placed on the appropriate nutrient medium to promote roots.

Stage III: Two or three weeks after the plant is placed on the rooting medium, roots form. This is the step in which transition is made from the laboratory environment — the operation in which rooted plants are transferred from test tubes to the greenhouse. In order for the plant to adapt to its new environment (varying temperatures, moving air, and higher light intensities), the plant must go thru a re-adjustment period. This growing-on stage, in a sense, is similar to transplanting tender, fragile seedlings. Our procedure is to dibble them into small peat pots and place them under mist for 1 week or less; the survival rate is 100%. As plants quickly develop, they are shifted up to larger containers, or planted into stock beds for normal propagation procedures.

Micro-propagation (tissue culture) is a tool the modern plant propagator should not fail to use for producing plants, both for economic reason and for product-improvement. It is a must today. Not all plants respond to this system, but for those which do, we feel should be managed via tissue culture. As research continues there is promise many of the woody species will also be produced via tissue culture.

TISSUE CULTURE PROPAGATION OF DAYLILIES

CHARLES W. HEUSER and JOHN HARKER

Department of Horticulture
The Pennsylvania State University,
University Park, Pennsylvania 16802

The Liliaceae is composed of many herbaceous perennial plants. It includes lilies, iris and other commercially important ornamentals. One flowering ornamental group, the *Hemerocallis*, known in the trade as daylilies, have been popular as herbaceous perennials for many years. The standard method of propagation is through division (1,2,4,6). This procedure, while it yields plants that are true-to-type, is a slow method of asexual propagation. The slow nature of propagation by division results in the better new cultivars never reaching the commercial market but remaining in collector and breeder gardens. We now describe a tissue culture method for rapid clonal multiplication of daylilies. When properly employed, the method yeilds uniform plants without genetic deviation.

MATERIALS AND METHODS

Tissue Source and Preparation. Flower buds measuring 0.5 to 1.0 mm in height are best as explants. Smaller buds were found to be less reliable and larger buds split open at the apex allowing the entry of microorganisms and thrips. Buds at this stage have a hard waxy covering and are free of most microbial contaminants. The buds are dipped for 2 to 3 sec in 95% ethanol prior to disinfection for 10 to 15 min in a 20-fold dilution of Clorox. After disinfection the buds are removed from the Clorox solution and rinsed three times with autoclaved water. From this stage on, all procedures should be performed under aseptic conditions. After sterilization, the buds are disected into individual parts. The three outer sepals and three inner petals are transferred to the nutrient medium. If tissue is in short supply, the six filaments can also be used for callus initiation.

Media. Three different media are necessary for the tissue culture of daylilies, one for each of the three steps in vitro: (1) the establishment of the initial callus from the bud explant; (2) the subsequent shoot multiplication step; and (3) the development of roots on the multiplied shoots. The Murashige and Skoog salt mixture (5) forms the basis for the media used in the three steps. Table 1 lists the other constituents employed at each step.

Table 1. Nutrient addenda employed in *Hemerocallis* tissue culture propagation.

Step I		Step II		Step III	
Compound	mg/l	Compound	mg/l	Compound	mg/l
Thiamine HCl	0.5	Thiamine HCl	0.1	Thiamine HCl	0.1
Nicotinic Acid	0.5	Kinetin	0.1	Nicotinic Acid	0.5
Pyridoxine HCl	0.5	Nicotinic Acid	0.5	Pyridoxine HCl	0.5
2,4-D	1.0	Pyridoxine HCl	0.5	IAA	1-10.00
Kinetin	1.0	i-Inositol	100.0	i-Inositol	100.0
i-Inositol	110.0	Adenine Sulfate	160.0	Agar	8,000.0
Casein Hydrolyzate	1,000.0	KH_2PO_4-H_2O	300	Sucrose	30,000.0
Agar	8,000.0	Casein Hydrolyzate	1,000.0		
Sucrose	30,000.0	Agar	8,000.0		
		Sucrose	45,000.0		

Agar formulations are utilized for the tissue culture of daylilies. The use of liquid is not recommended because of poor results. The tissue culture medium after adjusting the pH to 5.6-5.8 is autoclaved 15 min at 120°C (18 lbs pressure). In step I, the nutrient medium is placed in 4 oz french square bottles in 25 ml aliquots. For the shoot multiplication step 125 ml Delong or Erlenmeyer flasks with 50 ml of nutrient solution are utilized. Step III, the rooting stage, is carried out in 16 × 150 mm culture tubes with 5 ml of nutrient solution.

The shoots, after rooting, are treated as young seedlings and planted in a soil, peat and perlite mixture (1:1:1). The

plantlets should be placed under shade approximately 2 weeks. Mist was found to be detrimental and should not be utilized.

Environmental Conditions. Step I, callus initiation, is carried out at 29±1°C without light. Cultures in the multiplication and root initiation steps are illuminated 16 hr daily with approximately 100 ft-c. Temperatures in steps II and III are held at 26-27°C.

RESULTS AND DISCUSSION

Since our initial report (3) on the multiplicaiton of the daylily cultivar 'Chipper Cherry' the method has undergone considerable change. The revised procedure forms the basis of this report. The cultivars (consisting of both diploids and tetraploids) successfully propagated are found in Table 2.

Table 2. *Hemerocallis* cultivars propagated through tissue culture.

Cultivar	Ploidy Level*
'Chipper Cherry'	D
'Ed Murray'	T
'Hope Diamond'	T
'Little Wart'	D
'Mary Todd'	T
'Purple Robe'	T
'Red Siren'	D,T

* D = Diploid, T = Tetraploid

Callus initiation in stage I is slow and may take 8-12 weeks. At this time, the callus may have the appearance of organized masses. In stage II the concentration of applied hormones greatly influences the formation of shoots. Varying the kinetin concentration from a low of 0.1 to 30.0 mg/l results in a strong inhibition of shoot production as the kinetin concentration is increased. The best shoot production occurred at the lowest (0.1 mg/l) concentration. The auxin compound and concentration are also changed in step II. Initial experiments utilized 2,4-D but the production of some aberrant plants, as indicated by the presence of variegated foliage, resulted in a change to NAA. As with kinetin, lower levels of auxin were more promotive of shoot production. An NAA concentration of 0.5 mg/l gives satisfactory shoot production. Roots sometimes develop at the same time as shoots are formed. The roots, however, are often not attached directly to the shoots but to an intervening piece of callus. When this condition exists, the shoots should be separated from the callus mass and rooted. Attempts to establish plants in soil without roots attached directly to the shoot often leads to failure.

The initiation of roots on the shoots (stage III) is promoted by the presence of an auxin in the medium. As shown in Table

3, the shoots will develop roots without auxin but the presence of auxin substantially increases the root number. As is also shown in Table 3, the concentration of auxin is not a critical factor.

Table 3. Rooting response of hemerocallis plantlets to indoleacetic acid.

IAA Concentration	Roots/Shoot
0	1.6
1.0	3.8
5.0	4.0

LITERATURE CITED

1. Apps, D.A. and C.W. Heuser. 1975. Vegetative propagation of *Hemerocallis* — including tissue culture. *Proc. Intr. Pl. Prop. Soc.* 25:362-367.
2. Bailey, L.H. and E.Z. Bailey. 1930. *Hortus*. Macmillan Co., N.Y. 188.
3. Heuser, C.W. and D.A. Apps. 1975. In vitro plantlet formation from flower petal explants of *Hemerocallis* cv. 'Chipper Cherry'. *Can. J. Bot.* 54:616-618.
4. Morrison, B.Y. 1928. The Yellow Daylilies. *U.S. Dept. Agric. Circ.* 42.
5. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco culture. *Physiol. Plant.* 15:473-497.
6. Stout, A.B. 1934. Daylilies. MacMillan Co., N.Y. 102-103.

RAPID IN VITRO GERMINATION OF IMMATURE, DORMANT EMBRYOS

MARK R. ZILIS and MARTIN M. MEYER

University of Illinois
Urbana, Illinois

The germination and development of embryos *in vitro* on a defined medium is one of the oldest techniques of *in vitro* culture of plants for propagation purposes. Embryo culture was first shown to have promise for rudimentary embryos of orchid when Knudson (3) developed a nonsymbiotic method of germinating orchid seeds on a sterile medium. Up to that time, orchid seeds were germinated in conjunction with a fungus which gave a very low percentage of plants.

Plant breeders, particularly of fruit trees, were some of the early advocates of test-tube culture of plant embryos. They found that some crosses, which normally did not set seeds due to embryo abortion, would produce seedlings if the embryo was excised and grown *in vitro*. Tukey (7) made considerable use of this technique and developed media for fruit tree embryos. Lammerts (4) produced dramatic increases in the breeding programs of fruit trees, camellias, and roses using embryo culture.