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TISSUE CULTURES OF CALLUS DERIVED FROM AVOCADO FRUIT

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The technique of tissue cultures is frequently used to study the effect of growth substances and nutrients on tissue growth and development. Tissue cultures of soybean callus and tobacco pith are widely used for determining the level of cytokinins in plant tissues.

The avocado mesocarp was the first fruit tissue mentioned in the literature as being capable of undergoing proliferation under conditions of tissue culture (6). However, the tissue could not be successfully maintained for a long period in tissue culture (7). On the other hand, callus originating from avocado cotyledons could be successfully grown for several years (7).

Several years ago we started a broad research project dealing with the relation between the level of endogenous growth substances and the development of avocado fruit. In the course of the study, we attempted to grow cultures of avocado fruit tissue and to determine their response to the addition of growth substance. It was hoped that in this way we might learn something about the special nature of the avocado fruit, in whose pericarp cell division is constantly occurring, even after attaining maturity.

For four years we have been growing callus of avocado derived from mesocarp and cotyledons, which is transferred every two-three months to a new nutrient medium, and have encountered no difficulty in obtaining and growing new callus of this type.

OBTAINING THE CALLUS

The callus was obtained from fruits of the Fuerte variety which were disinfected with sodium hypochlorite, washed in sterile water and burned with alcohol. All the work was conducted under sterile conditions to prevent contamination. Cylinders of mesocarp, 20 mm in diameter, were removed from the fruit with a cork borer and cut into discs 3 mm thick. Cotyledons were cut into slices of similar size. The tissue discs were then placed on a solid nutrient medium.

The mesocarp discs generally swelled within a few days, as described by Schroeder (6), after which a white granulation appeared on the surface of the discs. After approximately 3-4 weeks, callus growth began on the sides of the discs (Figure 1). The discs were transferred to a new nutrient medium, and after a further period of growth,

the callus was removed, and every two months thereafter it was divided and replanted.

Callus from Cotyledons was obtained similarly, but in this tissue the callus also developed on the surface of the tissue (Figure 2).

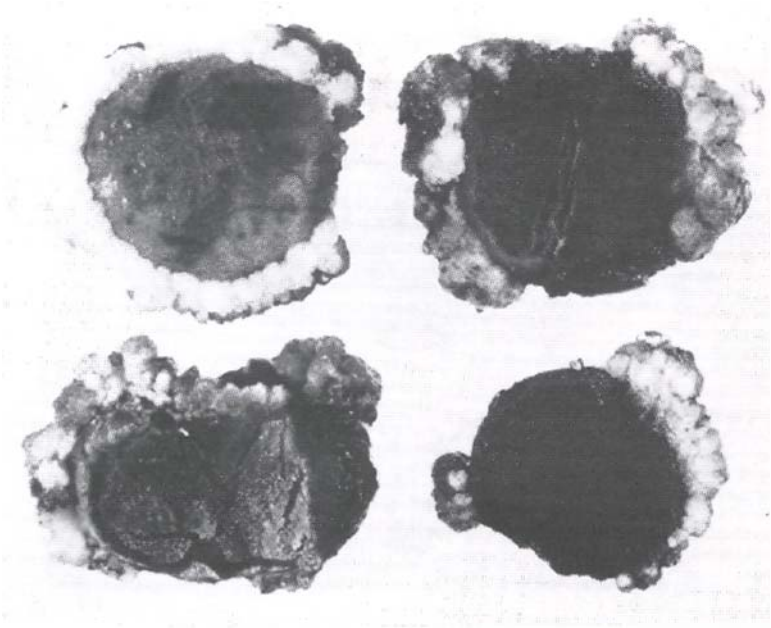

Figure 1. Growth of callus around mesocarp slices.

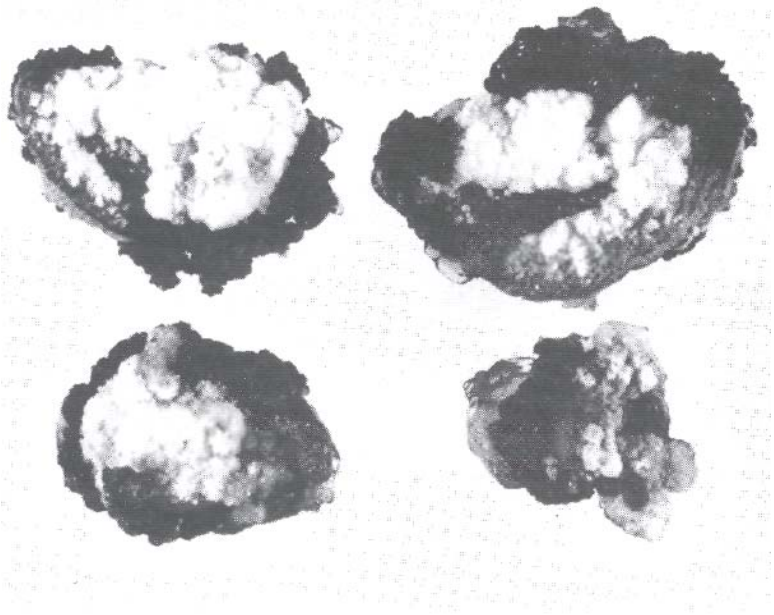

Figure 2. Growth of callus on cotyledon slices.

MAINTAINING THE CALLUS

In order to achieve good callus growth, we studied a number of relevant factors: composition of the nutrient medium, temperature, and light. We examined callus growth on a series of nutrient media (2, 3, 4, 5); it was found that the best growth was obtained on a medium prepared according to Miller (4). When temperatures of 20, 24, and 27 °C were compared, the best callus growth occurred at the highest temperature. Slightly better development was recorded in darkness than in light (40 f.c.). Consequently, on the basis of these results, we grew the callus under conditions of darkness, using a nutrient medium according to Miller, at 27°C and 80% R.H.

NATURE OF THE CALLUS

The nature of mesocarp callus growth is unique. The growth is not uniform throughout the entire tissue volume as in the case of soybean or tobacco callus growth, but occurs only in certain regions. As a result of this, there are protuberances on the callus, especially on that part facing the nutrient medium (Figure 3). In cross-section, the callus appears as if it were composed of many lobules with inlets between them (Figure 4). This callus is similar in its external shape to that obtained on mist-propagated avocado cuttings. The rate of callus growth was rather slow compared to that of soybean or tobacco callus; every two months the callus weight increases approximately ten-fold.

Cotyledon callus differs in its external appearance from that of mesocarp callus (Figure 5). It is softer, lighter in color, its growth rate is more rapid than that of the mesocarp-derived callus, and it is capable of increasing its weight approximately twenty-fold in two months.

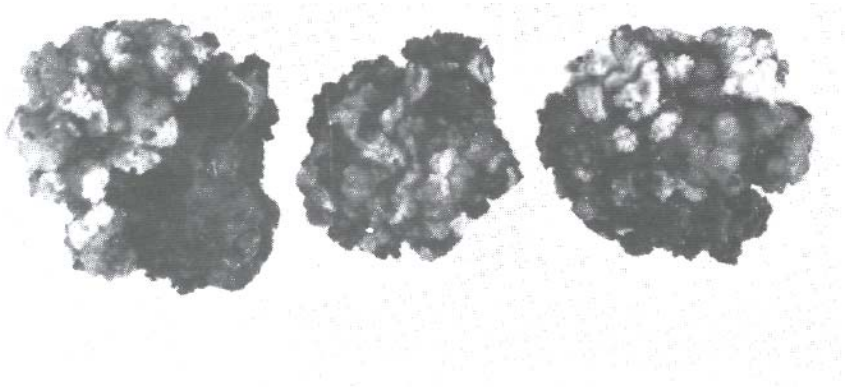

Figure 3. Callus derived from mesocarp.

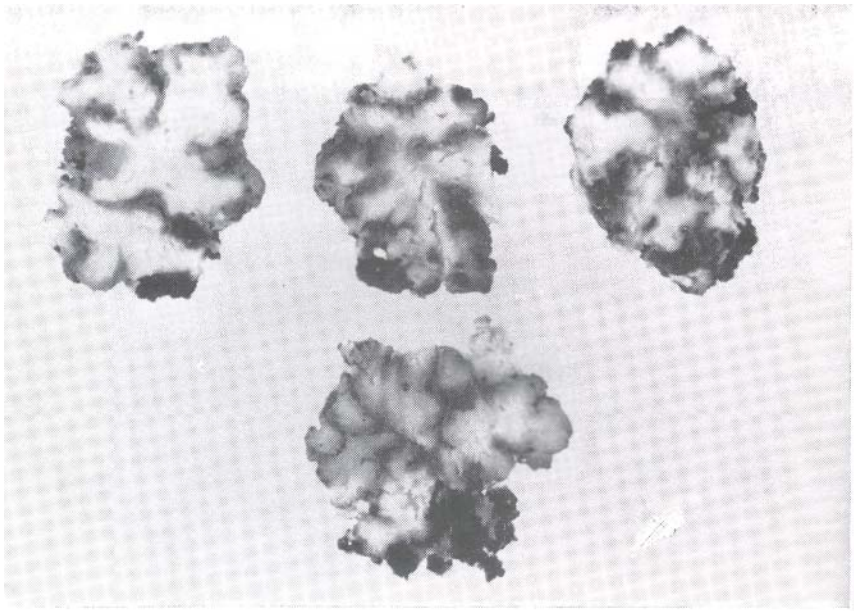

Figure 4. Cross-section through callus derived from mesocarp.

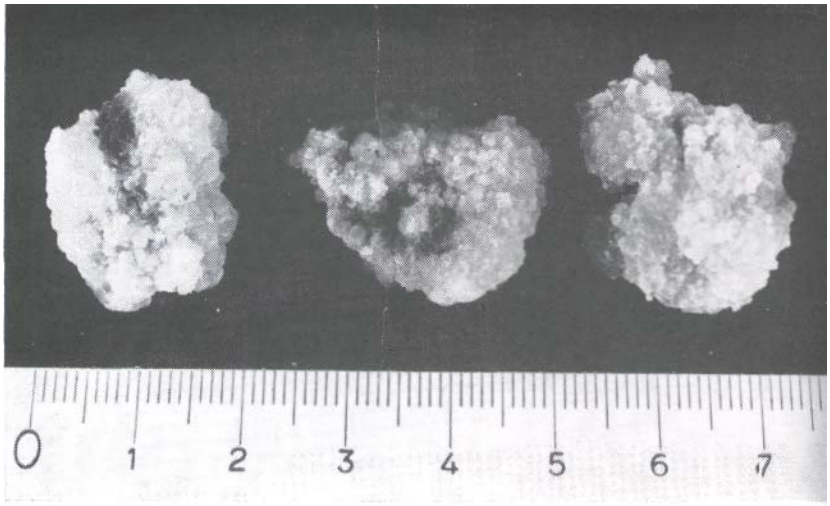

Figure 5. Cotyledonous callus.

When mesocarp and cotyledon callus were grown on a nutrient medium lacking cytokinins (a group of growth substances generally considered essential for tissue development), we found that the mesocarp callus desiccated and died, while the cotyledon callus was able to continue growing properly, even without the presence of these cytokinins in the medium. Endogenous cytokinins were found in cotyledon callus grown for a number of generations in cytokinin-free nutrient medium (unpublished data), and from these results we concluded that this callus is capable of producing its own cytokinins.

In the course of related research work we found that the cotyledons in avocado fruit contain high amounts of free cytokinins (1)

On the basis of these findings, it can be assumed that at least some of the cytokinins present in the avocado embryo are produced in the cotyledons themselves, and that the regulating effect of the embryo on fruit development may be due to this ability, whereas the mesocarp apparently depends on the supply of cytokinins from the external source—the roots or perhaps the seed.

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