles (Fig.6-A) whereas numerous pollen tubes were attached in SP micropyles (Fig.6-B) after 48 hr of pollination.

Discussion

To improve the breeding efficiency of lily, it is very important to develop highly productive pollination methods in interspecific crosses. The cut style pollination method is still the most successful method to produce distantly-related interspecific hybrid lilies⁽¹⁰⁾ albeit the efficiency be low.

Van Roggen *et al.* observed that most pollen tubes stopped in front of the micropyle in CSP in interspecific crosses⁽¹¹⁾. Janson *et al.* revealed that a higher pollen tube penetration was observed when longer styles were left in CSP in compatible crosses of *L. longiflorum*⁽¹²⁾. Hua *et al.* also reported a lower seed set in CSP in comparison with SP⁽¹³⁾. The present work shows that a shorter style produces smaller numbers of seed in six combinations of com-

patible crosses in 'Asiatic' hybrids. These results indicate that the lower seed set in CSP is caused by the pollination method itself.

In CSP pollen tubes may be not prepared to penetrate the micropyles because of short growth 'distance' or 'time', or ovules may be not prepared to accept pollen tubes because of a lack of pollination signal(s) through styles. If the ovules require the pollination signal through style to be active (receptive), pre-pollination would be effective. Hua *et al.* reported that pre-pollination with CSP enhanced seed set to some extent⁽¹⁴⁾ whereas Janson *et al.* could not find any positive effect of pre-pollination⁽¹²⁾. In LCSP no clear decrease was observed in a shorter distance from pollinated position to the ovary. The result may indicate that the existence of the style plays a role in the production of a pollination signal. Alternatively some 'length' of style may be required to establish the interaction between pollen tube and style, which induces pollen tube penetration to the ovule. LCSP and CSP with a rather longer style may be useful to enhance the efficiency of interspecific crosses in lily.

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(Received September 29, 2000)

ユリの種子形成に及ぼす花柱の影響

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要 約

アジアティックハイブリッドの和合性の品種間交雑において、花柱の長さを変えて花柱切断授粉を行うと、花柱が短いほど得られる種子数は減少した。一方、花柱を縦に切り様々な位置へ花粉を挿入すると必ずしも受粉位置が子房に近くても種子数の明確な減少は見られなかった。柱頭に授粉された花粉は24時間後には発芽して柱頭から花柱へと侵入したが、長さ1 cmに切断された花柱に授粉された花粉は24時間後には発芽はしたものの花粉管の伸長が見られず、48時間後によりやく花柱基部まで達した。