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LONGEVITY OF PLANT TISSUE CULTURES

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The culture of excised tissue pieces from many species of fruiting and ornamental plants has provided an important method to investigate several problems of plant propagation and plant physiology (2). Since the inception of the modern aspects of plant tissue culture, which possibly can be dated with the classical investigations of Philip White in 1939 (8), many advancements have been made and much literature has developed as the result of extensive research in this area of plant physiology.

The adaptation of tissue culture techniques to problems concerned specifically with the avocado has resulted in a number of literature citations (1, 3, 4, 5). The present account is given as a progress report on some studies concerned with various aspects of propagation and fruit and plant physiology of several plant species, including the avocado. Short term experiments to answer questions of immediate concern have been reported (1, 5) and previous reports on the continued propagation of callus tissue (4, 5) indicate that some of these tissues can be maintained indefinitely as a growing, undifferentiated callus culture. The objective in the present line of investigation is to determine how long avocado tissues can be maintained alive-after separation from the plant or fruit.

The general tissue culture technique consists of selecting explants of tissues of various dimensions from parenchymatous or living active cellular tissue from almost any actively growing portion of a plant body (7). The excised tissue is separated from the parent plant and placed on sterilized agar nutrient tissue in glass vials. All operations are carried out in a sterile room under carefully controlled conditions. The appropriate nutrient media and the subsequent correct environmental conditions of light and temperature determine to considerable extent the success of the experiment. In the present series of experiments tissue callus cultures were established with and have been maintained on a basic culture media developed by Nitsch (3). The callus in each instance has been kept at 25-27°C under a light intensity of approximately 90-150 foot candles. The callus grows well but not excessively under these general conditions. When the callus mass covers the agar surface of the 6-dram vial or if the agar media begins to dry excessively, the callus is subdivided into two to four or more pieces each of which is transferred to a freshly prepared culture vial.

The callus which results from such technique continues to undergo cell division at a slow rate. There is little evidence of cellular differentiation, the resulting callus mass becoming rather uniform in internal structure and texture and in surface appearance.

Avocado callus tissue has been established from several sources such as the pericarp or fruit wall, the firmer cotyledon or seed tissue with its massive starch content, and the

more delicate tissue such as the leaf petiole or the stem of the inflorescence which is termed the peduncle. Any portion of the plant or fruit which is not dead or which has not differentiated into fiber or vascular vessel generally can be induced to undergo cellular proliferation to produce an amorphous callus mass.

TABLE I
LONGEVITY OF PLANT CALLUS CULTURES IN VITRO

Tissue source	Origin	Number of transfers (as of Nov. 15, 1977)	Callus texture-color
Avocado cotyledon	June 22, 1960	36	fluffy, smooth white
Avocado (etiolated) stem tissue	Sept. 7, 1976	3	fluffy, smooth white
Avocado (peduncle) (stem of inflorescence)	April 22, 1971	10	smooth, white
Avocado (petiole of leaf)	Sept. 1971	9	fluffy, white
Rose (stem pith)	Nov. 2, 1967	12	smooth, tan
Lime (*Citrus aurantifolia*) fruit pericarp	Aug. 2, 1960	34	fluffy, white
Karne (*C. aurantium*) fruit pericarp	July 25, 1960	32	fluffy, white

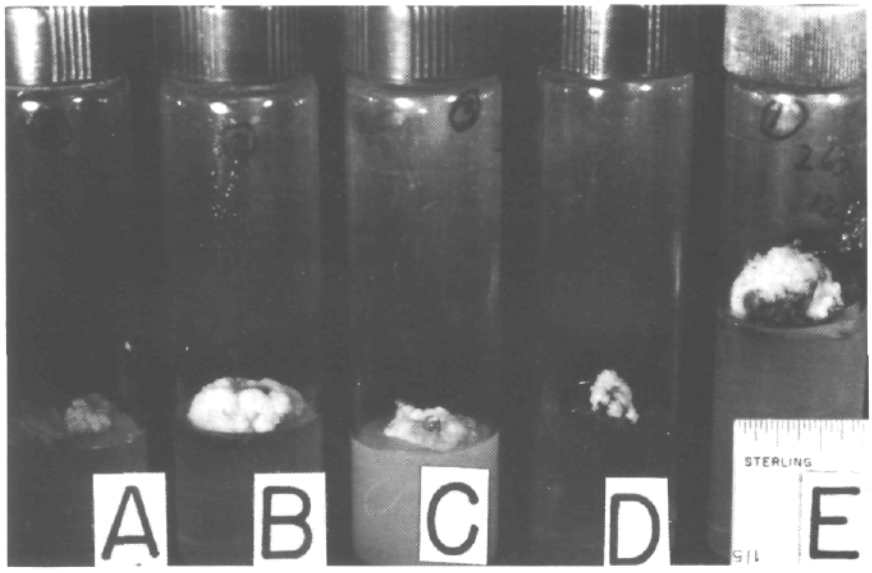

FIGURE I
Plant tissues derived from various plant parts maintained as callus in vitro for various periods. Photograph made November 15, 1977.
A. Rose pith tissue, 10 years old.
B. Tissue from avocado cotyledon 17 years old after 36 subcultures.
C. Tissue originally derived from rind of lime fruit in 1960 and carried through 34 transfers in vitro.
D. Tissues derived from peduncle of avocado inflorescence in 1971 after 10 subcultures.
E. Callus established in 1975 from stem of etiolated avocado seedling.

Inspection of Table I shows that tissues derived from several sources within the avocado plant and fruit have been established successfully as callus carried through several subcultures. The oldest tissue clone in the present experiment was established more than 17 years ago by cutting a disk of tissue from an avocado seed and placing this disk on agar nutrient media. Now after 36 transfers or subcultures of the original callus mass, the present tissue is a fluffy, smooth white callus. Apparently this callus can be maintained indefinitely under the conditions of the experiment. No attempt is made to speed up the rate of callus growth nor has the maximum reproductive capacity of the callus been ascertained. Avocado callus originally derived from peduncle tissue has been carried through 10 subcultures during the past six and a half years. The resulting tissue mass is essentially identical in appearance to that derived from other avocado tissues, possibly showing a slightly smoother surface. Another explant originally developed from a cylinder of stem tissue of an etiolated seedling maintained in darkness for several months prior to cutting the tissue, is growing well following three transfers during the past year. The petiole tissue of an avocado leaf originally planted six years ago indicates satisfactory proliferation and subsequent callus development during the nine subculture divisions and transplants of the tissue.

Tissues from other plant species such as the rose pith originally established in 1967 now are growing well after ten years and twelve subcultures. The appearance of the rose tissue is distinguished from that of avocado by a smoother, darker surface. The rose tissue is generally slower in its general growth habit. Two other tissues derived from the rind of a lime fruit *(Citrus aurantifolia)* and a Karna orange *(Citrus aurantium)* were originally excised in 1960. Both tissues are white and comparatively smooth in surface character and of moderate vigorous growth following 34 subcultures in the case of the lime tissue and 32 subcultures in the Karna callus. It would appear that these tissues can be maintained indefinitely as callus under the present experimental conditions.

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