California Avocado Society 1976 Yearbook 60: 160-163

RESPONSES OF AVOCADO STEM PIECES IN TISSUE CULTURE

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The techniques developed for the establishment of avocado clones by grafting, budding and by the rooting of leafy stem cuttings are well described in the literature (2, 4) and are presently widely utilized by nurserymen throughout the world. Standard nursery practices involve the use of germinated seed for rootstock source onto which are budded the selected clonal materials. Rooted cuttings can be obtained directly from some old clones by use of leafy cuttings assisted by root promoting hormones. Other old avocado clones usually are more difficult to root, as cuttings can be established on their own roots by the special techniques utilizing a treatment of etiolation or growing the clonal tissue in the dark for at least a portion of time before the roots are induced to form (2).

The objective of developing techniques to obtain clonal material of avocado by apical meristem culture for rootstock or other purposes has been the focus of attention by many investigators. Attempts to approach this problem by the use of tissue culture techniques have been encouraged by the success attained with other plant materials, usually those more succulent or herbaceous in nature, such as chrysanthemums, pelargonium, carnations, etc. (1, 5, 6). Fast growing species are comparatively easy to propagate by conventional methods, namely the rooting of leafy cuttings, and thus have proved feasible to propagate by culture of the apical meristem, which, if accomplished under appropriate conditions results in virus-free plants. Woody plant species on the other hand are usually slower in their growth responses and, likewise have proved more difficult to establish as clonal material from apical meristem or from small apical tips (3).

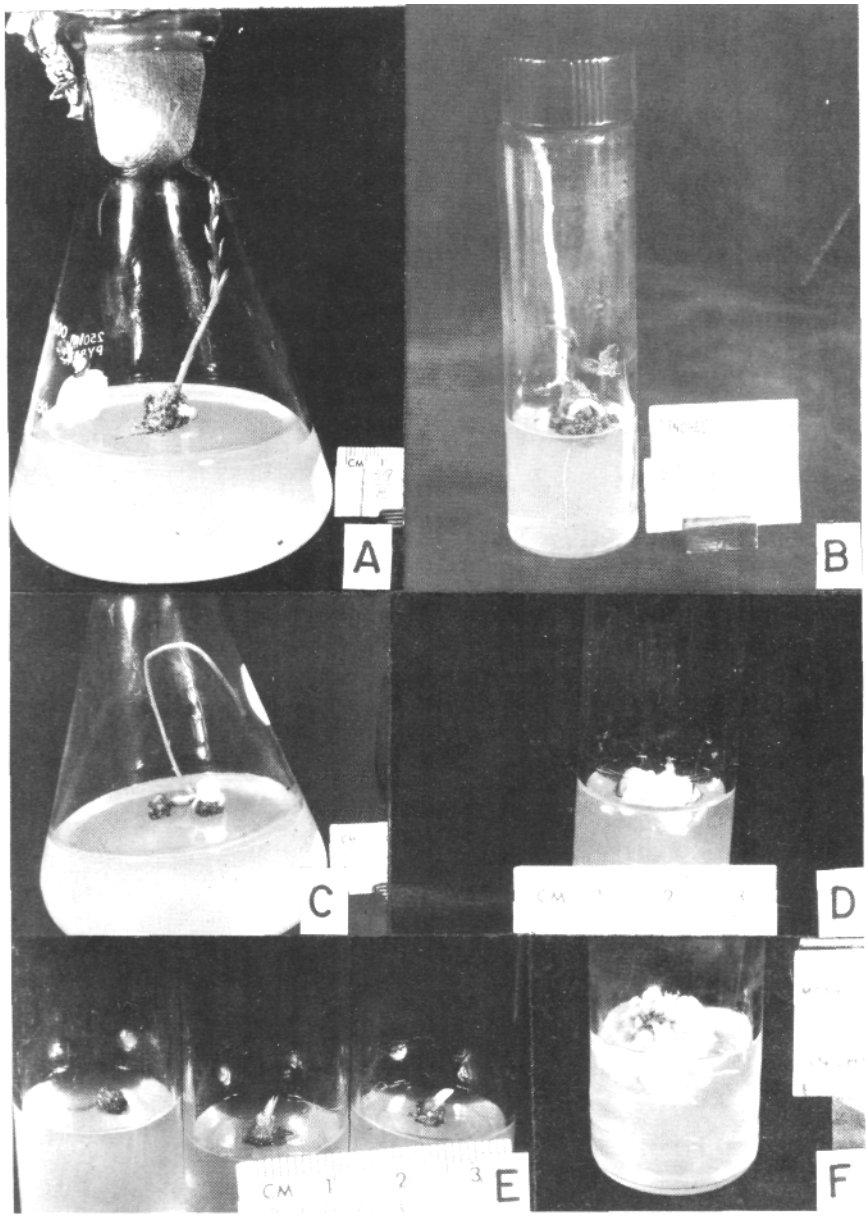
The investigations reported here are presented as a progress report on some studies which have been conducted on avocado tissues grown in vitro. Previous reports (7, 8, 9, 10) have been made which indicated that tissues from nearly all parts of the avocado plant can be cultured as callus masses in vitro. These materials have been studied from several points of view and with different objectives in mind. Some of the results attained thus far are only encouraging and do not necessarily point to definite conclusions but do suggest that useful techniques might be developed which may be helpful in propagation procedures. It is quite possible to maintain cultures of callus tissue derived from avocado the cotyledon for periods of fifteen years or more according to observations made at present. It is conceivable that callus derived from other tissues likewise, might be maintained indefinitely. Such tissue does not exhibit obvious conditions of differentiation but is merely continued in culture as a callus mass by a series of divisions into smaller segments and transfer of these segments to fresh media at various intervals of time, usually one to three months. Differentiation of this apparently homogeneous callus mass into definite, recognizable structures such as roots has been

observed in many experiments. The development of shoot structures, per se, from callus tissues has not yet been noted under the conditions of the present investigations. While the reproduction of an entire and normal plant from callus tissue derived from a relatively pure specific tissue of avocado has not yet been achieved, such an objective has been attained in several species such as tobacco (4), carnation (6) and others (1). It appears to be theoretically possible to accomplish the re-constitution of an avocado plant from any of its cells or a small group of cells, but the practical problems have not been overcome at this time. Therefore attempts are under way to reproduce avocado plants from more organized tissues, such as, apical meristem or small buds.

Repeated attempts have been made to culture in vitro what are considered as normal apical or lateral buds obtained directly from trees in the field. These buds did not indicate any evidence of length growth when collected but were "plump" and otherwise in a healthy condition. The buds at this stage are about three to four millimeters in length. They consist of many small bud scales and a few larger protective bud scales which cover the delicate apical meristem and sub-apical tissues. The selection of tissue in this condition is not considered as a meristem culture technique. The materials have been selected at this stage in an attempt to establish in vitro the more gross structure of an intact bud. True apical meristem tissues less than one millimeter in length have been excised from buds under the microscope, but, have failed to survive under a limited number of conditions which have been tried. This aspect of the study will require more refinement of techniques and must be repeated under a wider range of conditions.

The general behavior of material taken as "intact buds" when planted on a wide range of nutrient agar media, has been noted to proliferate as a callus from the cut surface or more usually from the periphery of the cut surface, when the bud is placed with the cut surface on the agar media. Massive callus formation, the result of extensive cell division, will generally cover the agar surface within the culture vial. This is followed by a considerable increase in the thickness of the callus mass which grows upward, generally enveloping and hence "inundating" the original bud mass, such that it cannot be detected within the callus. Occasionally some of the buds will exhibit some length growth and will eventually protrude from the callus mass to a distance of four to five millimeters. This length growth appears to be limited or inhibited in some manner, hence, the culture makes little progress except to increase the size of the callus.

Another approach to the problem of establishment of lateral or apical buds in vitro, which has provided more response in the development of length growth, has been the pretreatment of the bud system before excision of the bud. The general practice has been to place small grafted trees or seedlings in half gallon or gallon cans in a dark walk-in room. The leaves are removed from the stems and the new lateral shoots are allowed to grow in the dark as etiolated tissues. Considerable length growth of a lateral bud up to half a meter (18 inches) or more gives rise to a thin white lateral shoot with many small white leaves, each usually less than one centimeter in length. This etiolated stem will have a bud in the axil if each leaf, such that 20 to 25 lateral buds may be obtained from a half meter length of stem. The stem is generally cut into small cylinders, five to ten millimeters in length. Each cylinder may or may not contain a lateral bud and subtending leaf.



TISSUE CULTURE

A. Shoot elongation from avocado bud which had been grown in dark.
B. Shoot elongation and root development from callus of bud which had developed in dark.
C. Stem section which had been etiolated showing callus and bud elongation.
D. Etiolated stem section showing start of callus formation and no bud elongation.
E. Etiolated avocado buds just starting to elongate.
F. Non-etiolated avocado bud showing extensive callus formation but no elongation.

These etiolated cylinders of tissue are placed on their side on the agar nutrient media of several compositions and under various conditions of temperature and light. The first response of the tissue is that of massive proliferation at the cut ends to produce a callus. There is, however, a stronger tendency for the lateral bud, when present, to elongate and produce measurable length growth. The extension of the bud results in the normal development of small leaves, hence, a normal but small plantlet is established,

but with a massive callus at the base. Occasional roots will eventually develop in some of the cultures in which length growth of the apical portion has been achieved. Root differentiation and development has not been induced at will under the present experimental conditions.

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