

## Raising Seedlings of the Sasa lily (*Lilium japonicum*) in Vitro

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Native Japanese *Lilium* species, which have been often used in the breeding of Oriental hybrid lilies, are difficult to cultivate. The degree of difficulty of raising seedlings (from the most difficult to easiest) of the species is as follows:

- *Lilium noblissimum* (the Japanese name is tamoto-yuri) — the most difficult species;
- *Lilium japonicum* (sasa-yuri) and *L. auratum* (yama-yuri);
- *Lilium alexandrae* (uke-yuri) and *L. rubellum* (otome-yuri);
- *Lilium speciosum* (kanoko-yuri) — relatively easy.

All of the species mentioned above show good germination, but they grow very slowly. During the first year, they produce only small bulbs (ca. 2- to 5-mm diameter). They are also susceptible to pests and diseases. At the flowering stage (5 to 6 years after sowing) almost all plants lose their vigor because of infection by viruses and infestation by bulb mites. Therefore, we tried to raise seedlings of several Japanese lilies in vitro in order to conserve them for garden use and to prepare stocks for breeding with commercial cultivars of oriental and regale hybrids. In this report, we concentrate on the results obtained with the sasa lily (*L. japonicum* Houtt), but these methods can be applied to other species.

### MATERIALS

Sasa lily bulbs collected from Gifu Prefecture were cultivated in the Hakone Botanical Garden of Wetlands, and after open pollination, mature pods were harvested to use for the following experiments.

**Experiment 1. Seedling Growth in Vitro.** Seeds were sown on a medium containing half-strength MS medium supplemented with 0.3 mg liter<sup>-1</sup> IAA, 30 g liter<sup>-1</sup> sucrose, and 8 g liter<sup>-1</sup> agar (pH 5.7) on 27 Oct. 1995. After 4.5 months, the first subculture was carried out on the same medium. Then, on 2 July 1996, the seedlings were subcultured on an MS medium plus 0.3 mg liter<sup>-1</sup> IAA, 60 g liter<sup>-1</sup> sucrose, and 7 g liter<sup>-1</sup> agar (pH 5.7). After another 2 months, they were subcultured on a medium with the sugar changed to 90 g liter<sup>-1</sup> yellow soft sugar. On 21 Dec. 1996, 21 March 1997 and 25 Aug. 1997 (22 months after sowing), the seedlings were successively subcultured on the same medium.

**Results.** Sixteen and one-half months after sowing (4 March 1997), the average total fresh weight (FW) of the seedlings was 11.8 g. The average FW of the largest 31 bulblets was 8.06 g, and of the middle 3 and smallest 146, it was 3.66 g and 0.46 g, respectively. At 20.5 months after sowing (10 July 1997), the average total FW of the seedlings was 27.61 g. The 20 largest and 19 middle-size bulblets reached 11.47 g and 5.72 g, respectively.

**Experiment 2. The Effect on Germination of Cutting Away the Seed Coat.**

After harvest in Oct. 1996, the seeds were divided into four groups; 1) intact seeds (control); 2) intact seeds sown in vitro, but after 1 month of culture, one side of the seed coat was cut away; 3) just before sowing, one side of the seed coat was cut away; and 4) just before sowing, both sides of the seed coat were cut away. Seeds of each group were sterilized with 2% sodium hypochlorite solution for 20 min. After rinsing with distilled sterilized water, all of the seeds were sown on the medium as in Experiment 1.

**Results.** Seeds in the control group (no treatment) did not germinate within 4.5 months of sowing. However, seeds with one side of the seed coat cut away after 1 month from sowing, germinated well, i.e. 78%. The cutting treatments were very effective, seeds with the seed coat cut away on one or both sides showed more than 70% germination within 2 months.

**CONCLUSIONS**

Just before in vitro sowing, removal of a part of the seed coat proved very effective for rapid germination. After six subcultures with an interval of 3 months between, the seedlings grew to maturity (about 2 years after sowing). We think that autumn is the best season for transplanting the seedlings from in vitro to ex vitro in a greenhouse because the spring - summer period in Kanagawa Prefecture is too hot to grow *L. noblissimum*, *L. japonicum*, *L. auratum*, *L. alexandrae*, and *L. rubellum*.