

AVOCADO ROOTSTOCK DEVELOPMENT BY SOMATIC HYBRIDIZATION AND GENETIC ENGINEERING

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This research has been undertaken in order to develop a new generation of avocado rootstocks with a high level of resistance to *Phytophthora* root rot (PRR). The research has 3 major objectives: 1) somatic hybridization of avocado with PRR-resistant *Persea borbonia* and other wild *Persea* species by means of protoplast fusion; 2) genetic transformation of existing avocado rootstocks with antifungal genes; 3) developing an efficient *in vitro* protocol for propagating existing and newly developed rootstocks. All of these objectives are dependent upon the availability of an efficient system for regenerating avocado from cell and tissue cultures, i.e., somatic embryogenesis. Preliminary studies were begun one year before support was obtained from the California Avocado Society. The current status of this research is as follows:

1). Somatic hybridization. High levels of resistance to PRR have been identified in some of the wild relatives of avocado, including *P. borbonia*. Unfortunately, *P. borbonia* is sexually and graft-incompatible with avocado. This research was undertaken to force the hybridization (by protoplast fusion) of the two species in order to produce a potentially useful rootstock for avocado. This approach is dependent upon the ability to isolate viable protoplasts enzymatically from embryogenic avocado suspension cultures, to re-establish embryogenic cultures from the protoplasts and to regenerate somatic embryos and plantlets. We have successfully carried out this procedure with embryogenic cultures of genotypes 'T362', 'Esther', 'Thomas' and 'M25864', and have achieved an average plating efficiency of ca. 24%. Seedlings of *P. borbonia* have been germinated under sterile conditions and then leaves have been used as a source of mesophyll protoplasts. Protoplasts from *P. borbonia* leaves and from embryogenic cultures of either 'T362' or 'Thomas' have been co cultured in a plant growth medium containing polyethylene glycol in order to stimulate their fusion. Putative somatic hybrid cells have been identified and cloned. Embryogenic cultures have been recovered from the putative hybrid cells and somatic embryos are currently maturing; germination of somatic embryos in the 'T362' + *P. borbonia* cultures has been initiated. This study is being repeated with *P. cinerascens*.

During the next year we will recover a sufficient number of regenerated plants in order to evaluate their response to controlled infection with *Phytophthora cinnamomi*.

Thereafter, we will determine their graft-compatibility with avocado.

2. Genetic transformation of existing avocado selections. We would like to be able to modify existing avocado selections for a single horticultural trait, specifically resistance to PRR, by transferring a foreign resistance gene into an existing avocado rootstock or scion selection. Embryogenic 'T362' avocado cultures have been infected with a disarmed strain of genetically engineered *Agrobacterium tumefaciens*. The plasmid vector contained two bacterial genes: a selectable marker gene for kanamycin resistance (NPT II) and a scorable marker gene for P-glucuronidase (GUS). The genes were transferred to and were expressed in avocado. Putatively transformed cultures were identified that could grow on medium containing high levels of kanamycin, and somatic embryos were later regenerated from these cultures that tested positive for the presence of P-glucuronidase. The integration of NPT II and GUS into avocado somatic embryos was confirmed by Southern hybridization. We have therefore documented that avocado has been genetically transformed for these two bacterial genes.

During the next year, we are planning to transform 'Duke 7' and 'Thomas' with 2 antifungal genes, i.e., glucanase and defensin, that would confer a high level of resistance to PRR.

3. Micropropagating avocado rootstocks. Many tropical and subtropical fruits, e.g., *Citrus* and mango, reproduce vegetatively by the formation of polyembryonic seeds. The clonal embryos and seedlings develop from the nucellus, a tissue located within the integumental layers of the seed. We have demonstrated that it is possible to stimulate the formation of somatic embryos from the extracted nucellus of the following avocado selections: 'T362', 'Lamb', 'Hass' and 'Thomas'. During the spring of 1996, we will be attempting to induce embryogenic cultures from the nucellus of 'Duke 7'. Conditions for the efficient growth of embryogenic cultures and for the recovery of large numbers of somatic embryos have been determined.

We are currently developing protocols for the production of large numbers of high quality plants from these somatic embryos, and will be transferring the first regenerants into potting mixture during the coming year. This procedure should result in a low cost supply of clonal avocado rootstocks.

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