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Induction and Rapid Propagation of Shoot Primordia from Shoot Apices of *Lilium japonicum*

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Since *Lilium* is economically very important with tremendous popularity in the horticulture world, various tissue culture methods have been applied to develop new cultivars, virus-free, clonal micropropagation, and so on[1-9]. However, the cultured shoot primordium method of tissue culture discovered by Tanaka and Ikeda[10](Patents of Canada, France, and Germany; Patent of Japan officially announced) has not yet been studied in *Lilium*. The shoot primordium is a dome-shaped tissue which occurs during preformation of the shoot tip of a plant and which can be maintained without any regeneration into plantlets only in liquid media stirred in test tubes by rotary culture equipment (2 cycles/minute)[10]. This method can have the advantage of saving clonally genetic resources of endangered and threatened plant species[11] and economically important dioecious and annual plant species[12].

Lilium japonicum Thunberg leaves are highly variable with deep to shallow veins and the flowers vary in color from purplish-pink to white within the natural population. Since this species is difficult to grow in well-managed cultivated fields, the commercialized cut-flowers of *L. japonicum* have been harvested only from natural populations. This species is meeting with problems of decrease in the natural population, devastation of their habitat, and so on.

In this study, the shoot primordium method was applied to store the genetic diversity of *Lilium japonicum*.

Apical domes of shoot tips approximately 1 mm long were harvested from shoots that appeared from the sterilized bulb scales which were used for starting material for the culture and placed in test tubes (30 mm×200 mm, 25 m*l*) containing Murashige and Skoog media (MS)[13] supplemented with auxin [1-naphthaleneacetic acid (NAA)] at concentrations of 0, 0.02, 0.2, 2.0 and 4.0 mg/*l* and cytokinin [6-benzylaminopurine (BAP)] at concentrations of 0, 0.2 and 2.0 mg/*l* at pH 5.8 for shoot primordia induction. The cultures were incubated at 22°C under 10,000 lux illumination by mercury lamp for a whole day and shaken at 2 cycles/minute on a rotary culture equipment (1 m diameter). They were subcultured at two week intervals.

Masses of shoot primordia of *Lilium japonicum* were induced and proliferated in MS liquid media supplemented with NAA at concentrations of 0.02, 0.2, 2.0 or 4.0 mg/*l* and BAP at concentrations of 0.2 or 2.0 mg/*l* three months after the beginning of primary culture (**Table 1**). Thus, the best growth and shape of shoot primordia of this species were exhibited in MS liquid media containing 2.0 mg/*l* NAA and 0.2 mg/*l* BAP or containing 4.0 mg/*l* NAA and 2.0 mg/*l* BAP (**Table 1, Fig. 1A, B**).

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Table 1. Induction and proliferation of masses of shoot primordia of *Lilium japonicum* from apical domes in various MS media supplemented with auxin and cytokinin for primary culture and subculture

Cytokinin (BAP) (mg/*l*) \ Auxin (NAA) (mg/*l*)	0	0.02	0.20	2.00	4.00
0	F	F	F	F	F
2.0	F	F	F	SP	SPB
0.2	F	SP	SP	SPB	SP

SPB=best formation of a mass of shoot primordia
SP =formation of a mass of shoot primordia
F =failure
Four shoot apices were used in each experiment.

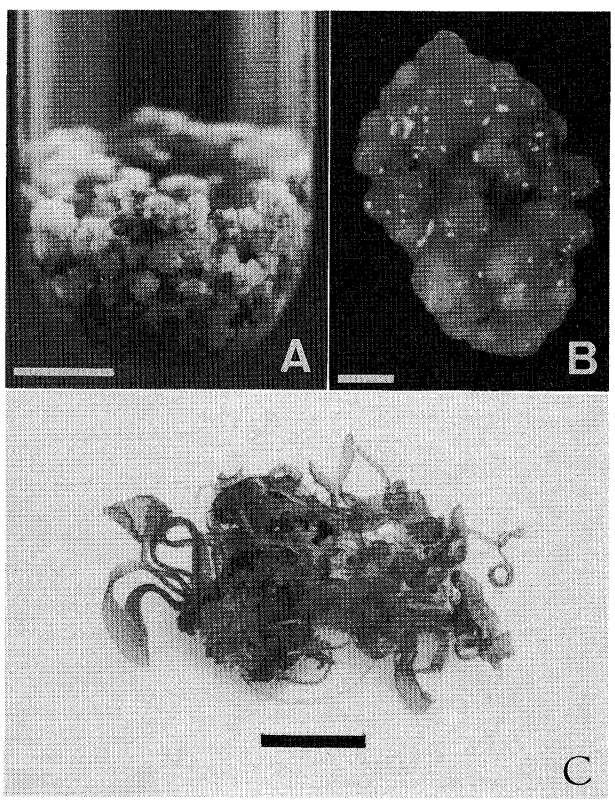

Fig. 1 Shoot primordia of tissue culture and regeneration of plantlets in *Lilium japonicum*. A. Well-propagated shoot primordia in MS liquid medium in test tube. Bar=1 cm. B. A mass of shoot primordia. Bar=1 mm. C. Regeneration of plantlets. Bar=1 cm.

Regeneration of numerous plantlets was easily made from the big-enough masses of green-colored shoot primordia (**Fig. 1C**; more than 25 plantlets per mass) of *Lilium japonicum* within ten days after they were transplanted to MS agar medium supplemented with 0.02 mg/*l* NAA at pH 5.8 in flasks (100 m*l*).

Thus, cultured shoot primordia of tissue culture of *Lilium japonicum* were readily induced and maintained.

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《和文要約》

ササユリの組織培養苗条原基作出と急速増殖

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ササユリの茎頂ドームを，2.0 mg/*l* NAA と 0.2 mg/*l* BAP または 4.0 mg/*l* NAA と 0.2 mg/*l* BAP を添加した MS 液体培地中で回転培養して，苗条原基の作出と急速増殖の好条件を得た。小植物体再分化には 0.02 mg/*l* NAA 添加 MS 寒天培地でよい結果が得られた。