Investigation No. 10.
In-vitro Culture of Strawberry (Fragaria spp.)

Background

The cultivation of plant cells, tissues, or organs in artificial culture medium (*in-vitro* culture) plays an increasingly important role both in the cultivation of plants and in the production of plants. This procedure is representative of a modern form of vegetative production in which numerous genetically identical individuals are asexually produced from a parent plant (clones). We can produce clones like these in the usual way, using cuttings from shoots and leaves. In the case of cell or tissue cultures, a leaf or a shoot is cut into suitable parts that are then sterilized and grown either in a liquid or solid culture medium. The cells and tissues must be supplied with all the substances that plants require for growth (mineral salts, sugar, amino acids, vitamins, plant hormones). Sterile working conditions are essential, otherwise the plant material is quickly overcome by micro-organisms. After a while, new shoots develop from the tissue, and the shoots can then be cultivated until the required amount has been produced. They must then be acclimatized to normal growth condition. *In-vitro* reproduction is relatively work intensive (sterile working conditions, suitable nutrients) and is therefore carried out by specialized firms (tissue culture laboratories) that supply nurseries and forestry companies. Because the process requires a relatively high investment, *in-vitro* cultures are only viable in the case of plants that are important from an economic point of view, more precisely for those that are difficult to produce from cuttings or exclusively from seed, those that produce only a limited number of seedings, or those whose yield is reduced if they are grown from seed, because of genetic variation.

This modern form of vegetative reproduction has been widely accepted above all in the area of ornamental plants, but also in the cultivation of fruit trees and in forestry.

Equipment

1 bent pair of tweezers (sterile)
1 scalpel (sterile)
1 kitchen timer
1 container for decanting liquids
4 glass beakers, 200 ml, sealed by a glass petri dish (sterile)
1 sterile tunnel
1 Bunsen burner
Adhesive tape
Transparent freezer bags
Wooden sticks ca. 10 cm

Numerous shoots develop from apical bud of the strawberry after 3 to 4 weeks, if the meristems are placed onto a medium containing cytokine (approximately 10 to 30 mg/l). If the shoots are then transferred to a medium supplemented with 0.5 to 1.0 mg/l auxin, they produce roots after about two weeks. The small plants can be cultivated further in plant pots. All of them produce leaves of the same shape and otherwise posses similar characteristics. Here the students experience the conspicuous production of clones. The work must be carried out in sterile conditions or else other micro-organisms might be produced that would overrun the pieces of plant tissue in a very short time. A 'sterile tunnel' can be used for this purpose.

Material

MS powder (basal salts with minimal organics)
Granulated sugar
Agar agar
Phytohormone solution

70% alcohol (denatured alcohol may be used), approximately 100 ml
96% alcohol (denatured alcohol may be used), approximately 100 ml
Diluted domestos solution (disinfectant), 20%, approximately 100 ml
Sterile tap water, approximately 1000 ml

Procedure

1. Rinse shoot appex strawberry runners in 70% alcohol in a sterile glass beaker for about 1 minute. The glass lid must be opened only for as short a time as possible and must be replaced immediately. Carefully decant the alcohol without removing the lid so that the objects do not slip out.
2. Add dilute bleaching powder solution and shake the glass beaker. Sterilization time should be 3 to 5 minutes. Decant the solution as in 1.
3. Rinse the shoot apex in sterile distilled water 3 times for 5 to 10 minutes; shake slightly with the lid closed. Carefully decant the last water used for rinsing.
4. Take the shoot apex out and place it onto an empty sterile petri dish. (The tweezers used for this step are sterilized in 96% alcohol before use.)
5. Dissect the meristem to explant (with the apical dome enclosed in several leaf primordia) removing outer leaves.
6. Gently press the meristem to explant onto the culture medium that contains cytokine.
7. Place the culture vessels in a well-illuminated place for about 3 to 4 weeks (room temperature).
8. When shoots form, transfer them to culture medium supplemented with auxine so that they form roots.
9. After approximately two weeks, as soon as small roots have been formed, transfer the plants to plants pots. These in turn are placed into freezer bags that are tied at the top. Place a small wooden stick in the soil.

Observations

Record your observations on the sheets provided.

Discussion

1. What is the purpose of rinsing in 70% alcohol and in bleaching powder solution?
2. What are the advantages of clonal propagation?
3. What are the possible disadvantages of clonal propagation of a plantation crop such as tea?

Investigation No. 11.

The Economics of Tissue Culture Propagation

Background

Plant tissue culture is a technique used to grow microbe-free plant material in an aseptic environment. The objective of this investigation is to determine the use of plant tissue culture as a plant production system. It is not necessary to produce all plants by tissue culture methods. Therefore we should first ask the following questions: How does the species concerned normally propagate in nature and how is it propagated by man? What are the advantages and disadvantages of using tissue culture methods for its propagation? Are the advantages more rapid multiplication, year round propagation, and elimination of viruses? Will the cost of using tissue culture for propagation be justified?

In a tissue culture laboratory one aspect of the research is to grow the plants in culture and another is production-line research aimed at cost cutting and maximizing performance. In 1985 it was reported that worldwide there were more than 200 commercial laboratories involved in plant micropropagation.

Crops	Percent of laboratories
Orchids	58
Ferns	19
Foliage and flowers	33
Woody crops	16
Tropical plantation crops	6
Vegetable crops	10
Fruit crops	15

In Sri Lanka, the Ceylon Tobacco Company and the Department of Agriculture have commercial laboratories in which orchids, anthuriums, potatoes and pineapple plantlets are produced. Orchids are also propagated by small holders. Woody plants are not yet produced on a large scale.

Aim of Investigation

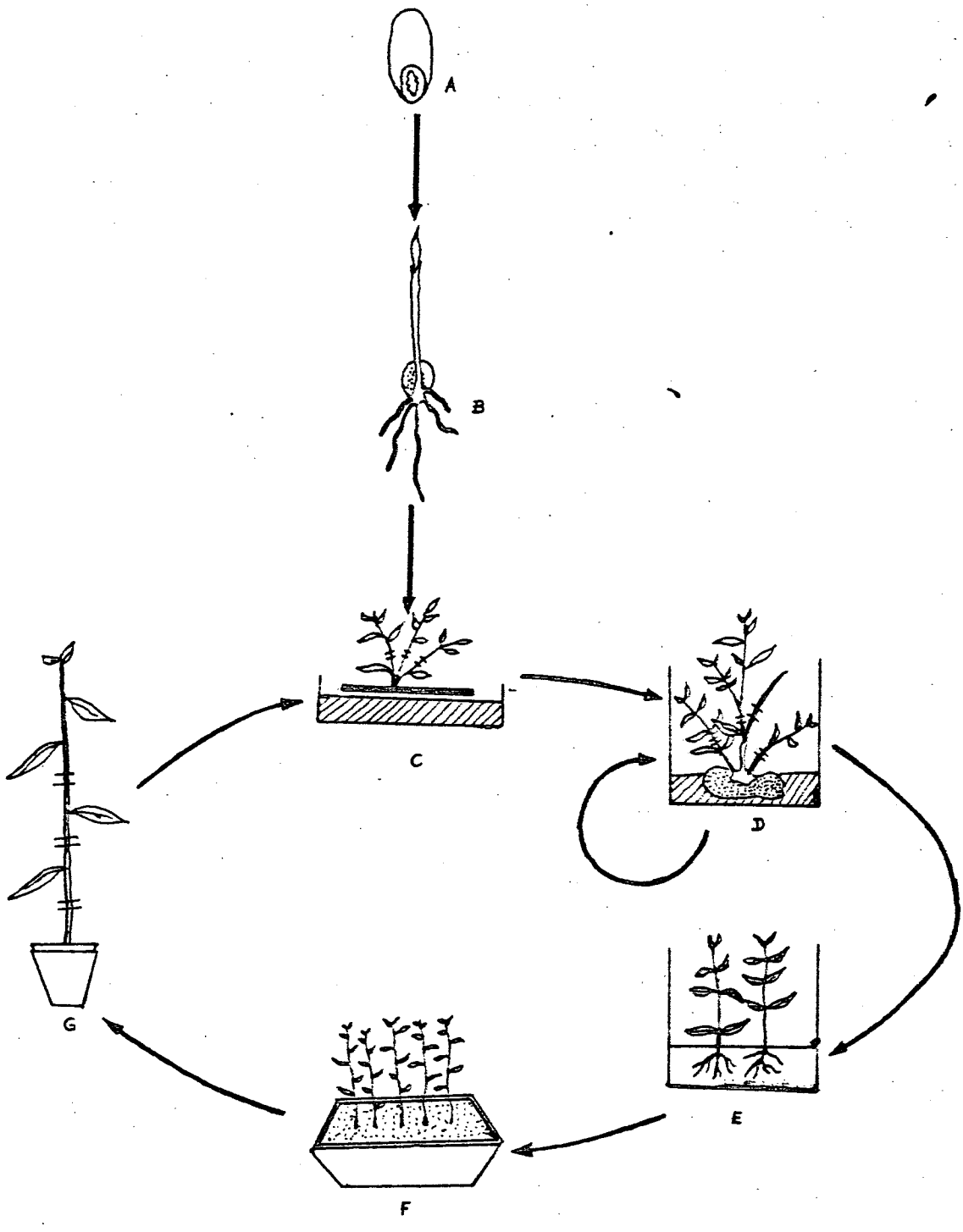
Bamboo, a useful plant, is difficult to propagate by conventional methods. Is it, therefore, feasible to propagate bamboo using tissue culture?

Information Required

The different methods of propagating bamboo are as follows:

1. From rhizomes by division: In eight hours a labourer may prepare only four rhizome divisions.
2. By cuttings: Only 2% of the cuttings are successful. In eight hours a labourer may plant 100 cuttings.
3. By tissue culture from seeds: Bamboo seeds are very difficult to find as bamboo plants flower very rarely. In India, they are able to produce bamboo plants from seeds at a cost of Rs.5.00 per plant. The breakdown of costs is typically 60 to 80% for labour, 5 to 10% for culture medium and the balance for miscellaneous expenses such as electricity. The Figure on page 27 illustrates how the seeds are cultured into plants.

If a labourer is paid Rs.30.00 per day, calculate the cost of production for 1,000 plants of bamboo by conventional and tissue culture methods.



Plant Propagation by tissue culture

A - Seed
B - In vitro Seedling
C - Bud-break
D - Shoot multiplication
E - Root initiation
F - Acclimatization
G - Plantlet

Results

1,000 plants by rhizome	Rs.....
1,000 plants by cutting	Rs.....
1,000 plants by tissue culture method	Rs.....

APPENDIX

1. Basal Agar Medium

Glucose	10.0gl^{-1}
Casein peptone	4.0
Meat extract	4.0
Yeast extract	0.5
Liver extract	0.5
NaCl	2.5
Agar-agar	15.0

2. Basal Broth Medium

Same as Basal Agar Medium without agar-agar.

3. Nutrient Agar Medium

Peptone from meat	5.0gl^{-1}
Meat extract	3.0
Agar-agar	12.0

4. Minimal Agar Medium

Dipotassium hydrogen phospate	3.4gl^{-1}
Sodium citrate $2H_2O$	0.5
Magnesium sulphate $7H_2O$	0.1
Ammonium sulphate	1.0
Glucose	2.0
D,L-Histidine	0.2
D,l Arginine	0.2
Thiamine-HCl	0.05
Agar-agar	15.0

5. Murashige-Skoog (MS) Medium

MS Basal salts with organics	4.7gl^{-1}
Granulated sugar	30.0
Agar-agar	8.0
Phytohormones	1ml

pH regulated with potassium hydroxide to 5.8

6. Nutrient Broth Medium

Same as Nutrient Agar Medium but without the agar.

Micro-organisms may be obtained from one of the following suppliers:

1. American Type Culture Collection (ATCC)
   12301, Parklawn Drive
   Rockville, Maryland 20852
   USA

   Telephone: (301)-881-2600
   Telex: 908768 ATCC ROVE
   Fax: (301)-231-5826

2. Deutsche Sammlung Von Mikroorganismen
   und Zellkuturen GmbH (DSM)
   Mascheroder Weg 1B
   D-3300 Braunschweig
   Germany

   Telephone: (0531) 61-87-0
   Telex: 531104 DSM
   Fax: (0531) 61-87-18