REGENERATION OF PLANTLETS FROM LEAF CULTURES OF *LILIUM LONGIFLORUM* THUMB.[1]

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ABSTRACT

Young leaf blades stripped off the floral stalk of *Lilium longiflorum* Thumb. c. v. 'Nellie White' were cultured in the dark at 25 C in solid Murashige and Skoog (MS) medium supplemented with all possible combinations of naphthaleneacetic acid (NAA) at 5 and 10 mg/1 levels and kinetin at 0, 0.1 and 1 mg/1 levels. Initiation of bulbs occurred directly at the base of the explanted leaves on all media. These bulbs, 1 to 3 per explant, developed into plantlets after transferral to the same basal medium without hormones. Root-tip chromosome counts made from 15 of the 112 plantlets showed no variation in number. Histological studies of the leaf bases revealed that no potential buds were present at the time of explanting, but reorganization of embryonic cells into bud primordia was noted as early as 15 days after inoculation. Occasionally, the explants initiated callus in the primary culture, and then differentiated into buds after being subcultured in solid MS medium containing 0.1 or 1 mg NAA/1.

This technique provides a rapid means of vegetative propagation which can be used by the breeder to shorten the cycle of propagation of this species, as well as increase valuable variants.

INTRODUCTION

Although embryogenesis was observed by Sheridan (1968) in stem cultures of the Easter lily, *Lilium longiflorum*, the development of plantlets in tissue culture of this species was not reported. Recently, cloning of the wood lily, *L. philadelphicum*, through tissue culture was achieved in this laboratory (Chen and Holden, 1975).

This communication describes the response of leaf cultures of the Easter lily in regeneration of bulbs and subsequent development of plantlets.

MATERIALS AND METHODS

The Murashige and Skoog (MS) basal formula (Murashige and Skoog, 1962) was adopted for preparing culture media. For initiating cultures, the basal medium was supplemented with a combination of 5 or 10 mg naphthaleneacetic acid (NAA)/1 and 0, 0.1 or 1 mg kinetin/1, making 6 hormonal treatments. The acidity of the media was adjusted to pH 5.8 with 0.1 N NaOH or HCl before autoclaving. Eight grams of granulated agar were added to each liter to gel the media, which were dispensed into 125 ml Erlenmeyer flasks, 40 ml each. The culture flasks were then capped with a double layer of aluminum foil. The media used in subculturing will be described in the results.

Potted plants of the Easter lily cultivar, 'Nellie White' furnished the culture materials. Young leaves, 2 to 8 cm long, were stripped off the stem immediately below the flower buds, disinfected according to Chen and Holden (1975), and explanted on media, one per culture flask. If a leaf exceeded 2 cm in length, a transverse cut was made and only the basal region was explanted. Each hormonal treatment consisted of 12 culture flasks. The cultures were kept in the dark at 25 C.

RESULTS AND DISCUSSION

A small translucent, swollen ring was first noted at the leaf base of most of the lily cultures 1 week after explanting (Figure 1A). The structure, in most cases, did not further proliferate, but appeared to organize adventitious roots or bulbs which were similar to those developed on bulb scales through vegetative propagation, 3 weeks after inoculation. Frequently, one to three bulbs initiaited from a leaf culture, as shown in Figure 1B. The explants which had bulb initiation were subcultured on solid MS basal medium free of hormones in a growth chamber with a photoperiod of 16 hr light (2,000 lux) and 8 hr darkness at 25 C. Chlorophyll formation usually became evident within 48 hr after exposure to light, while roots gradually initiated from the bulbs (Fig. 1C). Later, foliage leaves emerged from the centers of bulbs (Fig. 1D) and root systems were established. Each bulb gave rise to an independent plantlet. These plantlets were first transferred to vermiculite and nourished with one-quarter strength Hoagland mineral salt solution. Then they were transplanted to potting soil in the greenhouse. Several plantlets produced a single, full-sized bloom 11 months after the bulbs were initiated. The average plant height at the time of flowering was about 15 cm, making it disproportionate in size to its flower (Figure 1E). The response of the leaf cultures to the various hormonal treatments as recorded 3 months after explanting is summarized in Table 1.

All of the hormonal treatments tested were effective in bulb formation. No apparent difference in bulb initiation between the 2 composite NAA concentrations was noted. However, it appeared that the hormonal combination 10 mg NAA/1 and 1 mg kinetin/1 gave the best result in bulb formation but the least effect on initia-

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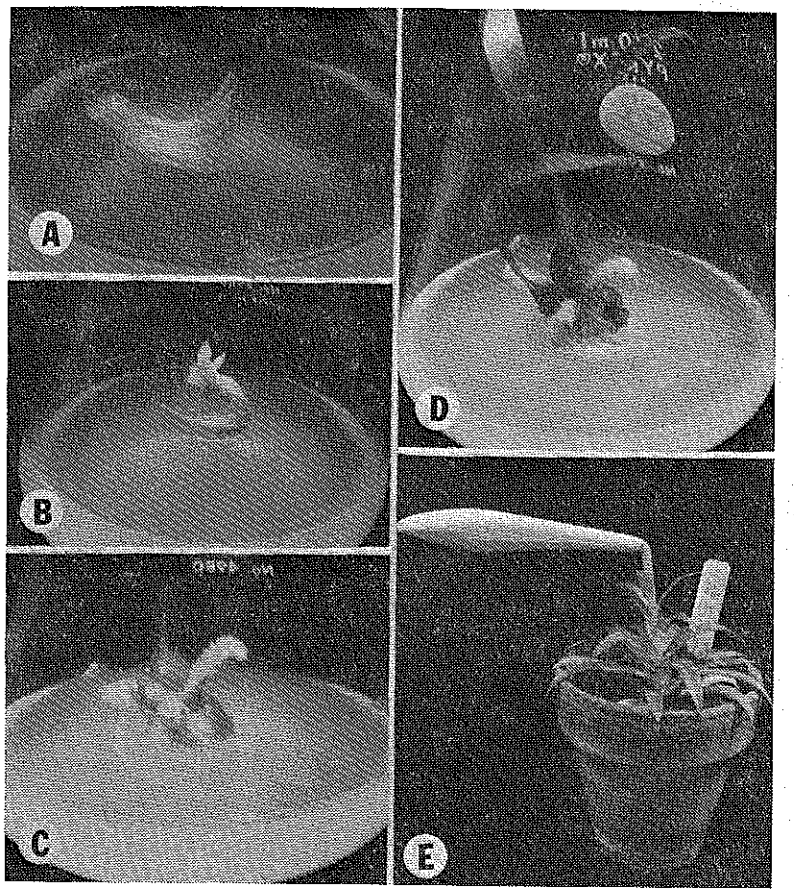

Figure 1. A sequential development of the leaf culture of L. longiflorum. (A) a swollen ring at the leaf base; (B) initiation of bulbs; (C) growth of roots from bulbs; (D) emergence of foliage leaves from bulbs; (E) a flowering plant derived from leaf culture one year after explanting.

TABLE 1

Response of *L. longiflorum* Leaf Cultures to Solid MS Media Supplemented With Various NAA and Kinetin Concentrations

NAA (mg/1)	5			10		
Kinetin (mg/1)	0	0.1	1	0	0.1	1
Bulbs	7	8	8	6	7	10
Roots only	4	2	3	4	5	1
Callus	1	0	1	1	0	1
No growth	0	2	0	1	0	0

*12 bottles per treatment.

tion of adventitious roots. Fox (1969) reviewed the inhibitory effect of kinetin on root formation and promotive effect on bud initiation.

The pattern of bud initiation in leaf cultures of the Easter lily appeared to be similar to the cultures of excised tissue from bulb scales of *L. speciosum* (Robb, 1957) but different from the cultures of the wood lily, *L. philadelphicum*. According to Chen and Holden (1975), adventitious roots generally initiated from the basal region of a wood lily explant and gradually transformed to callus tissue as affected by high NAA concentration in the medium. Numerous buds were then differentiated from the callus in an extended primary culture. On the other hand, the callus which occasionally perpetuated in the primary leaf cultures of the Easter lily did not differentiate into buds until subcultured on solid MS medium supplemented with 0.1 or 1 mg NAA/1. Similar effects were also obtained in the callus cultures derived from floral parts of the Easter lily (Stenberg, 1976).

The region in which the bulbs originated was histologically examined. Explants on solid MS medium containing 5 mg NAA/1 and 0.1 mg kinetin/1 were sampled at 0, 5, 10 and 15-day intervals of incubation. The triple stain technique described by Sass (1958) was used to prepare the serial paraffin sections of the samples. No potential buds were observed anywhere on the culture taken at the time of explanting (Figure 2A).

Sections made from the swollen base of 5-day cultured leaves indicate both localized meristem activity and single cell divisions, predominantly in a transverse plane (Figure 2B). Subsequent growth of these daughter cells account for the swelling.

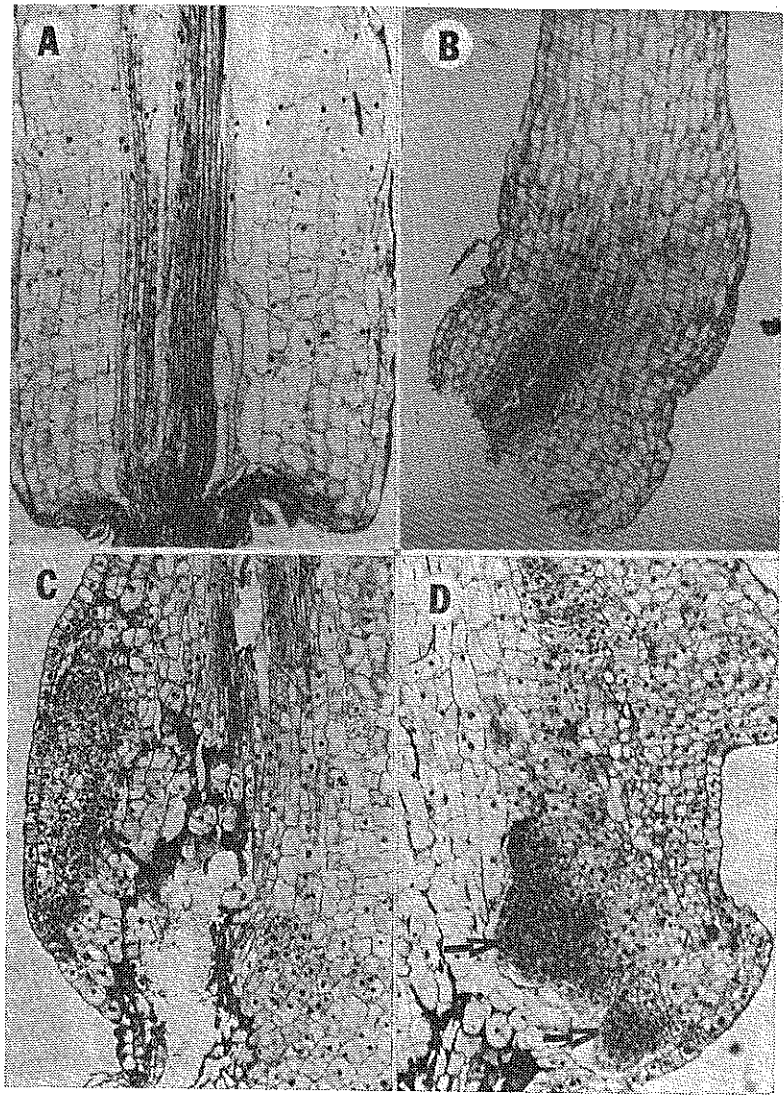

Figure 2. Longitudinal sections of L. longiflorum leaf cultures. (A) a section made at the time of explanting; (B) a section made 5 days after explanting showing localized meristem activity (arrow) near the leaf base; (C) a section made 10 days after inoculation showing a distinct meristematic region (indicated by arrow); (D) a section made 15 days following explantation exhibiting bud primordia (indicated by arrows). (34X)

By the 10th day of culturing (Figure 2C), the localized meristem was becoming embryonic tissue, as evidenced by the small cells with low cytoplasmic to nuclear ratio and the density of their cytoplasm. The single cell divisions on the opposite side of the localized meristem contributed to the swelling by growth following limited divisions.

Leaves cultured 15 days showed a distinct organizational pattern of organogenesis with a vegetative shoot apex and two bud primordia being initiated (Figure 2D).

The vegetative shoot has a definite outer mantle formed from the epidermis of the cultured leaf, a definite central meristem, and two leaf primordia. The flat apex is more similar in appearance to the vegetative apex of *Lilium longiflorum* Thumb. var. *eximium* than *L. longiflorum* Thumb. var. *giganteum* during storage (Pfeiffer, 1935). This clearly indicates that bud primordia were not present at the time of explanting but were induced by the culture conditions.

Successful induction of organogenesis from *in vitro* cultivation of young leaf or floral parts of monocotyledonous plants has been reported by Kaul and Sabharwal (1972) in *Haworthia,* by Chen and Holden (1972) in *Hemerocallis,* and by Chen, Stenberg and Ross (1976) in *Andropogon gerardii.* These structures not only provide a good source of intercalary meristem for rapid establishment of cultures, but also are easy to obtain and readily disinfected.

Chromosome counts on a random sample of 15 of 112 plantlets directly initiated from the primary leaf cultures revealed no deviation from 2n = 24.

The induction of bulbs, which thereafter develop into genetically normal plantlets through leaf culture, provides the lily breeder with a rapid means of vegetative propagation of valuable variants. Before bulb scales are available from a hybrid seedling, asexual reproduction may be achieved by *in vitro* culturing of the young leaves. This method not only avoids the risk of losing the original stock but also shortens the cycle of propagation of this species.

LITERATURE CITED

Chen, C. H. and D. J. Holden. 1972. Organogenesis in daylily callus. Proc. S. D. Acad. Sci. 51:146-149.

———— and ————. 1975. Cloning **Lilium philadelphicum** L. by tissue culture. Proc. S. D. Acad. Sci. 54:143-147.

————, N. E. Stenberg and J. G. Ross. 1976. Rapid cloning of big bluestem (**Andropogon gerardii** Vitman) by tissue culture. Agronomy Obst. p. 48.

Fox, J. E. 1969. The cytokinins. **In:** M. B. Wilkins, ed. Physiology of plant growth and development. McGraw-Hill, N. Y. pp. 85-123.

Kaul, K. and P. S. Sabharwal. 1972. Morphogenetic studies on **Haworthia:** establishment of tissue culture and control of differentiation. Am. J. Bot. 59:377-385.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco culture. Pl. Physiol. 15:473-497.

Pfeiffer, N. 1935. Development of the floral axis and new bud in imported Easter lilies. Cont. Boyce Thompson Institute. 7:311-321.

Robb, S. M. 1957. The culture of excised tissue from bulb scales of **Lilium speciosum** Thumb. J. Exp. Bot. 8:348-352.

Sass, J. E. 1958. Botanical Microtechnique. Iowa State College Press, Ames, Ia.

Sheridan, W. F. 1968. Tissue culture of the monocot **Lilium.** Planta 82: 189-192.

Stenberg, N. E. 1976. Cloning of **Lilium longiflorum** and **Andropogon gerardii** by tissue culture of leaf and floral parts. M.S. Thesis, South Dakota State University, Brookings.