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APICAL AND OTHER RESPONSES OF TISSUES OF AVOCADO IN ASEPTIC CULTURE

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Application of tissue culture techniques to the investigations on several problems associated with the avocado fruit have been reported (3, 6, 7, 8, 9, 10). Studies on tissue cultures of the vegetative plant parts have received less attention but some observations on these aspects have been made. The current interest in vegetative propagation of clonal materials from apical meristems or other vegetative parts of plants warrants a brief report concerning results of a number of experiments conducted in our laboratory over a period of years particularly related to this aspect of the avocado.

The feasibility of propagating clonal lines of plant materials from isolated segments or intact apical meristem segments under controlled sterile conditions in tissue cultures has been demonstrated repeatedly in many plant species (1, 2, 4, 5, 11, 12) particularly those kinds of plants having herbaceous or succulent stem structures such as carnations, chrysanthemum and asparagus. The cultivation of apical tissue from woody plants has been more difficult. A major objective in all these apical meristem investigations has been to culture only the extreme apical growing tissue of an old clone in the attempt to reproduce a virus-free new clone. The extreme apical meristem tissue of the plant axis is apparently free of virus particles because of the structural features of the developing tissues and the unspecialized cells in the apical dome. If the apical tissue freed from older subtending and differentiated cells, can be cultured and rooted much like a tip cutting the resulting new plant can be virus free.

There has been a need for pure clonal lines of avocado varieties which are virus free and of uniform genetical type for use in studies on rootstock responses, resistance to the root-rot fungus *Phytophthora cinnamomi* and for studies of transmission of viruses such as sun-blotch.

The present report is concerned with attempts to propagate small apical portions of avocado buds and infloresences with the objective of obtaining virus free clones and tissue clones of uniform genetical composition.

The establishment of tissue callus clones from any part of the avocado plant body or fruit structure requires transfer of small tissue pieces to a sterile agar nutrient medium under aseptic conditions. The basic techniques utilized in such investigations have been described in previous investigations (6, 9, 10). These earlier studies indicated the possibility of growing in vitro avocado tissues from various part of the plant such as root pith, stem pith, leaf petiole, cotyledon and the fruit pericarp. Some of these tissues which have been established as callus clones undergo cell division consistently and can be subcultured by taking parts some of the newly developed cells and transferring these

to new nutrient media. Such subcultures can be carried on indefinitely. Callus tissue clones derived originally from the cotyledonary tissue of the seed in July, 1960 has been maintained in tissue culture through 24 subcultures and is growing well as of January, 1972. Another avocado callus clone derived originally from the fleshy fruit wall now has been successfully subcultured three times since January, 1970. In most of these cases the resulting callus is a mass of tissue derived from extensive cell division with or as surface proliferation or internal meristem development. Such calluses are generally amorphous cellular masses with no particular form and without differentiation. Occasionally in established tissue clones derived from cotyledonary tissue there appears in the subcultures from certain original explants a tendency to develop roots which penetrate into the agar media (Figure I D) These roots exhibit the typical internal structure of normal roots with a well defined cortex, a vascular cylinder of normal lignified xylem elements and a rootcap. This observation is described in a previous publication (10). While roots appear frequently in some of the subcultures, the development of a shoot apex has not been observed in any of the cultures. It is possible that modification of the nutrient or physical environment could result in the development of shoot tissue but this specific condition has not yet been induced in the present series of investigations.

Segments of avocado leaf petiole placed horizontally on agar nutrient media will proliferate at the two cut ends to produce a dumbell-shaped structure with two massive calluses (Figure 1-A-B). The increase in fresh weight of such petiolar tissue can exceed 800 per cent within a period of two or three months. The use of avocado leaf petiole segments for physiological studies has been encouraged by these responses. It is of interest to note that the newly proliferated tissues which develop from the cut ends of the petiole do not penetrate into the agar media but rather spread over the surface in many instances. This particular response probably is related to the requirement of the tissue for a moderately high oxygen level. Previous experiments with liquid media cultures showed that aeration of the nutrient solution resulted in much greater tissue growth compared with non-aerated solutions.

Attempts to cultivate in vitro small apical and lateral buds from avocado shoots have resulted in the production of massive calluses at the base of the bud, but in all instances thus far there has been a failure to produce roots or to induce but very limited shoot elongation. This response is similar to that generally observed when woody shoot cuttings from old established horticultural varieties are placed in vermiculate or other rooting media. In this latter situation large leafy cuttings may maintain their leaves for long periods and massive callus frequently will form at the base of the cutting but root differentiation is not observed. Cuttings taken from young seedlings or from certain old horticultural clones, however, may form roots under such conditions. The relation of age from seed thus may effect root initiation in cuttings taken from clones.

Vegetative buds of avocado grown in-vitro have appeared to remain alive for long periods, but have failed to elongate under the experimental conditions described above. It is quite possible that modifications of either the composition of the nutrient media or the physical environment may eventually result in root development and shoot elongation. The apical and lateral buds utilized in the present investigations have included more tissue than desired for true apical meristem culture. Apical buds in the

present experiments have been approximately 3-8 mm in length and included much woody tissue and well developed bracts and leaf primordia. The ideal apical section for tip culture is probably 1 mm in length or less with few or no visible, identifiable leaf primordia. Until the gross technique for establishment of small tip cuttings is developed it appears more practical to use larger plant-tissue masses for these preliminary experiments.

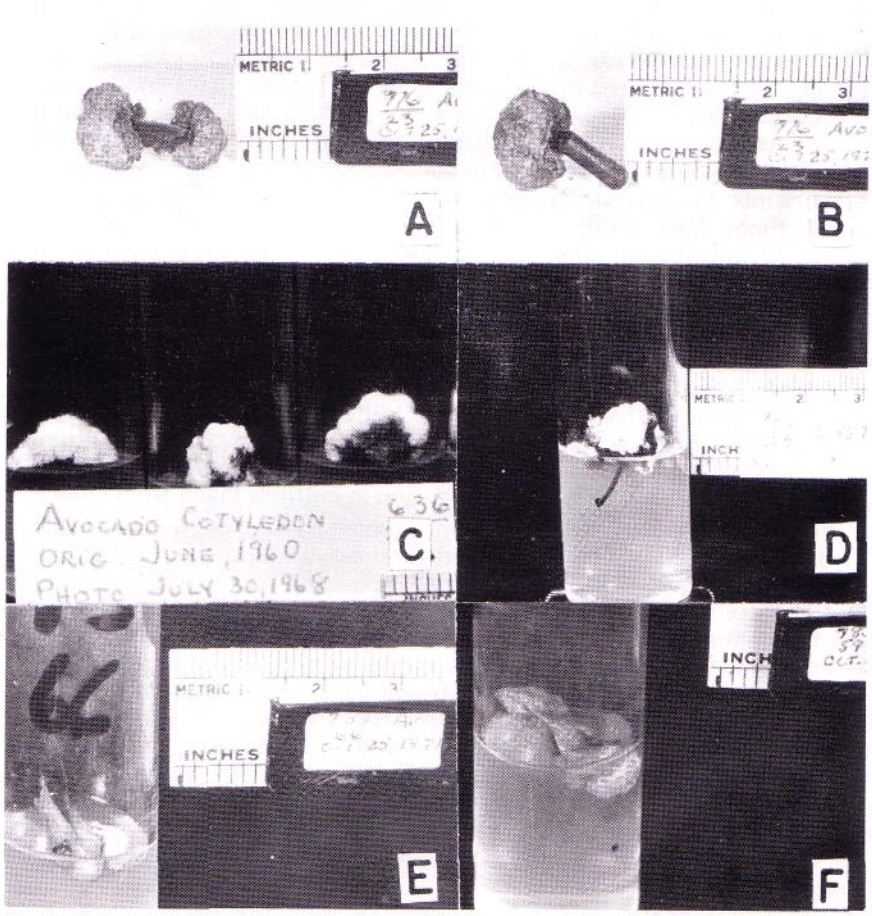

Figure 1. Avocado tissues *in vitro*. A-Tissue proliferation from cut ends of leaf petiole, B-Tissue proliferation from one end of petiole piece, C-Three callus tissues derived from avocado cotyledon in 1960 and grown in vitro through several subcultures, D-Callus tissue originally derived from cotyledon showing root development, one root in agar and one root in air. E-F-Callus formation at base of avocado bud maintained in vitro.

A series of experiments was conducted in an attempt to maintain individual flowers and small inflorescences of avocado in vitro to study their behavior in vitro. The response of such excised floral tissue on agar nutrient medias has been a proliferation of cellular tissue at the cut basal end but no elongation of the stem nor expansion of the flowers (Figure 1-E, F.). In some eases the extensive callus has practically enveloped the original floral bud. The flowers or flower buds did not develop beyond the stage at which they were taken. No roots appeared during the several weeks during which the callus developed.

This brief report indicates that apical shoot tissue and small segments of inflorescences from avocado can be maintained in tissue culture for extended periods and that the growth responses of such materials consist primarily of callus proliferation at the basal, cut end. There appears to be no tendency towards the production of root tissues under the conditions of the present experiments. Explants derived from the cotyledonary tissue of the mature seed and from the fleshy fruit pericarp, when subcultured in vitro, can give rise to normal roots. It remains to be determined by experimental procedure the necessary modification of the experimental conditions which will induce root formation. This may involve the variation of nutrient constitutents or manipulation of the physical environment perhaps by provision of a specific photoperiod or type of light source which might induce root formation in tissues originally derived from apical meristem. Investigations are under way toward this objective.

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