

Avocado Tissue *in vitro*

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The dramatic response of some plant species to environmental responses in tissue cultures has led to some very practical methods of reproduction of clonal materials for use as rootstocks, or more directly, to their use as clonal scions as means of reproducing fruit varieties (4). This method of clonal reproduction has been especially adaptable to several herbaceous or succulent types of plant materials such as certain orchids, carnations, and many vegetable species, though not entirely restricted to these types of plants. A number of woody species of both dicot and monocot plants, including the date and oil palm, have been successfully established as clones through tissue culture techniques. It is generally reported that woody species are somewhat more difficult to propagate by tissue culture procedures, hence special precautions or treatments must be developed such as pretreatment and preconditioning of the source material or very exact sequences of treatments and techniques must be followed to insure success.

Tissue of the woody avocado, *Persea americana*, has proved somewhat more difficult to handle under sterile tissue culture conditions, though some improvement of technique has been developed during the past several years. The production of clonal materials in avocado has been announced on several occasions but wide application of the methods has not been well demonstrated. The present report represents some aspects of the problems of avocado propagation techniques which might provide information for future investigations. It represents a progress report on some basic aspects of tissue culture technique applied especially to the avocado.

The utilization of sterile tissue culture techniques to investigate physiological responses of avocado tissues to such factors of the environment as light and temperature (2, 3, 4) has suggested basic information which can be applied to clonal propagation of woody species. Much of the tissue culture investigations have utilized the fruit pericarp as an experimental tissue. The present study is concerned only with the responses of tissues derived from stem segments or from the use of entire lateral or terminal buds of seedling plants.

Materials and Methods.

The present studies were made on avocado seedling materials grown in pots in a glasshouse. One might expect some variation between any two seedlings in respect to their responses because of genetics which probably prevail. Some of the seedlings were grown in a glasshouse for a period of three or four months until they attained a height of 24 to 30 inches. Some of the plants were then defoliated by cutting off the

mature leaves and were immediately placed in a dark chamber. Several lateral buds were produced and these elongated into side branches while the defoliated plants were maintained in the dark chamber. This gave rise to a series of "etiolated" branchlets. The latter were completely lacking in chlorophyll, and hence were white. The branchlets bore only vestigial white leaves, approximately 1/4 to 3/8 inch long. Small lateral buds, frequently pink in color, were developed in the leaf axils. Some of the lateral etiolated branches reached 5 to 6 feet in length. Some of the etiolated branches were dotted or streaked at random with pink tissue patterns.

The culture media used in these experiments were those of Nitsch modified by Schroeder (2, 3, 4) or that of Murashige and Skoog (1), again with some modifications as indicated in the specific treatments. The culture vials were 6 dram screw cap vials each containing 10 ml of agar media.

Tissues were collected and surface sterilized by dipping the stem piece in a 10 percent solution of Clorox for approximately 10 minutes followed by a quick rinse of distilled, sterile water. The selected tissue segments were excised under sterile conditions and transferred to the vials each containing 10 ml of nutrient agar media. The vials were then kept in a temperature controlled room at 25-27°C under approximately 100-200 foot candle light conditions.

While tangible results of rapid multiplication of avocado clonal materials are generally not available, the occasional induction of shoot and root tissues in an original callus segment suggests the possibility of tissue culture procedures which warrant continued investigations. Several aspects of the process of differentiation in avocado tissue sections and pieces *in vitro* have been reported (2, 3, 4). Avocado fruit tissues have proved to be satisfactory experimental materials for basic investigations on temperature tolerance and conditioning (2). Roots have been observed to develop from calluses of tissues originally obtained from fruit pericarp (3), but no shoot tissue has been observed nor reported in such investigations. The maintenance of entire flower buds and pieces of floral components *in vitro* has resulted in the production of a series of unusual proliferation of stamen and other floral structures. Some of these aberrant growths simulate those structures noted occasionally *in vivo* on some trees which result in the so-called "woody avocado."

Some basic information on the response of tissues derived from avocado stems (3) suggests that a pretreatment or preconditioning of the tissue by etiolation or development of tissue in the absence of light can be utilized to induce shoot elongation. Buds taken from shoots grown in the light and transplanted directly onto the culture media frequently will not produce shoots but remain in an apparent dormant state. The tissue subtending the inactive or dormant buds may exhibit extensive proliferation of cellular tissue resulting in a massive callus. Frequently roots will differentiate in this basal callus, but the apical bud system of the original explant remains inactive. When comparable buds are selected from plants grown in the dark, there is observed the intense cell division response which results in a massive callus; but additionally, there is observed the elongation of the included apical or lateral bud. This generally results in the development of a plantlet frequently with some root development, though roots cannot always be predicted or induced under the present conditions of the experiments.

The results of a typical response are shown in Figure 1. The etiolated tissue has produced massive callus on the cut end of the etiolated stems. Bud elongation is observed when a lateral bud has been included in the stem piece (Fig. 1A). When no lateral bud was included (Fig. 1B) a massive callus developed and sometimes roots appeared after a period of approximately three months in the growth chamber.

Nitsch media with isopentyl adenine (6 mg/l) and caseine hydrolysate (4 mg/l) induced callus to develop beneath the agar surface (Fig. 1C left) whereas Murashige-Skoog media with isopentyl adenine (30 mg/l) resulted in callus development above the agar surface (Fig. 1C-right).



Figure 1. Avocado seedling stem pieces from etiolated dark grown plants. A-shoot extension and root formation from included lateral bud. B-massive callus developed on both ends of stem section without included bud. C-development of submerged callus (left) and surface callus (right) on stem sections without buds. D-etiolated shoots of avocado; note reduced leaf size.

Literature Cited

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