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

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RAPID IN VITRO GERMINATION OF IMMATURE, DORMANT EMBRYOS

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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.

A recent development in embryo culture utilizes the *in vitro* technique to germinate seeds with particularly complicated dormancy mechanisms. The dormancy giving the greatest problems belong to seeds containing rudimentary or immature embryos. The embryo is small and must develop using food stored in the endosperm. Other dormancies may further complicate rapid germination. Hu (2) reported in 1974 that *Ilex* embryos grew rapidly and germinated precociously using embryo cultures. This technique saves several years in breeding programs by shortening the generation time.

Two genera of plants, *Paeonia* and *Viburnum*, whose seeds possess difficult dormancies, have been studied at the University of Illinois. The embryos of both are minute, remaining in the rudimentary stage of development when the seeds are dispersed. Peony seed must first undergo a warm, moist stratification for embryo and root development and moist chilling to overcome epicotyl dormancy. Many peony seeds may take 2 years to germinate.

Germination of *Viburnum* seed (1) follows a pattern similar to the peony — warm stratification for root development followed by a period of moist chilling. A few species, however, need only a cold treatment. Viburnum seed germination takes 2 or more years. Peony and viburnum seedlings have been produced in a relatively short time through embryo culture.

Excising the embryos of peony and viburnum seed follow similar techniques. First, the seeds are sterilized in a 10% Clorox solution for 15 to 20 minutes. The embryo half of the peony seed is removed by a wire stripping tool followed by seed coat removal. The endocarp is peeled off the viburnum seed without cutting it in half. The remaining seed parts containing thę embryo are soaked in 10% Clorox for 20 minutes. The seed is then placed in plastic petri dishes and moved to a sterile chamber where the final extraction fo the embryo takes place.

All tools for further extraction are sterilized in 70% ethanol. A scalpel is used to remove the endosperm on either side of the peony embryo. At this point, with viburnum seeds, the endosperm is cut in half and the remaining endosperm is scored above the embryo without cutting through the embryo.

Tweezers are used to grasp the seed on each side of the embryo. The endosperm is bent until the embryo is exposed. A small spatula tool or dental probe can be used to remove the embryo. If the endosperm does not break cleanly, the embryo can usually be teased from the endosperm without injury. Finally, the embryo is inserted into the test tube containing the sterile medium. The tubes are placed under cool white fluores-

cent lights at 300 ft-c. The temperature is maintained at 80°F.

A modified Linsmaier-Skoog (5) medium without hormones or optional constituents was used. Hu (2) found this worked well with *Ilex* embryos. The modified medium contains a higher sucrose (40 g/l) and salt level than the early workers used in embryo culture. It also contains inositol and thiamine HCl, not found in earlier media. The medium was found to be superior to Knudson's or Knopp's in the embryo culture of *Paeonia lactiflora* and *P. suffruticosa.* Only 6 g of agar were used to prevent suppression of growth of the embryo as found by Stoltz (5).

Peony embryos grow rapidly with cotyledons appearing and the root axis reaching 3 to 4 inches in 6 to 8 weeks. At this time, however, the seedling exhibits epicotyl dormancy. A cold treatment for 4 to 6 weeks is necessary to produce the first set of true leaves.

Viburnum seed embryos grow without a cold treatment. There are noticeable changes in embryo size within 24 hours. The radicle protrudes into the medium after 1 week. By the 44th day, the seedlings usually have grown one or two sets of true leaves and can be transplanted.

Peony and viburnum seedlings should be planted without delay. Leaving them in the tubes too long cuts down on survival. The small plants are pulled out of the agar with long forceps, washed off in distilled water to remove clinging agar and planted into a mix of 60% coarse sand, 20% soil and 20% peat moss. Peony seedlings are watered and moved to a greenhouse growing bench. Viburnum seedlings need 5 to 7 days on a mist bench to harden them before moving to a growing bench.

Embryos of Viburnum lentago, V. lantana and V. burkwoodii have been cultured and successfully transplanted. *Paeonia lactiflora,* a herbaceous peony, and *P. suffructicosa,* the tree peony, respond well to embryo culture.

Growing time for seedlings of both genera is cut down dramatically. *Viburnum lentago,* the nannyberry, may take 1 to 2 years to germinate under natural conditions. Seedlings of *V. lentago* can be produced in 45 days by culturing its embryo. This can shorten a breeding program many times.

Thus, *in vitro* embryo culture can be a handy tool for the nurseryman. From its earliest uses with orchids and abortive embryos, it has made possible the consistent propagation of many plants seldom grown by seed. Seeds with complicated dormancy problems caused by rudimentary embryos can produce seedlings easily by embryo culture.

If the excised embryos are raised with hormones in the

medium, masses of callus can be formed. This callus is useful for developmental studies of plants from true tissue culture. Herbaceous perennial plants have been developed from callus as *Iris*, *Hemerocallis*, and *Hosta*, in our laboratory. We hope to use callus to propagate large quantities of more woody perennials in a short time. The growth of embryos *in vitro* can be a valuable tool for studies of tissue culture propagation by other methods.

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ADVANCES IN TISSUE CULTURE: RAYFLOWER AND PROTOPLAST CULTURE[1]

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INTRODUCTION

Culture of organs, tissues, single cells and protoplasts has been used to solve many problems including improving propagation time and increasing clones, developing new clones, growth regulator and physiological studies and producing disease-free clones. In our laboratory these techniques have been used for reduction of time for propagation, increasing clones, physiological and growth regulator effects and plant

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