<Original>Micropropagation using Leaf Blades in Genus Lycoris

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Micropropagation using Leaf Blades in Genus *Lycoris*

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Abstract

To develop an efficient tissue culture method for *in vitro* bulb formation from leaf explants of *Lycoris*, regeneration conditions were studied. Higher regeneration rates were obtained using 1 mg/l 1-naphthaleneacetic acid (NAA) with 5 mg/l 6-benzylaminopurine (BAP) in modified MS medium of Huang and Liu (1989). Adventitious buds were induced from the proximal (young) end of explant without intervening callus. Regeneration capacity was greatly affected by ages and parts of leaf, and the basal parts of younger leaves gave higher regeneration rates. In culturing the basal parts of younger leaves on the above optimum medium, 5.0-56.7% regeneration rates with 1.0-11.4 bulblets/explant were obtained in 12 out of 13 tested genotypes. Regenerated bulblets developed into whole plants with bulb after transfer onto hormone-free MS medium.

Key Words: leaf blade, *Lycoris*, micropropagation, regeneration, tissue culture

Introduction

Lycoris is an important flower bulb commonly cultivated for garden uses in Japan, China and the United States (Hsu et al., 1994). Since *Lycoris* is naturally propagated by offset-bulbs, the multiplication rate is very low (1-2 new bulbs / mother bulb). For improving propagation efficiency, micropropagation using twin-scale as explant (twin scale method) has been developed in *Lycoris* (Ezura et al., 1990; Huang and Liu, 1989). Although a considerable improvement on propagation rate was achieved in twin-scale method (27-142 bulblets / flowering bulb) (Ezura et al., 1990), this method is not suitable for propagating new hybrids and rare species which are only available as single or small numbers of bulbs, because inflicting injury on the mother bulb has a risk of losing the mother bulb itself.

Micropropagation using leaf explant has been achieved in several bulbous plants, such as *Allium* (Inagaki et al, 1988), *Lilium* (Wickremesinhe et al., 1994), *Lachenalia* (Niederwieser and Vcelar, 1990), *Narcissus* (Hussey, 1982; Sage et al., 2000) and *Curculigo* (Suri et al., 2000). The multiplication rates are generally high as observed in *Curculigo* (Suri et al., 2000). In *Lycoris*, leaf tissue would be a desirable explant, because it could be isolated successively without damaging mother bulbs. In the present study, in order to develop an efficient

Table 1. Effect of BAP combined with 1 mg/l NAA on adventitious bud regeneration from leaf explants[1] of *Lycoris*.

enotype	Concentration of BAP (mg/l)		
	1	5	10
L. squamigera	+++[2]	+++	++
L. incarnata	+++	++	+
L. sprengeri	−	+	+
L. pumila[3]	−	++	+
L. aurea	−	+	++
L. radiata	−	−	−
L. traubii	−	−	−

[1] Twenty explants were cultured for each genotype.

[2] +++: regeneration was observed in more than 1/3 explants; ++: regeneration was observed in 1/10 to 1/3 explants; +: regeneration occurred sporadically, -: no regeneration was observed.

[3] *L. pumila* is originally noted as *L. radiata* var. *pumila* (Hsu et al., 1994).

Table 2. Plant regeneration from leaf explants of 13 *Lycoris* genotypes[1].

Genotype	Regeneration rate (%)	No. of plants[2] per regenerating explant
L. squamigera	53.3	2.5
L. incarnata	43.3	2.1
L. sprengeri	6.7	1.0
L. pumila	10.0	3.7
L. aurea	6.7	1.0
L. 'Sprengitsune'	13.3	1.0
L. 'Jacksoniana'	56.7	11.4
L. 'Satsumakaryu'	5.0	2.5
L. 'Akasatsuma'	37.5	1.5
L. 'Cherry Pink'	5.0	9.0
L. 'Oosumi'	25.0	3.0
L. 'Albipink'	5.0	7.0
L. 'Satsumabijin'	0.0	–

[1] Twenty to forty explants were cultured for each genotype
[2] Two types of plants are included: a, bulge bulb with leaf; b, slim bulblet with leaf.

method for in vitro bulb formation from leaf explants of *Lycoris*, the effects of growth regulator concentration as well as explant ages and parts of leaf on regeneration ratio were studied, and the developed method was applied to a wide range of genotypes.

Materials and Methods

Plant material

In vitro plants of seven *Lycoris* species and eight cultivars were used in this study (Table 1 and Table 2). Those leaf blades were cut into 5 to 8 mm long segments and used as explants (Fig. 1A). Leaf explants were embedded horizontally on medium with the abaxial side down.

Conditions affecting bulblet regeneration from leaf explants

Preliminary study showed that the addition of 1-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP) to the modified MS medium of Huang and Liu (1989) was satisfactory for adventitious bud induction from leaf explants in *Lycoris* species. For determining the optimal growth regulator concentration, three concentrations of BAP (1, 5 and 10 mg/l) were combined with 1 mg/l NAA in the modified MS medium and tested for seven *Lycoris* species. Twenty explants per species were cultured for each treatment. The basal parts of leaf blades were dissected and used as explant.

To test the effect of ages and parts of leaf, three ages of leaf (first: about 1/4-1/3 expended leaf; second: about 2/3 expanded leaf, and third: fully expanded leaf) and three leaf parts as showed in Fig. 1 were prepared from *L. squamigera*, since its higher regenera-

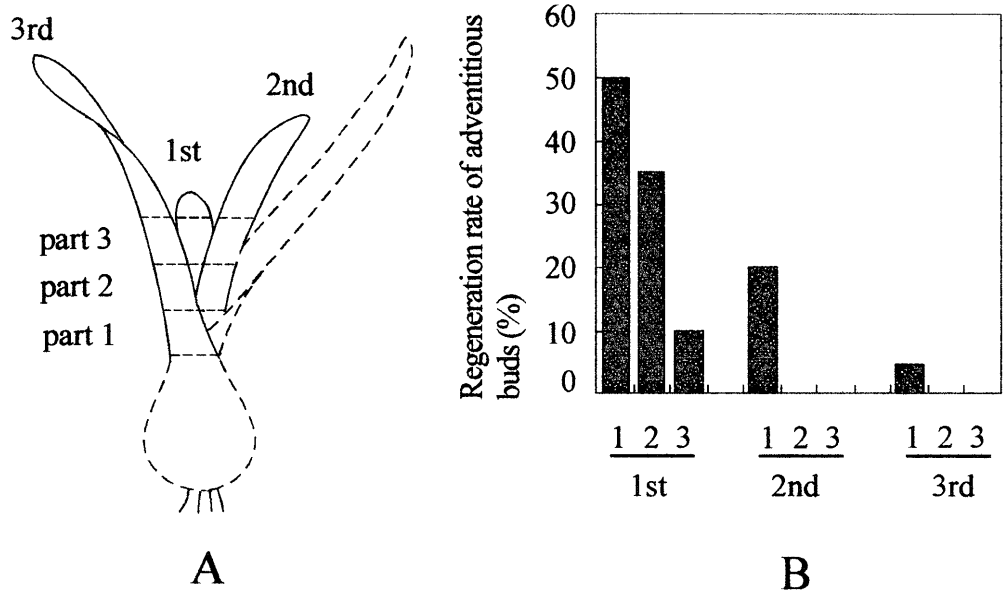

Fig. 1. Influence of ages and parts of leaf on adventitious bud regeneration from leaf explants of *L. squamigera* on modified MS medium with 1mg/l NAA and 5 mg/l BAP.

tion capacity was performed in preliminary study. Twenty explants were cultured for each treatment on the medium with 1 mg/l NAA and 5 mg/l BAP.

The basal medium of Huang and Liu (1989) contains MS medium salts with 300 mg/l NaH_2PO_4 instead of 170 mg/l KH_2PO_4 and is supplemented with 100 mg/l myo-inositol, 80 mg/l adenine sulfate dihydrate, 1 mg/l thiamine HCl, 0.5 mg/l pyridoxine HCl, 5 mg/l nicotinic acid, 2 mg/l glycine, 1000 mg/l casein hydrolysate (Difco), 30 g/l sucrose and 0.2% gellan gum (Wako Pure Chemical Industries, Ltd.). The pH was adjusted to 5.8 before autoclaving. The cultures were incubated at 25±1℃ in the dark. Regeneration rates of adventitious buds were recorded 8 to 10 weeks after culture.

Application of the in vitro bulb formation method

The applicability of our regeneration system was tested in 13 genotypes (Table 2). Twenty to forty leaf segments per genotype were excised from the basal parts of young leaves (the first and second ones in Fig. 1) and cultured on the modified MS medium with 1 mg/l NAA and 5 mg/l BAP. The cultures were kept at the same incubation conditions as in the above experiments. After 8 to 10 weeks of culture, regeneration rates and average numbers of bulblet per regenerating explant were recorded, and the regenerants (bulblets about 1.5 cm in length) were transferred to a fresh hormone-free MS medium with 6% sucrose at pH 5.8 and maintained at 25±1℃ under 16h photoperiod light for rooting and bulb development.

Results and Discussion

Conditions affecting bulblet regeneration from leaf explants

Growth regulator concentrations: At the beginning of culture, most explants enlarged 2 to 3 times in size within 1 to 2 weeks. Although callus formation was observed sporadically at higher BAP concentrations (5 and 10 mg/l) for all species, no regeneration was achieved from these calli. Without intervening callus, adventitious buds were induced directly from the proximal (young) end of explant in five species excluding *L. radiata* and *L. traubii*; the results are showed in Table 1. In L. *squamigera* and *L. incarnata*, higher regeneration rates were obtained at lower BAP levels, whereas in *L. sprengeri, L. pumila* and *L. aurea*, higher BAP levels (1 mg/l NAA plus 5 or 10 mg/l BAP) resulted in higher regeneration rates. From the above, the combination of 1 mg/l NAA + 5 mg/l BAP seems to be suitable for most genotypes. Thus the modified MS medium with 1 mg/l NAA and 5 mg/l BAP is used for the following investigations.

Ages and parts of leaf: As shown in Fig. 1, higher regeneration rates were obtained in part 1 and 2 of the first leaves and in part 1 of the second leaves. These results suggest that younger leaf tissues would have higher ability to form adventitious buds, namely, 10-16 mm long basal to middle parts of first leaves (yellow to yellow green in color) and 5-8 mm long basal part of second leaves (white green in color) are more suitable leaf explants for shoot differentiation in *Lycoris*. Similar results have been also observed in many plant species such as *Lachenalia* (Niederwieser and van Staden, 1992), carnation (van Altvorst et al., 1992) and apple (Yepes and Aldwinckle, 1994). Organogenic ability observed among different parts and ages of leaves would be due to different maturation levels caused by a gradient of hormones and nutrients in the leaf, as reported in Yepes and Aldwinckle (1994).

Application of the in vitro bulb formation method

In 13 genotypes, 5-8 mm leaf segments excised from basal parts of young leaves were cultured on the modified MS medium with 1 mg/l NAA and 5 mg/l BAP. Adventitious bud formation was observed about 6 weeks after culture, and non-regenerating leaf explants of all genotypes turned brown and necrotic within 4 to 6 weeks in culture. Adventitious buds grew vigorously and developed into bulblets (bulging bulblet with leaf or slim bulblet with leaf) on the same medium within 8 to 10 weeks of culture (Fig. 2A and B). Regeneration was achieved in all the 12 genotypes except for *L.* 'Satsumabijinn,' and differences in regenerative rates were observed among genotypes (Table 2). Higher

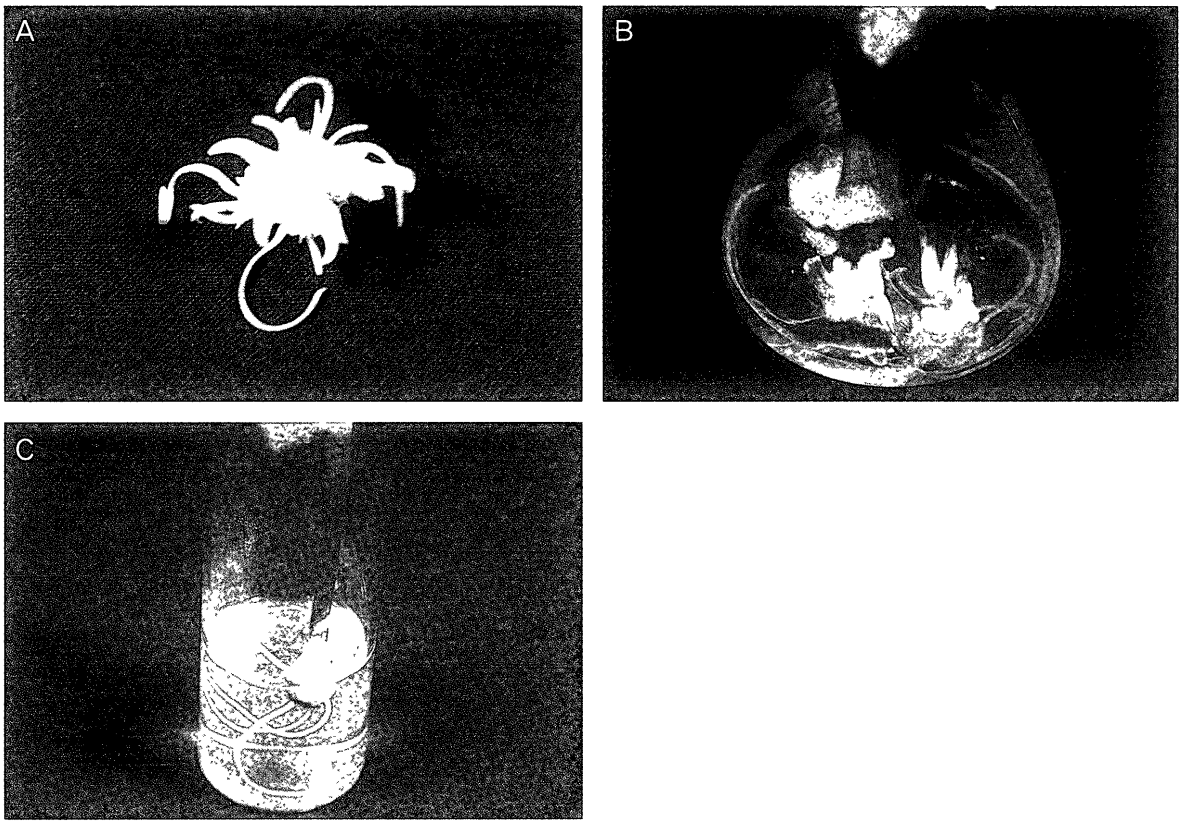

Fig. 2. Plant regeneration from leaf explants of *Lycoris*.

(A) Slim bulblets regenerated from leaf segments of *L.* 'Jacksoniana' on modified MS+1 mg/l NAA+5 mg/l BAP, after 10 weeks of culture, (B) Bulging bulblets regenerated from leaf segments of *L squamigera* on modified MS+1 mg/l NAA+5 mg/l BAP, after 8 weeks of culture; (C) A whole plantlet of *L squamigera* developed from regenerated bulblet on hormone-free MS medium with 6% sucrose

regeneration rates (25.0-56.7%) were obtained in *L. squamigera, L. incarnata, L.* 'Jacksoniana,' *L.* 'Akasatsuma' and *L.* 'Oosumi,' and lower regeneration rates (5.0-13.3%) were observed for the other seven genotypes. The number of bulblets per regenerating explant was varied from 1.0 to 11.4 according to genotypes (Table 2). More bulblet numbers (7-11.4 bulblets / explant) were obtained in *L.* 'Jacksoniana,' *L.* 'Cherry Pink' and *L.* 'Albipink,' whereas less bulblet numbers (1.0-3.7 bulblets / explant) were observed in other genotypes. It was found that the number of bulblets per regenerating explant was correlated with the size of regenerants, namely, as showed in Fig.1 A and B, smaller size of regenerants gave more numbers of bulblets per regenerating explant. The bulblets rooted readily on the hormone-free MS medium and developed into whole plants with bulb within 4 to 8 weeks of culture (Fig. 2C).

All the genotypes except for *L.* 'Satsumabijin' were successfully regenerated in this study. The above results indicate that the present method for *in vitro* bulb formation from leaf explants could be applied to a wide range of *Lycoris* genotypes. The regeneration rates in this study ranged form 5.0 to 56.7%. In other reports using leaf explant, the regeneration rates were 10 to 27% in *Allium* (Inagaki et al., 1988), 2.5 to 42% in carnation (van Altvorst et al., 1992) and 8 to 96% in *Rhododendron* (Iapichino et al., 1992). It is considered that the present method for *in vitro* bulb formation from leaf explants is one of efficient regenerating method using leaf explant. This system should provide a reliable method for mass propagation of new hybrids and rare species in *Lycoris*.

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