

RESPONSE OF AVOCADO FLOWER BUDS AND FLORAL PARTS CULTURED IN VITRO

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Observations on the behavior of specific and isolated tissues derived from a number of plant species have provided much useful information toward the understanding of some physiological responses within the intact plant and have added to the knowledge of the morphological and developmental sequences of the plant at various stages. Information thus attained on the factors which control or affect floral ontogeny and subsequent fruit formation can be of particular value in the understanding of some aspects of fruit development in the field. The isolation and culture *in vitro* of individual plant and floral organs and specific fruit tissues thus can provide data from which new experimental approaches to problems can be developed and may offer a reasonable explanation of some problems at hand.

Some of the following experimental procedures were originally designed to study the floral behavior of mature avocado flower buds under strictly controlled environmental conditions. It has proved difficult to induce the mature flower bud to undergo *in vitro* the normal sequence of development such as enlargement of the floral parts, development of receptivity in the pistil and anthesis of the stamens. Apparently an arrest of all morphological development results under the conditions of the experiment, but a number of other interesting responses of the excised tissues have occurred which are described in this report.

METHODS

Inflorescences of avocado with flower buds in various stages of development were divided into several categories of tissues such as mature flowers, partially developed flower buds and very small buds. Individual floral parts from nearly mature flowers were excised and planted individually as perianth parts, stamens, staminodes, nectaries and pistils. Portions of the peduncle and of pedicels were also planted. The nutrient agar media were of several basic types, namely White (18), Murashige and Skoog (2) and Nitsch (5) with some modification in the form of exogenous kinetin, indole-acetic acid (IAA), naphthalene acetic acid (NAA) or coconut milk (CMF) in various concentrations. Ordinary sterile-room and transfer techniques were employed. The floral buds were separated into size groups or dissected into their several parts and placed in 5 to 10 percent Chlorox solution for 5 to 10 minutes, rinsed in sterile distilled water, and held in sterile petri dishes until placed individually in the 6 dram screw cap vials containing the nutrient media and floral parts. These vials were then held in constant temperature chambers at $27^{\circ}\text{C}\pm 2^{\circ}$ in the dark. Periodic observations were made under the dissecting microscope.

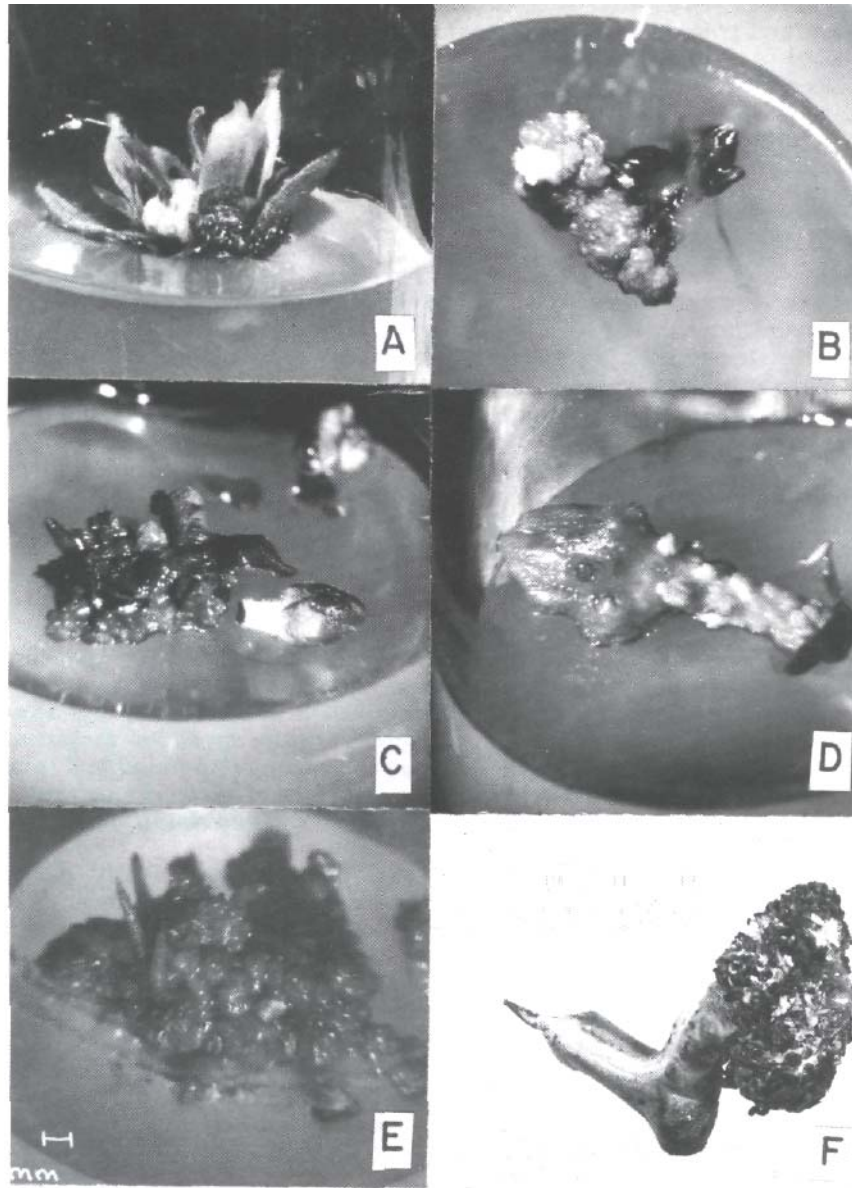


Figure 1. Avocado flowers and floral parts cultured in vitro on agar nutrient media. A—Mature avocado flower showing typical callus development at base of floral parts. B—Proliferation from inflorescence axis of immature avocado floral cluster. C—Callus mass from inflorescence axis showing separation of partially developed flower buds. D—Proliferation on outer surface of immature flower bud. E—Root formation from callus mass derived from flower buds in vitro. F—Woody avocado fruit borne on regular inflorescence stem.

Observations:

When a segment of inflorescence included a group of several very small immature flower buds together with the undeveloped inflorescence axis, the massive callus which sometimes developed at the cut base and later in the several abscission zones of the individual flowers proliferated to such an extent that the entire original tissue was completely enveloped by callus tissue.

A rather regular sequence of morphological developments was observed in the isolated, partially developed or unopened flower bud when placed on agar nutrient media and maintained in darkness. Two zones of meristematic activity were stimulated under such conditions. The first was the development of an abscission layer subtending the point of insertion of the floral parts and somewhat basal to the receptacle (Figure 2-C). Cell division activity became prominent in a plane perpendicular to the floral axis causing a separation of the floral receptacle from the pedicle. Another point of increased meristematic activity was at the base of the pedicle if the flower had been kept in a horizontal position on the agar (Figure 2A, C). If the flower was placed upright in its normal attitude with the pedicle submerged in the agar, then there was seldom any meristematic activity or callus formation at the basal end of the pedicle. Little or no growth of any kind and from any tissue was observed below the agar surface of the nutrient media. Limited aeration reduces meristematic activity and callus formation in such instances (9).

The development of the abscission layer in individually placed flower buds caused the floral bud to separate from the pedicel. The meristematic activity of the abscission layer continued in many cases, resulting sometimes in a massive callus several times the volume of the original bud. Frequently the bud was completely surrounded by the extensive callus mass which resulted from this activity.

Partially open and nearly mature flower buds exhibited the development of the abscission layer and in addition proliferated at irregular points on nearly all the attached floral parts (Figure 1-A, Figure 2-B). Thus meristematic activity produced small masses of callus most generally on the ventral side of the perianth segments (Figure 2-G), at the base of the stamens (Figure 2-D, E, F,) and from the receptacle at the point of insertion of the staminodes and nectaries (Figure 2-D). The stimulation of abnormal cell division in the basal regions of floral parts other than the ovary may be of particular significance as this response could be related to the development of abnormal fruits which will be discussed later. Foci of cell division activity also were noted at irregular positions all along the outer pedicle surface (Figure 1-D), giving rise to a "warty" appearance on the surface.

Attempts were made to culture very immature, nearly mature and open flower buds, the latter not yet in anthesis, with the objective of observing some sequential late stages of development, particularly as related to anthesis. Under the conditions of the experiments and following 5 to 10 minutes exposure to 5 to 10 percent Chlorox solution, there appeared to be a cessation of floral bud enlargement from the younger stages and a failure of the anther to continue development to the anthesis stage in the nearly mature flowers. The treatment with sterilizing solution did not appear to affect the subsequent though somewhat later development of callus tissues from the pedicle region or from the interior of the perianth parts, especially at their bases (Figure 2-G). Initiation of proliferating centers among the basal portion of the staminodes and stamens in some cases contributed a considerable volume of callus.

In some instances floral buds in various stages of maturity were cut in a vertical plane into two identical pieces and placed on agar media. Proliferation was prominent from nearly all cut surfaces and again also from among the basal portion of the filaments and staminodes.

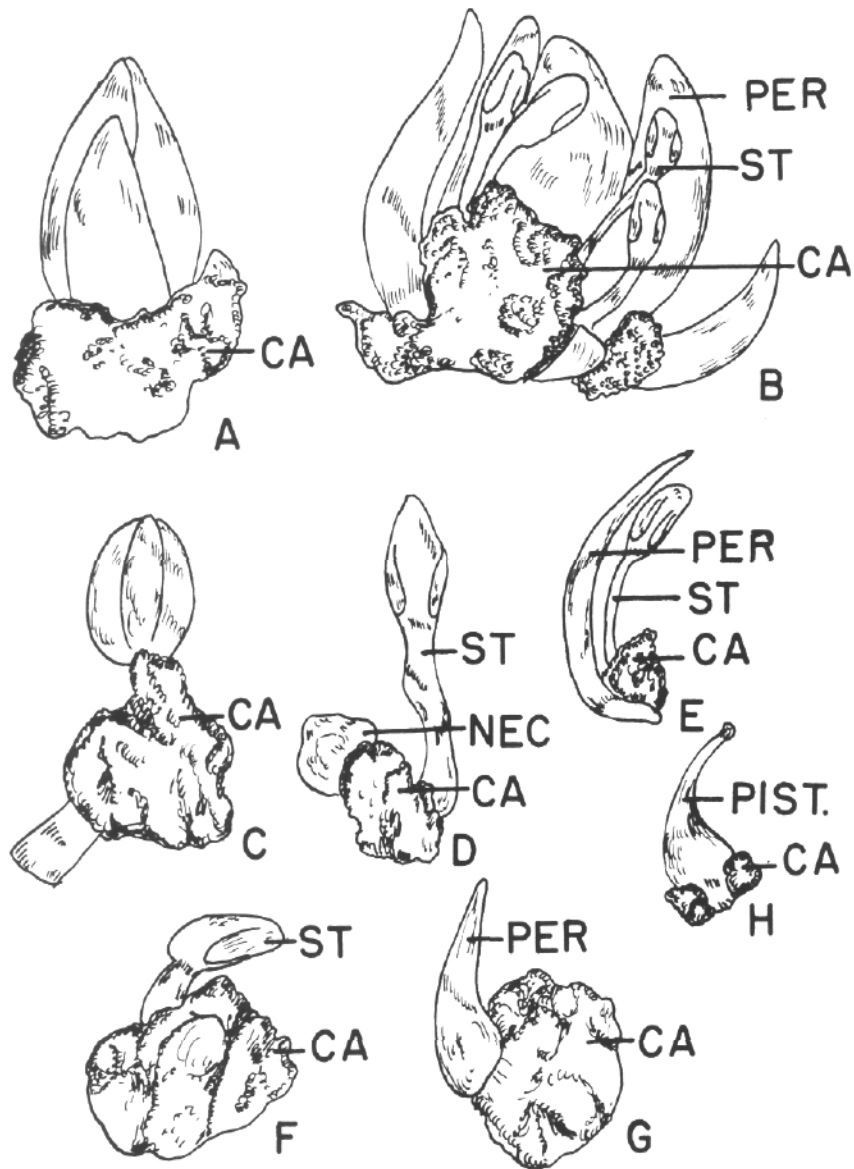


Figure 2. Sketches showing extent of callus from flower buds and floral parts cultured in vitro on agar nutrient media. A—Proliferation of callus at base of unopened, small flower bud. B—Mature flower cultured in anthesis stage showing massive proliferation from base of floral organs. C—Immature, small flower bud showing callus at abscission region of flower stalk. D—Stamen and nectary cultured together with callus formation at base. E—Stamen and perianth part cultured together showing callus formation at base. F—Small immature stamen cultured in isolation with massive callus at base. G—Callus development at base of individual perianth part cultured on agar media. H—Callus at base of pistil cultured in vitro.

Regeneration of callus from individual floral parts

Individual floral parts placed on nutrient agar in separate vials showed a wide range of responses. Generally the major site of meristematic activity was evident at the base of the specific organ where detachment from parent tissue had occurred. Thus callus

development is first noted generally at the basal end of the anther filament and in the lower portion of individual perianth parts (Figure 2-D, F, G). Perianth parts which were placed in a horizontal position on the nutrient agar surface developed a massive callus over nearly all the upper, exposed surface but this meristematic activity was initiated in the basal region of the perianth part and progressed in development toward the apical portion. Occasional perianth parts with their apical portion immersed into the agar media developed massive callus on the exposed basal end.

Individual excised pistils placed upright in a normal position or on their side showed occasional proliferation generally near or at the cut basal end (Figure 2-H). Some excessive cell division and callus formation was noted on a few stigmas.

Root formation in callus from flower buds

A rather striking example of tissue differentiation within the callus mass which developed at the base of intact individual flower buds was the occasional appearance of root structure in some specimens (Figure 1-E). Several buds which had been growing on agar media for approximately four weeks developed callus masses nearly six or seven times the volume of the original flower bud. Distinctive root structures then appeared growing outward and frequently upward from the lower surface of the basal callus mass.

DISCUSSION

Many reports in the literature are concerned with attempts to culture entire floral buds or individual floral organs *in vitro* (1,2,4,6,11,12,15,16,17). Vasil (14) has provided an excellent review of anther physiology in which isolated anthers on agar media provide the basis for several investigations. Tepfer (13) mentioned that floral buds of *Aquilegia* grown on agar with IAA at .05 ppm caused early abortion of carpels; at 2-5 ppm IAA the entire bud was abnormal and callus was induced. They did not study the callus. Raste (7) using inflorescence segments of *Mazus pumilus* obtained callus from which developed roots, shoots or flower buds.

The potentiality and tendency of floral parts of avocado buds to proliferate at their points of insertion on the receptacle provides an interesting and possible explanation of the development of occasional so-called "woody" avocado fruits which are reported from many areas and from many varieties (8). These aberrant structures have appeared infrequently usually singly on a tree but occasionally several have been observed on one tree. The woody structure is borne on an inflorescence stem much like a normal fruit and is frequently subtended by persistent perianth parts and simulates a typical fleshy fruit except that the surface is highly irregular in texture and the form is extremely variable (Figure 1-F). The bulk of such fruit tissues consists of highly lignified cell masses of sclerenchyma or stone cells and wood vessels. Thus the texture is completely different from the soft, thin-walled parenchyma which comprises the major portion of the soft pericarp wall tissue of the normal avocado fruit. Such structures may weigh several pounds and attain considerable and abnormally large size. The surface of the woody fruit may be relatively smooth or suggestive of a rosette type of vegetative development. Previous morphological investigations (8) indicate that this unusual fruit-

like structure is not derived from the abnormal development of the carpellary wall of the ovary but instead from proliferation of tissues outside the ovary, possibly from the base of a stamen, staminode or nectary. The present observations concerning the tendency of floral parts other than ovary to proliferate *in vitro* indeed suggest the possible origin of "woody" fruits from a stimulation of receptacular or floral tissue which results in a massive callus. Differentiation of vascular elements, sclerids and lignification of many kinds of cells then could proceed much as has been demonstrated in previous investigations on callus derived from avocado fruit pericarp tissue (9).

The appearance of roots from otherwise undifferentiated callus tissue of avocado has been reported (10). Such callus was originally derived from the fleshy pericarp wall. Other observations on the development of roots *in vitro* were made on tissues derived from avocado cotyledons which have shown a strong tendency to develop well differentiated roots as the callus have been periodically transferred since the original explant made more than eleven years from their original excision. Roots also have been observed on callus derived from petiole segments (unpublished data). The present observation on isolated flower buds was made on callus masses from which root structures have developed within a period of six to eight weeks from the time of planting *in vitro*.

While the development of root structures appears to be common from callus derived from almost any tissue of the avocado, the production of shoot structures *de novo* has not yet been in evidence in any of the many observations made thus far.

The potentiality of nearly all tissues from the avocado plant, flower and fruit to undergo intensive cell division and proliferation when placed under favorable environmental conditions *in vitro* provides an excellent opportunity to study the many factors which may be concerned with floral development of this species. One might speculate on the possibility of producing complete plantlets from such floral tissue in the future. Such plantlets could possibly be free of virus diseases hence could be of value in many research endeavors. The establishment of plantlets from avocado tissue cultures has not been achieved as yet.

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