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Growth and Flowering of *in vitro*-Propagated *Lilium auratum* Bulbs in Soil

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The cultivation in soil of bulblets of *Lilium auratum* propagated by tissue culture was investigated. *In vitro*-produced bulblets of *L. auratum* grew well in soil and required 3 growing seasons for normal flowering. Flowering time and flower quality were uniform and the plants revealed no virus symptoms. Abnormal flowers (four or five tepals) were observed when the bulbs were less than 15 g, while all flowers were normal when the bulbs were over 15 g. Flower opened within a week and the size distribution of flower tepals revealed a normal distribution curve.

Lilies and their hybrids are among the most important bulbous ornamental plants together with the species *Tulipa Freesia*, and *Narcissus*. Since *Lilium* bulbs are difficult to propagate under natural conditions, artificial propagation methods such as bulb-splitting, scaling, or stem-cutting are used.[1] However, these methods are problematic because production of a large number of genetically homogeneous plants is difficult, and moreover, deterioration occurs in cut flowers infected with viruses.

Lilium auratum is the most slowly growing species and the species most infected with viruses among 15 wild Japanese *Lilium* species.[2] It was introduced to Europe in 1861,[2] and became popular because of its large flower and strong fragrance. *L. auratum* bulbs are produced commercially by collecting wild bulbs in their natural habitat and growing them in the field for one year; then fully grown bulbs are harvested and shipped. Recently, however, the natural resources of this species have rapidly disappeared. The substituted bulb-production methods of *L. auratum* are propagation by scaling, seeds, and bulbils. Unfortunately, few bulbils are produced; furthermore, their size is too small. Scaling is widely used in propagation of various *Lilium* species, but in the case of *L. auratum*, scales easily deteriorate during scaling. The use of seeds is the only practical method for propagation, but this method is problematic because of the phenotypical variation by genetical segregation and because of the extraordinarily slow growth of the bulbs until flowering in practical terms for 4 to 5 years.

Given this background, a practical mass propagation method for *Lilium auratum* was developed, using tissue culture techniques.[3-5] In the present report, bulb growth and flowering characteristics of *in vitro*-propagated bulbs of *L. auratum* in soil are described.

Materials and Methods

Bulbs of *Lilium auratum* Lindl. cv. No. 10 selected from wild bulbs[6] were propagated through *in vitro* culture methods.[5] These bulbs were transplanted to soil (volcanic soil-bark=3 : 1, v/v) on September 7, in the first year after low temperature treatment at 4℃ for 60 days, and then grown in a greenhouse at 30-15℃ day-night temperatures under natural solar radiation. The subsequent cultivation in soil was performed according to similar schemes. The bulbs were harvested on April 1 in the

second year, weighed, chilled at 4℃ for 60 days, transplanted into the soil on May 31 in the second year, and grown in the greenhouse with the same conditions as mentioned above. The bulbs were harvested on November 20 in the second year and weighed. Then the bulbs were transplanted into the soil on December 1 in the second year, chilled under natural conditions, and grown in the greenhouse as mentioned above(bulbs less than 8 g) or in an isolation net-house in the field (bulbs over 8 g). The bulbs flowered in the end of July in the third year.

Results

Establishment and growth of tissue-cultured Lilium auratum plants in soil

Most bulbs produced by tissue culture methods were less than 1 g (**Fig. 1**). After the first year of cultivation in soil, the mean weight of the bulbs increased to 3.0±1.8 g, but no flowering plants were

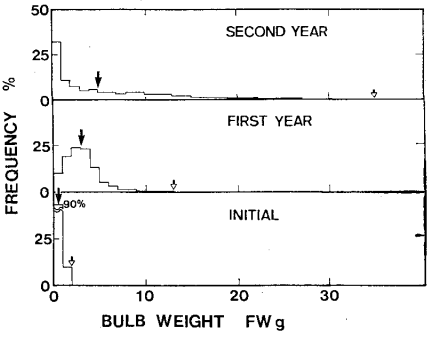

Fig. 1. The enlargement of *Lilium auratum* bulbs after cultivation in soil.
INITIAL : Bulbs produced by tissue culture. FIRST YEAR : Weight distribution of bulbs after cultivation in soil for the first year. SECOND YEAR : Weight distribution of bulbs after cultivation in soil for the second year. ↓ =mean weight and ⇩ =maximum weight (FW) of bulbs.

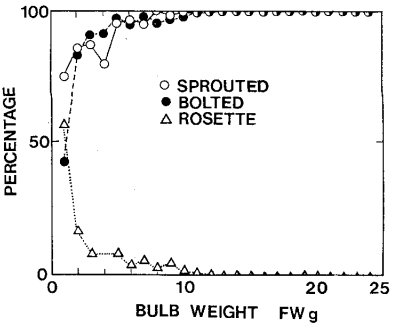

Fig. 2. Relationship between initial bulb weight and type of growth of *Lilium auratum* plants in the third year of cultivation in soil. The percentages of bolted and rosette plants were calculated against the number of sprouted plants.

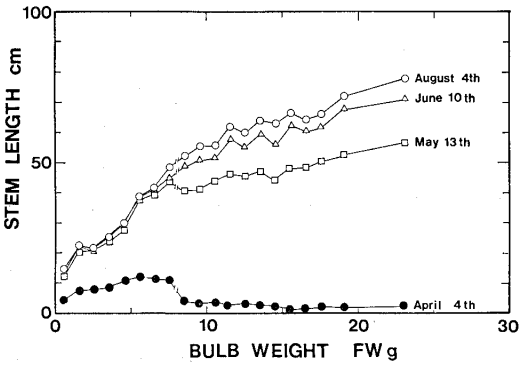

Fig. 3. Relationship between initial bulb weight and pattern of growth (stem length) of *Lilium auratum* plants in the third year of cultivation in soil. The bulbs were transplanted into soil on December 1, 1981, and plant growth (stem length) was measured on the dates in 1982 as indicated in the figure.

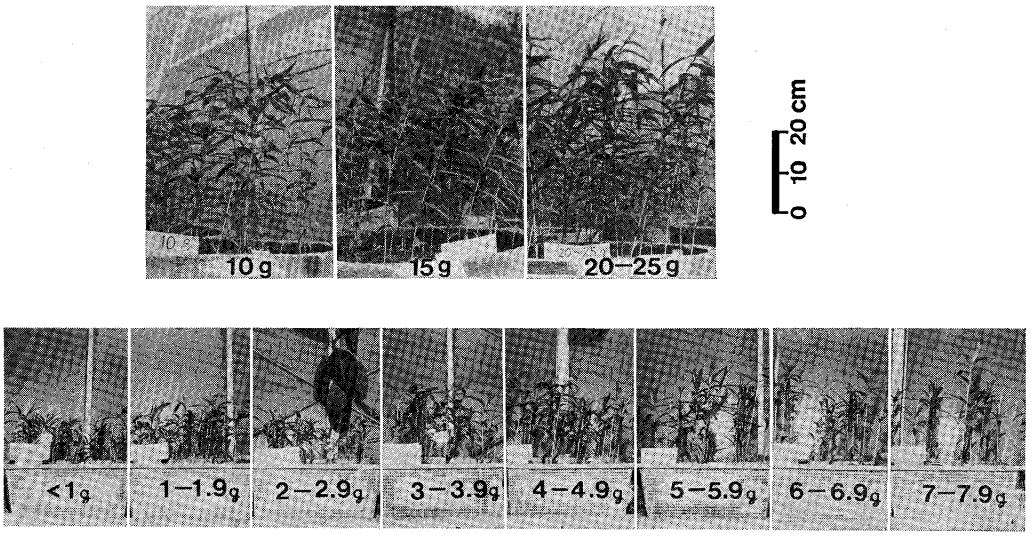

Fig. 4. Growth characteristics of *Lilium auratum* plants in the third year of cultivation in soil. Photographs were taken on April 7 in the third year for bulbs of 0 to 8 g (lower row), and on May 13 in the third year for blubs over 8 g (upper row). The bulbs were transplanted into soil as indicated in Fig. 3, and photographs were taken in 1982. Values in the photographs indicate the bulb weight.

observed. In the second year, bulbs grew to 5.0±5.2 g, and a few plants flowered. In the third year, plants grown from bulbs over 10 g flowered.

Only 80 to 90% of plants sprouted when bulbs less than 5 g were planted (**Fig. 2**). Sprouting percentage rose to 95% when the weight of planted bulbs was 5 to 10 g, and reached 100% when the bulb weight was more than 10 g. Bolting was also related to the weight of bulbs. When the bulb weight was less than 1 g, only 40% of bulbs bolted. The percentage of bolted plants rose as the bulb weight increased, and reached 100% when the bulb weight was more than 10 g.

The bulbs smaller than 7 g grown in a greenhouse sprouted on March 15 in the third year. The most rapid increase in plant height was observed from April to May in these bulbs; after that, the growth rate decreased (**Fig. 3**). The bulbs larger than 8 g grown in an isolating net-house in the field sprouted early in April, and then grew rapidly during May to June; after that, growth rate decreased. Plant height reached maximum at anthesis (**Figs. 3** and **4**).

During the third year of cultivation of *L. auratum* plants in soil, not all plants survived (**Fig. 5**). The death of the plants was closely related to the initial bulb weight. When bulbs of less than 1 g were planted, only about 35% still survived by August 4th. The percentage of surviving plants increased as the initial bulb weight increased, and almost all plants survived when the bulb weight was over 10 g (**Fig. 5**). The observation on June 20 in the third year revealed that flower buds were formed only in the plants whose initial bulb weight was over 5 g (**Fig. 6**). However, development of flower buds stopped and failed to open in the plants whose initial bulb weight was 5 to 8 g. Most flower buds developed and flowered normally whose initial bulb weight was over 8 g, especially over 10 g.

Flowering

Flower opening (anthesis) of *L. auratum* plants during the third year of cultivation began on July 25 in the third year (**Fig. 7**). The number of flowering plants increased day by day, reached a maximum on July 31 in the third year, and the flower opening ended on August 6 in the third year. Within the week from July 28 to August 4, 80.3% (448 out of 558) of the plants flowered.

Flower size (tepal length) was about 16 cm regardless of the initial bulb size and their size distribu-

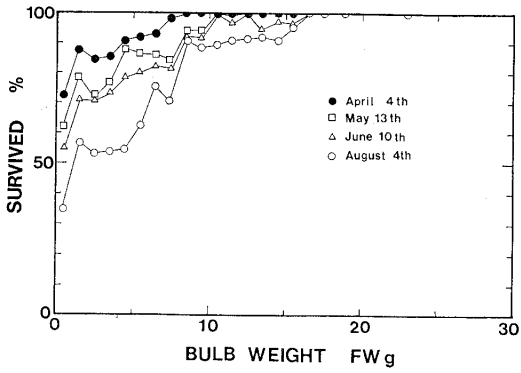

Fig. 5. Effect of initial bulb weight on % survival of *Lilium auratum* plants at four dates during the third year of cultivation in soil.

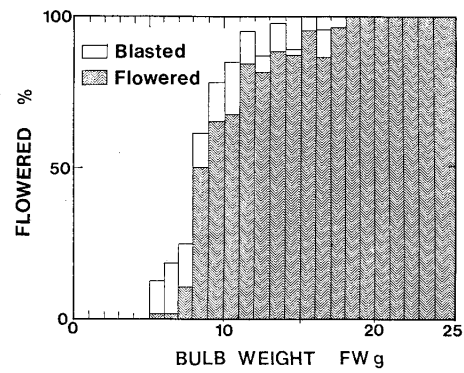

Fig. 6. Flowering (%) and blasting (%) during the third year of cultivation in soil of *Lilium auratum* plants in relation to initial FW of bulbs.

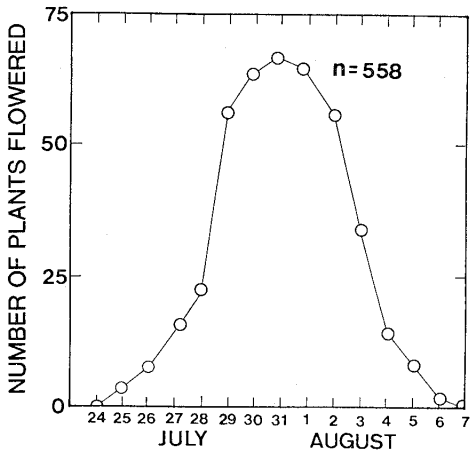

Fig. 7. Flower opening of *Lilium auratum* plants at different dates during the third year of cultivation in soil.

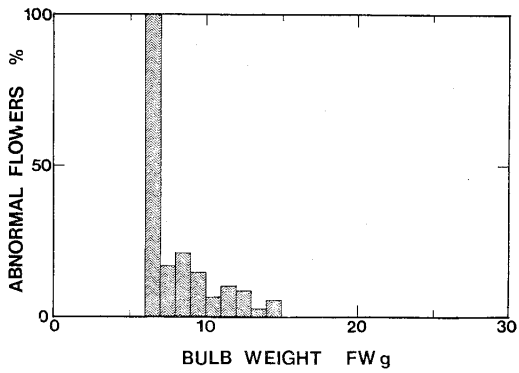

Fig. 8. Relationship between initial bulb weight and formation of abnormal flowers with 4 or 5 tepals in *Lilium auratum* plants in the third year of cultivation in soil.

tion was similar to a normal distribution curve (data not shown).

Some abnormal flowers consisting of four or five tepals were observed when the bulbs were less than 15 g, while all flowers were normal when the bulbs were over 15 g (**Fig. 8**).

Discussion

Some important results have been reported concerning tissue culture propagation of *Lilium* bulbs. *Lilium* viruses are eliminated or disinfected using shoot tip cultures,[7-11] and their micropropagation using serial subculture of bulb-scales dissected from *in vitro*-produced bulblets[12-14] or using callus.[15,16] In spite of these results, bulbs propagated by tissue culture techniques were smaller and difficult to establish in soil or compost.[17] In practice, virus-free bulbs produced by shoot tip culture are propagated using conventional propagation techniques.[17] Takayama and Misawa (1983)[5] studied the possibility of mass propagation of bulbs using tissue culture techniques, and proposed a mass propagation scheme

for lilies, especially for *L. auratum*. The plants of *L. auratum* grown in soil flowered at the third year of cultivation in soil. This is about two to three years faster than conventional methods of growing from seeds, which requires 5 or more years. This indicates the practical use of established tissue culture methods as a tool for vegetative propagation of lily bulbs.

The phytopathological and morphological examinations proved the excellent quality of these flowers. Flowering time and flower quality were uniform and the plants revealed no virus symptoms.

Notwithstanding the uniform and healthy characteristics of the bulbs, some flowers became morphologically abnormal with four or five tepals. However, the result in this report shows that this abnormality occurred in the flowers of bulbs less than 15 g, which are immature for flower opening. Therefore, stimulation of bulb maturation will be indispensable for commercial mass propagation of *Lilium* species. The temperature in mid-summer in Kanagawa Prefecture, where the plants were grown, seemed to be too hot for *L. auratum* plants, though the optimum environmental conditions for the growth of *L. auratum* have not yet been established. The control of environmental conditions for large-bulblet production *in vitro* will be an important research area for mature bulb production of *L. auratum*.

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≪和文要約≫

組織培養で増殖したヤマユリ（*Lilium auratum* L.）の育成と開花特性

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組織培養で増殖したヤマユリを土壌に移植栽培し，開花までの球根の育成経過と開花特性について調べた．組織培養で増殖した球根は，栽培3作目に開花した．球根の生育には大きな差があり，栽培2作目の収穫時には1gから最大35gまでの重量の差が生じた．栽培3作目の開花時期および花の品質は均一であり，ウイルス病斑もみられなかった．花被が4枚あるいは5枚の異常花が観察されたが，これは球根重量 15g 以下の未成熟の球根でのみ発現し，15g 以上ではすべて正常であった．