California Avocado Society 1968 Yearbook 52: 128-130

THE LONGEVITY OF AVOCADO TISSUE IN VITRO

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The continuous culture of tomato roots through 1600 passages and for more than 28 years by White (6) indicates the practicability of maintaining plant tissues for very long periods in vitro. This phenomena also supports the theory that under appropriate environmental conditions plant tissues can be maintained in living conditions for an indefinite time. Plant tissues other than roots such as tobacco pith have been established as clones. Through the process of successive transfers to new media these materials have been multiplied many fold in some cases and appear to have established the ability to maintain a degree of vegetative function and to undergo cell division indefinitely.

The early report on the establishment of citrus pericarp tissue as a clone of continuously dividing cells and callus forming tissue (3, 4) is presently verified as some of these materials now have been maintained through continuous culture by periodic transfer over a period of eight years. Tissues originally obtained from the rind or albedo of Valencia orange *(Citrus sinensis),* the lemon (C. *limon)* and Citron *(C. medica)* have been maintained in continuous culture on artificial agar media of known inorganic nutrient composition and appear to thrive.

Explants from avocado pericarp were among the first fruit tissues described to undergo proliferation in vitro (2). While successive transfers and continued callus formation has been achieved over short periods of time up to three or four months, these particular tissues have not yet been fully established as clones. Short term experiments up to periods of eight weeks have involved studies on optimum temperature and light requirements, nutritional responses and high temperature tolerances. The establishment of true reproducible clonal lines for cytological and other studies has not been achieved.

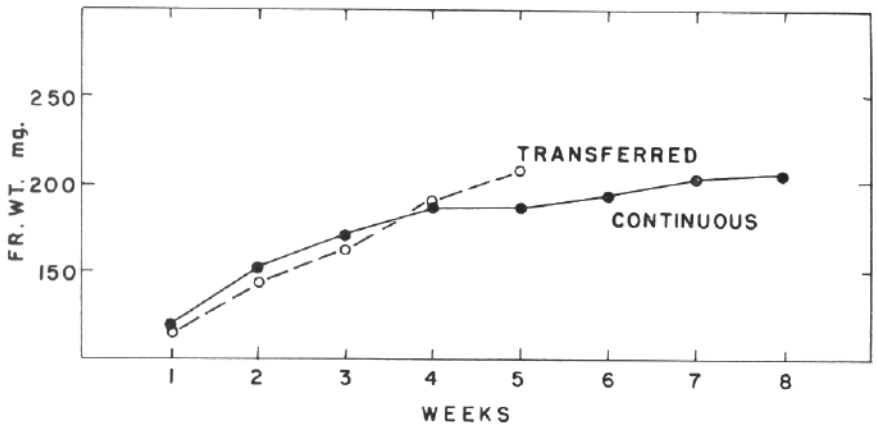

Figure 1. Growth curves of avocado pericarp tissue grown in vitro under continuous culture compared with tissue transferred to fresh media each week.

A typical growth curve of avocado tissue grown continuously on a standard media without periodic transfer compared with a series of sections which were transferred to fresh media each week is shown in Figure 1. Period transfer appears not to markedly increase the growth rate or total growth of tissue over the short term experiment. The typical growth response shown by sections grown continuously without transfer on agar media indicates that maximum size or weight is attained about four weeks following the planting and that increase in fresh weight is probably continued but at very slow rate after this period. Short time experiments are generally terminated after four weeks of observation.

Tissues originally obtained from the large fleshy cotyledons of the avocado seed, however, appear to be more favorably adaptable to continuous culture and subsequent subtransfer and thus have become established as true tissue clones. One set of such clones was first established on agar media on June 22, 1960 and through a series of 21 periodic subcultures has been maintained in excellent growing conditions until the present time, August, 1968. This callus remains essentially undifferentiated and proliferates irregularly as a white callus mass. The nutrient agar media which is employed in these studies is that suggested by Nitsch (1) with the substitution of FeEDTA (Geigy 138) for iron citrate and the addition of exogenous Indole-3-acetíc acid (10 ppm).

It is theoretically possible to induce plant cells from any source to undergo division and exhibit proliferation if the original tissues are not too highly differentiated when selected for explanting. The major problem in the establishment of new tissue clones from any source is to determine the optimum or essential nutritional environmental conditions required for continuous tissue growth. The formulation of the inorganic and organic nutrient supply probably is the major difficulty which limits the establishment of such cultures. The composition of the media must be determined experimentally as presently there is very little knowledge from which we can predict the nutrient requirements of untried tissues except in a very general manner Other environmental conditions of temperature, light and pH of media also may be important factors which may limit

success of such cultures, hence these factors must be determined experimentally.

Figure 2. Avocado cotyledon tissue which has been maintained on agar nutrient media and subcultured over a period of eight years.

Morphological modifications have been described for avocado tissues grown in vitro (5). There has been little evidence thus far of the development of oil or starch in the clonal callus tissues, although the tissue from which it was derived originally was characterized by high oil content. It may be altogether possible under some specific conditions not yet known to induce the explanted tissues to produce oil and other substances in vitro and function much as the normal intact tissues.

LITERATURE

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