VEGETATIVE PROPAGATION OF HEMEROCALLIS — INCLUDING TISSUE CULTURE[1]

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Mass production with improved colors, and better flower forms of *Hemerocallis* cultivars has been limited because of slow natural increase. Many of the newer cultivars produce only 1 or 2 new fans yearly under natural conditions in temperate zone climates. Using Traub's (12) improved propagation technique, only 20 to 30 plants can be obtained per year in greenhouse environments. Because of slow increase most of the best new cultivars remain in breeder and collector gardens and are unknown to the general gardening public. A better propagation method will increase both garden and commercial potential of this plant.

This paper discusses 1) current propagation techniques practiced by a limited number of commercial growers and breeders; 2) a new propagation method involving the application of kinetin compounds to freshly cut crowns; and 3) propagation by tissue culture.

CURRENT PROPAGATION METHODS

Until the late 19th century, *Hemerocallis* consisted of unimproved species types. In the 1890's, George Yeld (6) made the first interspecies crosses and selected improved cultivars. Many of these new cultivars contained genes from rhizomatous types (those that multiply by underground stems) which propagate freely.

Further improvements within the genus resulted from research work conducted by Stout (6) at the New York Botanical Garden between the years 1920-1940. Stout obtained many species and cultivars directly from China and in a well documented research program was able to produce the forerunners of today's true pinks, reds and purples. However, with the improvement in colors and garden quality, the rhizomatous genes were largely eliminated, further reducing the natural vegetative propagation potential.

In early work, Morrison (7), Bailey and Bailey (1), and Stout (10) described dividing compound rhizomes and multiple stems. Since the crown division method produces only a few new plants each year more rapid propagation techniques have been sought. Traub (11) reported two successful propagation methods that are still used today. One method involved making 4 vertical cuts through leaves and crowns such that four sections were formed. Both the roots and

[1]Authorized for publication as Paper No. 5005 in the journal series of the Pennsylvania Agriculture Experiment Station.

leaves were trimmed to 2 to 3 inches and the sections rooted in a sand medium. Eventually each newly rooted section formed a new shoot. If crowns were divided into more than four sections new plantlet formation was greatly reduced. Traub also reported a method whereby crown tip cuttings were cut from a plant such that a small portion of the stem was included with adhering leaves of each shoot. The crown tip cuttings were rooted and the mutilated plant remaining in the ground subsequently produced another shoot.

Various other authors have reported similar methods (2, 3, 5). Norman (9) mentions the feasibility of producing 12 to 30 plantlets from cuttage, the method Traub used. He also suggested the possibility of 20 to 40 plantlets from an undescribed method he labeled "spooning."

PROPAGATION WITH KINETIN COMPOUNDS APPLIED TO FRESH CUT CROWNS

In February of 1974, a clump of *Hemerocallis* 'Sea Gold' was brought into the greenhouse. The fans were divided and potted in 15 cm clay pots such that the crowns were above soil level. Greenhouse temperatures were maintained at 21°C nights. Plants were lighted to provide a long day (14 hrs) for this otherwise dormant cultivar. In 2 weeks, after active growth had started, the tops were cut off with a sharp knife. The cut was made directly through the crown perpendicular to the shoot growth. The fresh cut crowns were treated with various concentrations of kinetin once daily for 3 days in succession (approximately 2 ml/plant). The kinetin treatments were applied with an eye dropper using the tip to spread the liquid to cover the entire crown without running off.

Approximately 7 days after the cuts were made new shoot formation was visible at the sides of the crown and sparingly in leaf axils across the top of kinetin and SD 8339[1] treatments. Crowns treated with distilled water produced new shoots but usually either at the side of the crown or the center terminal shoot. Four weeks after the treatments began, shoots 10 cm in length (Figure 1), were torn from the mother crowns and treated with Hormodin No. 2 and rooted under mist. New shoots were taken from each crown as they reached the 10 cm size every 2 weeks for a total of four times. At the eighth week one shoot was allowed to remain on each plant. Later the old crown and roots were removed and the new shoot repotted and grown on. All of the plantlets from these treatments were rooted and grown to flowering size during the summer and fall of 1974. With ample plant material obtained from a single cultivar a future experiment was planned.

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[1]SD 8339 is a synthetic cytokinin produced by Shell Development Company, Biological Science Research Center, Modesto, California 95352.

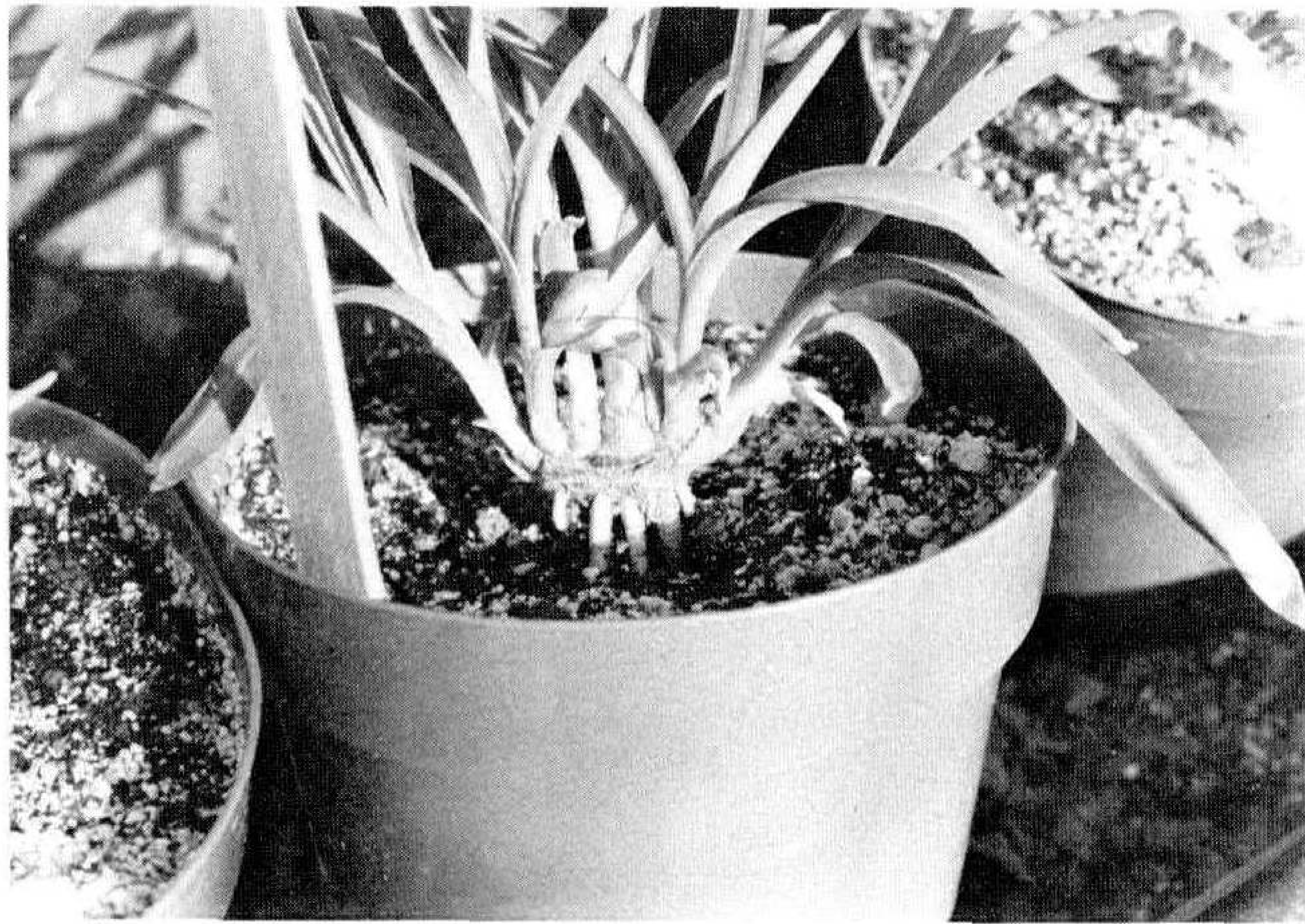

Figure 1. Daylily plantlets approximately 10 cm in length formed on the crown of *Hemerocallis* 'Sea Gold'.

Materials and Methods

'Sea Gold' plants obtained from the preliminary study were treated with distilled water (control), 25 ppm kinetin plus 4% DMSO, 50 ppm kinetin plus 4% DMSO, 400 ppm SD 8339 or 800 ppm SD 8339. The experiment was initiated January 16 and terminated April 16, 1975. Nine plants were used for each treatment (each treatment contained three replications with three plants each which were randomized throughout the block). Each plant was removed from the soil, the top severed, and the plant repotted such that the crown was again above the soil level. Treatment application technique was similar to the preliminary study. Since it was difficult to keep SD 8339 in solution new formulations were prepared each day.

Results and Discussion

All of the treated crowns produced a greater mean number of new shoots than the control (Table 1). The site of new shoot development varied from the control plants and that of the kinetin and SD 8339 treatments. New shoots always occurred at the extreme outside of the crown or at the center of control plant crowns. Kinetin and SD 8339-treated plants often had shoots arising from the axil of leaves midway between the terminal and lateral buds. Some crowns produced as many as 23 new shoots in the 3 month period of time. Although the mean number of shoots was greatest for the kinetin or SD 8339 treatments, the data were not significant at the 0.05 probability level. We attribute this to difficulty in cutting each crown at the most desirable level — a problem which could be overcome with further refinement of the technique.

Table 1. The effect of kinetin and SD 8339 on shoot production in *Hemerocallis* 'Sea Gold'.

Treatments	Total number of new shoots from 9 ramets	Mean number of new shoots per ramet
Control	78	8.6
Kinetin 25 ppm plus DMSO	105	11.6
Kinetin 50 ppm plus DMSO	96	10.6
SD 8339 400 ppm	105	11.6
SD 8339 800 ppm	113	12.5

Our results indicate that the method of severing the top shoot through the crown appears to have considerable merit in vegetative propagation of *Hemerocallis* cultivars. Further, this method appears to be enhanced with cytokinins.

TISSUE CULTURE PLANTLET FORMATION

Materials and Methods

A complex inter-species hybrid 'Chipper Cherry' which divides slowly in gardens was the source of flower petal explants. Immature flower buds (5-7mm) were removed from the flower stalk and surface sterilized by immersing in 95% ethyl alcohol for 5 sec, soaked for 15 min in 10% sodium hypochlorite, and washed three times with autoclaved double distilled water. Petals and sepals were aseptically removed from the sterilized buds and placed on the culture medium.

The culture medium was essentially that of Murashige and Skoog (8) with 0.5% casein hydrolyzate added; 2,4-D (1 mg/l), and kinetin (1 mg/l) were added in combination or individually depending on the treatment. The pH was adjusted to 5.7 before autoclaving. For culture induction, 25 ml of a medium containing 2,4-D and kinetin were placed in 60 ml bottles with loosely fitting plastic caps. Plantlet formation was induced by transferring the callus to an agar medium minus 2, 4-D but containing kinetin at 1.0 mg/l and IAA at 0.5 ml/l.

Callus initiation occurred in a controlled environment room at 29±1°C without lights. Plantlet development was carried out at 26±1°C with a 16 hr photoperiod.

Results and Discussion

Callus was induced on flower petals and sepals. It was compact, yellowish-green in color, and had what appeared to be organized cell masses. Development of numerous rooted plantlets was accomplished by transferring the callus to a medium without 2.4-D but containing kinetin and IAA (Figure 2).

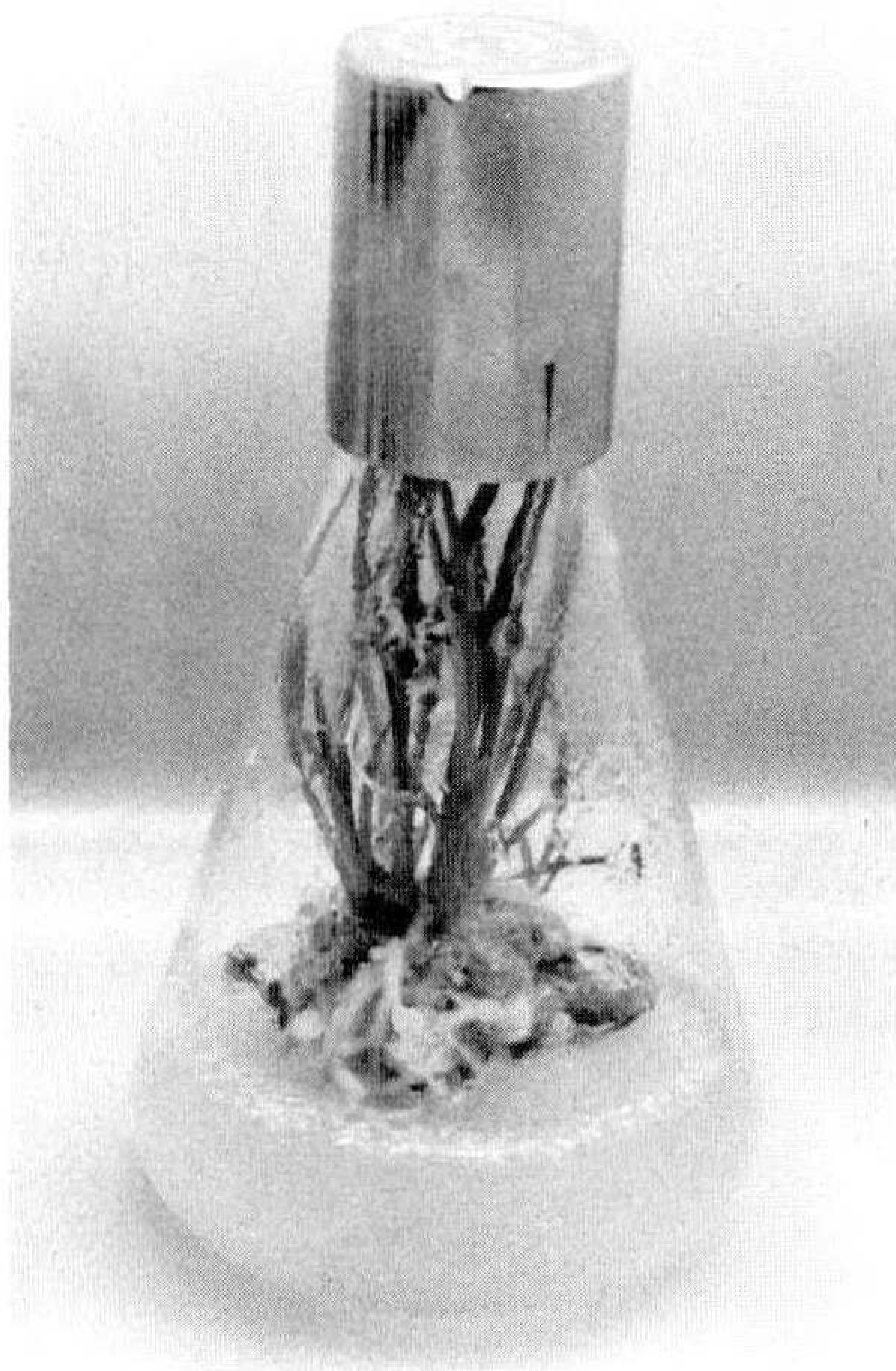

Figure 2. Daylily plantlets formed in tissue culture.

In this experiment, we observed the development of callus directly from petal and sepal explants. Chen and Holden (4) previously have initiated plantlets from callus of *H. flava (H. liliosaphodelus)*. However, they first developed adventitious roots on petal explants and then initiated callus from the root sections.

The plantlets were then removed from the 60 ml bottles, separated into individual plantlets and planted directly into 6 cm clay pots. When they had grown to sufficient size (3 months), they were transplanted to 15 cm plastic pots.

At this writing, the tissue-culture plants have not flowered, however the foliage appears identical to the parent plant. If the procedure is to be of value for rapid propagation the resulting plants must have the same genotype and phenotype as the parent. This portion of the study will be documented as flowering occurs.

LITERATURE CITED

1. Bailey, L. H. and Ethel Zoe Bailey. 1930. *Hortus.* Macmillan Co., N. Y. 188.
2. Baker, S. Houston. 1960. Rapid propagation of *Hemerocallis. Am. Hemerocallis Soc.* 14:96.
3. Benzinger, Fred M. 1962. Propagation of 'Captain Reid' tetraploid with gibberellin. *Am. Hemerocallis Soc.* 16:39.
4. Chen, C. H. and D. J. Holden. 1972. Organogenesis in daylily callus. *Proc. S. D. Acad. Sci.* 51:146-149.

5. Foret, T. A. Jr. and Ira S. Nelson. 1967. Propagation of Hemerocallis by ramet cuttage. *Am. Hemerocallis Jour.* 21:44-48.

6. Hu, Shiu-Ying. 1968. An early history of daylily. Daylily Handbook. *Am. Hort. Mag.* 82-85.

7. Morrison, B. Y. 1928. The Yellow Daylilies. *U.S. Dept. Agric. Circ.* 42.

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

9. Norman,, Alex. 1964. Increasing plants. *Am. Hemerocallis Soc.* 18:70.

10. Stout, A. B. 1934. Daylilies. MacMillan Co., N.Y. 102-103.

11. Traub, Hamilton. 1937. Methods of propagating daylilies *(Hemerocallis)* vegetatively. *Herbertia* 4:205-207.

12. Traub, Hamilton. 1936. Propagation of *Hemerocallis* (daylilies) by crown cuttage.*Herbertia* 3:123.

VOICE: How long does it take from putting the tissue on the medium until you get plants formed?

DARREL APPS: It is about 3 to 5 months but this varies considerably depending upon the cultivar.

VOICE: Have you been able to get the callus to proliferate?

DARREL APPS: Yes we have and we have lots of callus and therefore lots of potential for plant production.

VOICE: What type of medium do you plant the young plantlets into from the flasks?

DARREL APPS: We use the Penn State mix which is 2:1:1 soil, perlite, peat. We had to be very careful with the plants at this stage and we kept them in the growth chamber until they could be satisfactorily established to be moved out of there onto the greenhouse bench.