INDUCTION OF TETRAPLOID *LILIUM LONGIFLORUM* THUNB. PLANTS BY COLCHICINE TREATMENT OF CULTURED BULBSCALE DISCS

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ABSTRACT

Tetraploidy in cultured bulbscale discs of *Lilium longiflorum* Thunb cv Nellie White was induced by colchicine. Bulbscale discs mitotically activated on MS medium supplemented with 0.1 mg NAA and 3 mg kinetin per liter for 10 and 20 days were transferred onto the MS medium plus 0, 10, 20, and 40 mg/liter colchicine for 2 and 4 days, respectively, before being returned to the colchicine-free MS medium for bud initiation. Cultures were incubated in the dark at 25°C until adventitious buds were visible. Bud-forming discs were then transferred onto MS medium containing 0.1 mg NAA per liter and incubated in a lighted growth chamber at 25°C completing plantlet development.

The treatments where bulbscale discs were mitotically activated for ten days and exposed to colchicine for two days produced effective polyploidization. Tetraploid plants regenerated from 10-, 20-, and 40-mg/liter treatments were 23%, 42%, and 30% of total regenerants, respectively. On the other hand, bulbscale discs cultured for 20 days in the bud-initiation medium before being exposed for 4 days showed polyploidization only in the two higher concentration treatments. Of 63 regenerants from the 20-mg/liter colchicine treatment, 6 (9%) were tetraploid, whereas in the 40-mg/liter treatment, 24 (19%) of 125 plants were tetraploid. Some of the plants were cytochimeras. All 203 plants regenerated from the bud-initiation medium without colchicine treatment in the two experiments had the diploid number, 2n = 24. Some characteristics of the tetraploids are described.

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INTRODUCTION

Historically, tetraploid plants in *Lilium* were induced in low incidence by soaking either the apical meristem of a floral stalk or the bulbscale bases prior to scaling in an aqueous colchicine solution (Emsweller and Brierley, 1940; Emsweller and Ruttle, 1941; Emsweller and Lumsden, 1943). With the development of tissue culture techniques for rapid propagation by culturing bulbscale discs (Stimart and Ascher, 1978; Chen et al., 1980), *in vitro* production of tetraploid lily plants on a large scale has been possible.

In this paper, we report a method for induction of tetraploid Easter lily plants by colchicine treatment of mitotically activated bulbscale discs in culture.

MATERIALS AND METHODS

Bulbs of the Easter lily (*Lilium longiflorum* Thunb cv Nellie White) harvested from three-year old plants were used for preparing explants. Outer scales were discarded and inner, healthy ones were stripped off the bulbs and cleaned in non-toxic detergent solution for 10 minutes. The scales were then rinsed in running water for 1 hr, dipped in 100% ethanol for 10 seconds and disinfected in 10% commercial bleach for 15 minutes. After being rinsed three times in sterile water, the lower half of a scale was sliced at a thickness of approximately 2-3 mm. Three slices of bulbscales were inoculated into each 125-ml culture flask containing 40 ml of culture medium. MS basal formula (Murashige and Skoog, 1962) supplemented with 0.1 mg naphthaleneacetic acid (NAA) and 3 mg kinetin/liter and gelled by 8 g/liter agar was proved to be effective for bud initiation (Chen et al., 1980). The cultures were incubated in the dark at 25°C.

Colchicine treatments were started on the tenth day after explanting on the bud initiation medium since at this time onset of mitotic activities had been noted through histological observations of the explants. The discs were transferred onto the bud initiation medium plus 0, 10, 20 or 40 mg/liter filter-sterilized colchicine for two days before being returned to the colchicine-free bud initiation medium. At the mid-point of colchicine treatment, bulbscale discs were inverted to insure that both sides of the discs received colchicine. Cultures were incubated in the dark at 25°C until adventitious buds were visible. Bud-forming discs were then transferred onto MS medium supplemented with 0.1 mg NAA per liter and incubated at 25°C in a lighted growth chamber at a 16-hr photoperiod (cool-white fluorescent light at 1 klx) for completing plantlet development. Another set of treatments in which the differentiating bulbscale discs were grown on colchicine media for 4 days was done 20 days after explanting. The handling of cultures was the same as described above.

Ploidy identification in regenerated plants was made by stomate and pollen grain measurements and root-tip chromosome counts, since these tissues were respectively derived from the histogenic layers L-I, L-II and L-III (Arisumi, 1972).

RESULTS AND DISCUSSION

Both stomate size and pollen grain diameter of diploid and tetraploid plants fell into two distinct groups (Table 1). Based upon these two parameters and root-tip chromosome counts, ploidies in various degrees were identified in all regenerated plants. Twenty (23%), forty-four (42%) and nineteen (30%) tetraploid plants were recovered from bulbscale discs which were precultured for 10 days followed by colchicine treatment at 10, 20 and 40 mg/liter, respectively (Table 2). Among the tetraploids regenerated from 10-mg/liter colchicine treatment, three (3%) were cytochimeras. Two of these were sectorial, retaining a segment of diploid tissue in the root tips. The third was affected by colchicine only in the L-II histogenic layer, as only pollen grains of haploid and diploid sizes were observed. All twelve cytochimeras regenerated from the 20-mg/liter colchicine treatment had 4X stomate size. The three cytochimeral plants regenerated from the 40-mg/liter treatment maintained a segment of tetraploid tissue in their roots.

Table 1. Stomate and pollen sizes of diploid and tetraploid forms of Easter lily.

	Chromosome number	Stomate Length μM ± S.E.	Pollen Diameter μM ± S.E.
Diploid	24	103.6 ± 0.50	97.8 ± 0.30
Tetraploid	48	144.0 ± 0.73	140.4 ± 0.25

Table 2. Occurrence of tetraploidy in regenerated plants from bulbscale discs of Easter lily grown on bud initiation medium containing various concentrations of colchicine for two days after ten days of culturing.

		Colchicine conc (mg/liter) 0	10	20	40
No. plants regenerated		102	87	104	63
No. tetraploid plants		0	20	44	19
Pure tetraploid plants	No.	0	17	32	16
	%	0	18	31	25
Cytochimeral plants	No	0	3	12	3
	%	0	3	11	5

Bulbscale discs precultured for 20 days and then exposed to colchicine in the medium for 4 days showed polyploidization only in the two higher concentration treatments (Table 3). All 176 plantlets regenerated from discs treated with 0 and 10 mg/liter colchicine were diploid. Four (6%) of the 63 plantlets regenerated from the 20-mg/liter colchicine treatment were pure tetraploid and two (3%) were cytochimeras. One of the two cytochimeras was sectorial. It had 2X and 4X cells in the root-tips but only tetraploid stomata and diploid pollen grains. The other one was periclinal, bearing tetraploid stomata but haploid pollen grains and diploid roots. Fifteen of the 125 plants regenerated from the 40-mg colchicine per liter treatment were pure tetraploid, and nine (7%) were cytochimeras. Eight of the cytochimeras were periclinal, having tetraploid stomata, haploid pollen grains and diploid roots, and one was a sectorial cytochimera with 2X and 4X chromosomes in the roots.

Table 3. Occurrence of tetraploidy in regenerated plants from bulbscale discs of Easter lily grown on bud initiation medium containing various concentrations of colchicine for four days after twenty days of culturing.

		Colchicine conc (mg/liter)			
		0	10	20	40
No. plants regenerated		101	75	63	125
No. tetraploid plants		0	0	6	24
Pure tetraploid plants	No.	0	0	4	15
	%	0	0	6	9
Cytochimeral plants	No.	0	0	2	9
	%	0	0	3	7

There is considerable rationale for the higher occurrence of tetraploidy when colchicine treatments were performed 10 days after the bulbscale discs were explanted on bud initiation medium. At the time, the discs were just mitotically activated, but organogenesis, in most cases, had not occurred. Polyploidization by colchicine in the meristematic cells followed by initiation or reorganization into adventitious buds would create a high chance of polyploid plant regeneration. *In vitro* induction of tetraploid plants in high frequency by colchicine treatment of differentiating daylily calli was reported by Chen & Kallemeyn (1979). On the other hand, bulbscale discs receiving colchicine treatments 20 days after explanting had organized either meristem masses or even bud primordia. The structure of these tissues would hinder colchicine penetration. The treatment would thus be less effective, resulting in more cytochimera production. The developmental stage of an explant in culture is therefore crucial for

the success of polyploidy induction by colchicine. No evidence of spontaneous chromosome doubling was observed in plants regenerated from untreated bulbscale discs, implying that tetraploidization was not affected by the presence of a high kinetin concentration in the medium.

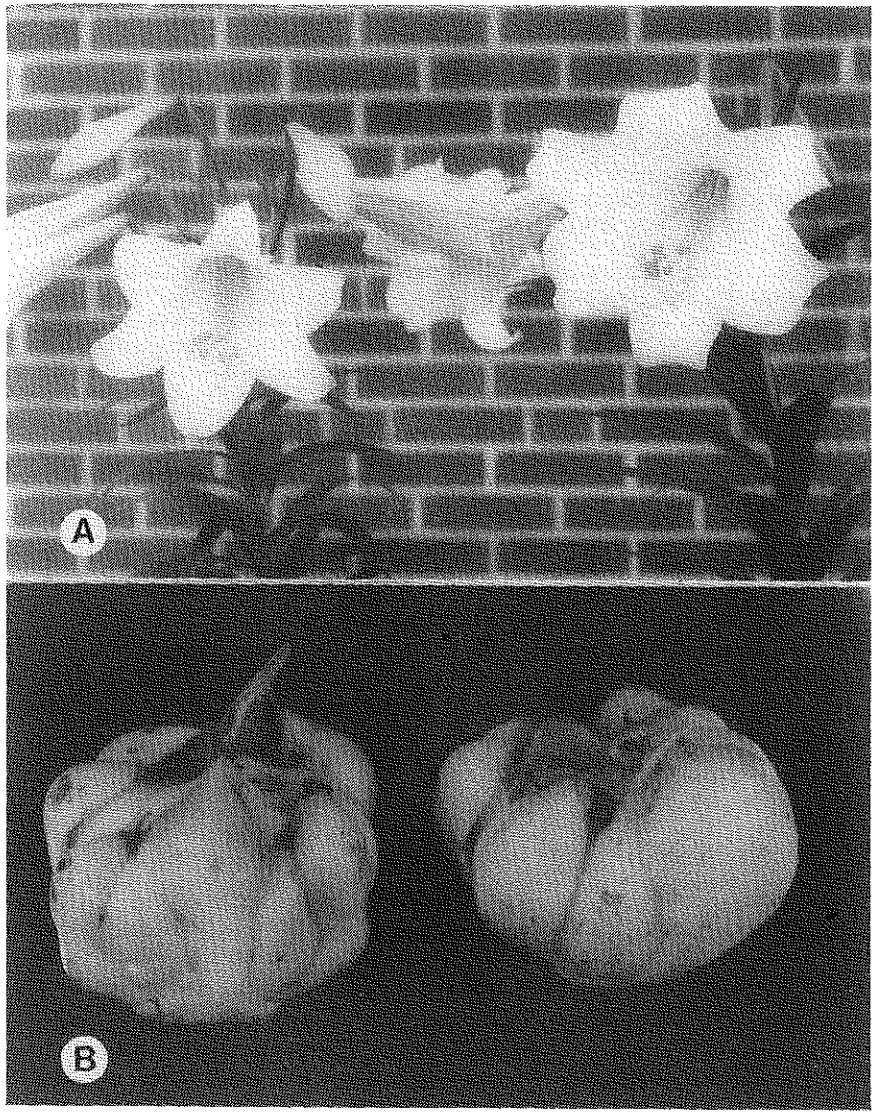

Figure 1. Comparisons in flowering spikes (A) and bulbs (B) between diploid (left) and tetraploid (right) Easter lily plants evolved from colchicine-treated bulbscale discs.

In vitro induced pure tetraploid lily plants exhibited gigantic characteristics, such as leaves, flowers and bulbscales, but bore fewer blossoms than corresponding diploids (Figures 1A and 1B). Earlier, however, Sagawa (1958) reported an improvement of the floriferous nature and maintained other desirable traits of tetraploids by creating triploid plants through hybridization between the tetraploids and the corresponding diploids. Tetraploids bloomed slightly later than diploids even though plants of both ploidy forms were developed from similar sizes of bulbs. No significant difference in height between the two ploidy forms was noted (Table 4). *In vitro* colchicine treatment of mitotically active bulbscale discs followed by plant regeneration could provide a highly efficient means of producing showier Easter lilies.

Table 4. Comparison of selected traits between ten diploid and ten tetraploid Easter lily cv Nellie White.

	Diploid	Tetraploid	
Bulb weight (gm)	62.8 ± 8.3	60.0 ± 8.0	
Plant height (cm)	57.7 ± 2.9	50.0 ± 1.8	
Days to flower	86.4 ± 0.9	89.5 ± 0.8	*
Corolla length (cm)	15.2 ± 0.3	17.3 ± 0.3	**
Corolla width (cm)	11.5 ± 1.0	14.1 ± 0.2	*
Blossoms per plant	3.2 ± 0.3	1.8 ± 0.2	**

* and ** indicate significant differences between ploidies at 5% and 1% probability levels, respectively.

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