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Root Cultures

Liquid media have been employed in tissue culture research mainly in the culture of excised roots and cell suspension (see chap. 9). In addition, various kinds of microcultures, e.g., hanging-drop preparations and protoplast cultures, are special applications of liquid media. Aside from experiments on the formation and development of embryoids, relatively little has been done on the cultivation of organized plant structures other than roots in liquid media. The use of a liquid medium has some advantages over the use of nutrients in an agar-solidified matrix. When a callus is grown on an agar medium, diffusion gradients of nutrients and gases within the callus will lead to modifications in growth and metabolism. The agar itself releases contaminants to the culture medium, and metabolites secreted by the growing callus accumulate in the agar matrix.

One consideration given to plant material growing in a liquid medium is the availability of oxygen and the extent to which the isolated cells or organs require agitation or forced aeration. In regard to root cultures, the importance of oxygenation is unclear. Although Street (1957, 1969) has stated that the availability of oxygen is not a limiting factor in tomato root cultures, this view disagrees with the findings of Said and Murashige (1979). These workers found that continuous and gentle agitation of tomato root cultures resulted in a doubling of root elongation compared to root growth in stationary cultures, and the production and elongation of lateral roots were considerably improved.

There are certain advantages in achieving the continuous culture of an isolated root of a plant. This technique provides information on the nutritional requirements of the root, i.e., removed from the interchange of compounds with other plant organs. We have a detailed account of the nutritional requirements for isolated tomato roots (Street, 1957). In addition to nutritional studies, root cultures from herbaceous species have provided experimental material for investigations on lateral root and bud formation, initiation of cambial activity, and nodulation (see Torrey, 1965). Another advantage of employing sterile cultures is the elimination of the complicating effects of microorganisms. Root clones have a rapid growth rate, and there are no difficulties in multiplying the

have a rapid growth rate, and there are no difficulties in multiplying the clone in order to yield any desired quantity of plant material (Butcher and Street, 1964).

A question has been raised about the relationship between cultured excised roots and similar roots produced by an intact plant. Although excised roots and "intact" roots are alike in many anatomical and metabolic ways, certain differences have been reported. Excised roots gradually lose the capability of forming secondary vascular tissues during culture. Cultured tomato roots, in contrast to seedling roots of the same plant, fail to show a normal geotropic response (Butcher and Street, 1964). In addition, the biochemical composition of cultured roots may differ significantly from that of seedling roots (Abbott, 1963). Studies with microbial symbionts associated with roots have suggested that cultured roots may differ in some respects from the roots of axenically grown seedlings (Torrey, 1978). In spite of these differences, research results obtained from root cultures are relevant to the physiological activity of the roots of higher plants; for example, the production of alkaloids, anabasine and nicotine, continues undiminished in *Nicotiana* root cultures (Solt, Dawson, and Christman, 1960). Although *Solanum* callus synthesizes only traces of glycoalkaloid steroids, the rootlets contain 5.2% (Staba, 1982). Root cultures may become important in the future in the commercial production of useful compounds.

An interesting root culture technique was devised to study the infection of legume roots with *Rhizobium* (Raggio and Raggio, 1956; Raggio, Raggio, and Torrey, 1957). The excised roots were provided with organic nutrients via the basal end of the root inserted in a medium contained in a small vial. The apical portion grew into an inorganic medium in a Petri dish. This arrangement simulated in vivo conditions, i.e., organic metabolites normally enter the root base from the shoot system, whereas minerals are mined from the soil by the tip region. Since *Rhizobium* infects the root via root hairs, the inorganic medium was inoculated with the microorganism. Isolated roots from *Phaseolus vulgaris* and *Glycine max* were used in these studies, and later the method was improved by Cartwright (1967). Torrey (1978) reviewed the use of this technique, as well as other symbiosis studies involving root cultures.

The first successful organ culture, i.e., potentially unlimited growth of the isolated organ, was reported by White (1934) with excised tomato roots. Several modifications of White's medium are currently employed in plant tissue culture studies (Thomas and Davey, 1975; Said and Murashige, 1979). After the initial experiments with tomato, the excised

roots of numerous herbaceous species were placed in culture (Butcher and Street, 1964). Less success has been achieved in starting root cultures from woody plants, although there are reports on cultures from *Acacia* sp. (Bonner, 1942), *Robinia* sp. (Seeliger, 1956), *Acer rubrum* L. (Bachelard and Stowe, 1963), and *Comptonia* sp. (Goforth and Torrey, 1977). Also the roots of several species of gymnosperms have been cultured (Brown and Sommer, 1975). In some cases, the minimum growth requirements were met with the essential minerals, a carbon source, a vitamin supplement, and a few amino acids. Some responded favorably to the addition of auxin and other growth regulators. Inositol is an effective growth stimulant for some isolated roots, and this cyclitol plays a role in secondary vascular tissue formation in excised roots of radish (*Raphanus*) (Loomis and Torrey, 1964; Torrey and Loomis, 1967).

The technique employed to initiate and subculture roots requires some explanation. Root tips approximately 10 mm in length are removed from young seedlings produced during the axenic germination of seeds, and these apical tips are transferred to an aqueous culture medium. After about 1 week the primary root produces lateral roots. The main root axis is subdivided into "sectors," and each contains a portion of the main axis plus several lateral branch roots. Each of these sectors is transferred separately to a fresh medium for an additional period of incubation. The lateral roots subsequently produce laterals themselves. Each of these sector cultures provides the investigator with a constant supply of lateral root tips for experiments, as well as a source of material for the propagation of the clone. This technique can be used only when the excised root produces laterals in some sequential order. In some species this does not occur.

Some general comments can be made about the nutrition of cultured roots. A marked improvement over White's medium is the substitution of a chelated form of iron for $Fe_2(SO_4)_3$, because use of the latter salt leads to an iron deficiency during prolonged culture. During root growth an alkaline shift in pH occurs, which results in the precipitation of $Fe(OH)_3$ (Guinn, 1963) and possibly $Fe_2(PO_4)_3$ (Dalton, Iqbal, and Turner, 1983). The sodium salt of ferric ethylenediamine tetraacetic acid (NaFeEDTA) is generally used as a chelated form of iron. Sucrose is the carbon source of choice, although some monocot roots grow equally well with glucose. A sugar level of 1.5–2.0% (w/v) is sufficient, and higher concentrations may alter root metabolism (Guinn, 1963; Fig. 8.1). Al-

though several amino acids have been tested, most of them inhibit the growth of cultured roots. The vitamin requirements vary slightly for different species, although all species require the addition of thiamine. Cereal roots have a higher auxin requirement in comparison to dicot roots. Street (1957) reported that Petkus II rye roots required either auxin or an auxin precursor for continued growth. The effects of light on the growth of cultured roots, however, appear to vary with the species and the cultural conditions.

In the present experiment, sterile seedling roots obtained from the axenic germination of seeds taken from a fresh tomato fruit will be excised and cultured in a modified White's medium. After approximately 7–10 days of growth, sufficient lateral root development will have occurred to permit the first subculture with sector inocula (Fig. 8.1). Through periodic subcultures the student should maintain the root culture over a period of several weeks. Various experiments can be devised in order to determine the optimum conditions for growth and development of the isolated roots.

PROCEDURE

Preparation of White's medium (see formulations of tissue culture media at the end of the book). In general, use the same procedure outlined in Chapter 4 for the preparation of the MS medium, i.e., first prepare stock solutions of iron, micronutrients, and vitamins. Because of the small amount of molybdenum involved, weigh 10 mg MoO_3, dissolve it, and add DDH_2O for a final volume of 1,000 cm^3. Pipette 1 cm^3 to deliver 0.01 mg MoO_3 to the micronutrient stock.

White's (1963) formulation required $Fe_2(SO_4)_3$. Because ferric sulfate precipitates easily from solution, a chelated form of iron is more desirable. The concentration of iron employed in the MS medium can be used satisfactorily in White's medium. Prepare and use the stock as directed in Chapter 4.

The vitamins are dissolved in DDH_2O in the order indicated, and a final stock volume of 100 cm^3 is prepared.

Add approximately 400 cm^3 DDH_2O to a 1 liter beaker. Weigh and dissolve each of the macronutrient salts given in the table of formulations. From each of the stock solutions add by pipette to the macronutrient solution: 5 cm^3 iron, 10 cm^3 micronutrients, and 1 cm^3 vitamins. The final medium is adjusted to pH 5.5, and sucrose is supplied at a concentration of 20,000 mg/l of final medium. (*Note:* A possibility exists of some degradation of the B vitamins during autoclaving.)

Culture procedure

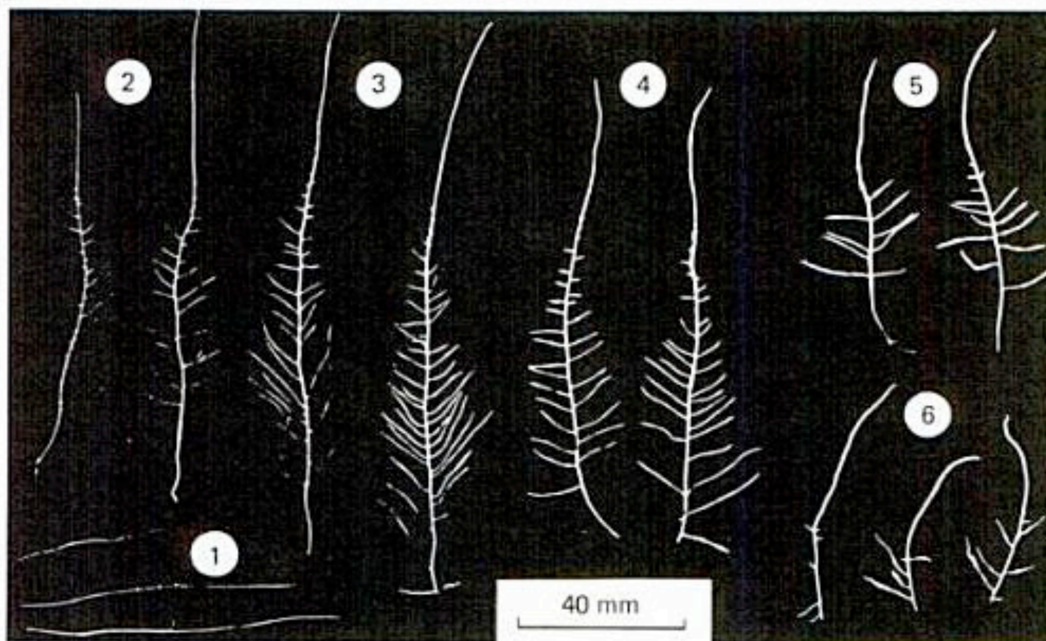
The working area of the transfer chamber is lined with paper toweling, previously sterilized by autoclave. The folded toweling is enclosed in a heavy paper mailing envelope for sterilization. Wash the tomato fruit with tap water. Surface sterilize the skin of the fruit with ethanol (70% v/v) in the transfer chamber. Using sterile instruments, cut the skin and slice the fruit into four sectors. Pull the sectors apart exposing the seeds. Remove the seeds with sterile forceps, and carefully remove the fruit pulp around each seed. Place approximately 5 seeds in each of the Petri dishes lined with Whatman No. 1 filter paper. Moisten the filter paper with about 8–10 cm^3 of sterile DDH_2O delivered by pipette. Wrap the dishes in aluminum foil, and place them in the incubator (25°C). The seeds will germinate within a few days. The Petri dishes must be checked frequently for signs of drying of the filter paper. If necessary, a few drops of sterile water may be added. Root tips approximately 10 mm in length are excised with a scalpel or iridectomy scissors and transferred to the flasks containing White's medium. White (1963) suggests that the root tips be transferred individually, i.e., one per culture flask. After about 1 week, sufficient lateral root development will be evident, and sector inocula (Fig. 8.1) can be removed and subcultured to a fresh medium. The student should perform weekly transfers and attempt to maintain the tomato root culture over a period of several weeks.

RESULTS

Although cultured roots are difficult to measure in situ during culture, an attempt will be made to approximate the linear growth rate. Daily measurements can be made on a single culture, within the flask, without exposure and risk of contamination. Place the flask on a sheet of graph paper, and align the root with the markings on the paper. Compare the growth rates of root cultures that have been stationary to the growth rates of other cultures that received agitation on a shaker. An interesting

comparison can also be made on roots cultured in the dark versus other roots that received illumination. The incubation temperature will also influence the growth rate. The growth rates of tomato roots cultured under various conditions have been reported by Boll (1965).

Fig. 8.1. Excised tomato (*Lycopersicon esculentum*) roots cultured 7 days at 27°C in White's medium containing sucrose at a concentration of (1) 0.5%, (2) 1.0%, (3) 1.5%, (4) 2.0%, (5) 3.0%, and (6) 4.0%. Note the formation of lateral roots from the main root axis. In order to subculture roots, the root is cut into sectors. Each sector, transferred to a fresh medium, contains a portion of the main root axis plus several lateral roots. (Courtesy of H. E. Street.)



QUESTIONS FOR DISCUSSION

1. What are some advantages of using an aqueous medium in comparison to an agar-solidified medium? Can you think of any advantages that were not mentioned in this chapter?
2. In addition to providing a source of oxygen, what are some other possible effects of agitating cultured plant cells, cell aggregates, or organs?
3. What are the nutritional requirements for the culture of isolated tomato roots? Do all isolated roots have approximately the same nutritional requirements?