# *IN VITRO* CULTURE OF AVOCADO: A MODEL SYSTEM FOR STUDYING THE BIOCHEMISTRY OF FRUIT GROWTH

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## ABSTRACT

The avocado (*Persea Americana* Mill.) is considered by many to be a horticultural problem, Cultivars in use today have several drawbacks associated with yield, disease susceptibility and fruit quality. Breeding programs to counter these disadvantages have been met with limited success due mainly to the crop's heterozygosity, outbreeding nature and long juvenile period. Furthermore, genetic information regarding current commercial scions and rootstocks is limited and crosses are made based on parental phenotypic characteristics which are not always additive. It is proposed that the development of a protoplast-to-plant system for avocado would not only provide a means for plant breeders to overcome these problems, but would also present researchers with a useful tool for studying biochemical and physiological mechanisms operating within the plant. An investigation into the development of an *in vitro* system for use in metabolic studies was carried out. This technology was then used as a model system for studies into the metabolic control of cell growth.

An attempt was made at developing a protoplast system from the mesocarp tissue of 'Hass' avocado. It was found that the purity and activity of the cellulase preparation in the protoplast isolation medium was critical. Failure to generate a protoplast system from mesocarp tissue prompted an investigation into the development of cell cultures. Mesocarp, seed and embryo tissue was subjected to various treatments in an attempt to induce callus for use as a source material for cell cultures. Callus derived from nucellar tissue of 'Hass' avocado seed at high concentrations of a-naphthalene acetic acid (NAA)(5 $mgL^{-1}$) and isopentenyladenine (iP)(5 $mgL^{-1}$) in Murashige and Skoog media (MS) proved to be the most amenable to subculture into liquid medium. Cell suspensions initiated from this callus grew fastest in MS media supplemented with NAA (5 $mgL^{-1}$) and iP (1 $mgL^{-1}$). These cell suspensions were maintained through subculture and were selected for use in metabolic studies.

Cytokinin-dependent cell cultures from avocado seed callus were used to study the involvement of isoprenoid products in cell division. Addition of mevastatin, a competitive inhibitor of the key enzyme in the isoprenoid pathway, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), caused a reduction in cell growth at low concentrations (0.01 µM, 0.1 µM and 1 µM) and cessation of growth at higher concentrations (10 µM

and 40 µM). Cotreatment with the isoprenoid compounds mevalonic acid lactone (MVL)(6 mM) and farnesyl diphosphate (FDP)(10 µM) completely reversed the effects of mevastatin at the 1 µM and 40 µM levels. The addition of stigmasterol (10 µM) to cell cultures treated with mevastatin (1 µM and 40 µM) resulted in a slight positive growth response indicating partial alleviation of inhibition. However, the response was not significantly different from the control suggesting that sterols played a minor role in cell division. It was concluded that isoprenoid-derived products played a critical role in the regulation of the cell cycle. Furthermore, it was suggested that mevastatin-induced HMGR inhibition gave rise to a response, most likely ABA-mediated, that acted antagonistically to regulatory mechanisms controlled, in part, by isoprenoid compounds.