

Generation and Selection of *Phytophthora cinnamomi* Resistant Avocado Rootstocks through Somaclonal Variation

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Introduction

Root rot, caused by the soil fungus *Phytophthora cinnamomi*, is the most serious disease affecting commercial California avocado production (Coffey, 1987). Avocado root rot has affected an estimated 9,000 acres of trees in California (Zentmyer and Ohr, 1981). Control measures in commercial avocado production fields include: 1) careful isolation of infested plots and monitoring of irrigation and runoff; 2) proper site selection; i.e., soils with increased drainage or the incorporation of surface and subsurface drains; 3) application of soil fungicides, which would be costly; 4) crop rotation with plants not at risk, such as citrus, vegetables, or flower crops; 5) use of tolerant rootstocks (Zentmyer and Ohr, 1981). All of these described methods of control are still at best only methods to confine the spread of root rot or limit its severity once it is present in an avocado orchard. However, the ultimate method of choice for control of avocado root rot is the development of resistant rootstocks. Although rootstocks with increased tolerance to root rot have been selected, such as 'Duke 7', no commercially acceptable, true resistant rootstocks currently exist.

Although resistance has been found in related *Persea* species, these species can contribute commercially undesirable traits upon sexual hybridization, leading to a heterozygous population from which selections must then be made. In the case of a tree species, this evaluation process requires many years of growth to be able to truly evaluate a particular selection's field performance and commercial potential. For example, the production of the rootstock 'Martin Grande' (G755) by crossing *Persea americana* with *Persea schiedeana* has resulted in increased root rot tolerance (Coffey, 1987), but more recently, associated problems with chlorosis and winter root dieback have been observed.

Project Objective

As will be described in greater detail later, the objective of the proposed study is to generate and select for resistance to *Phytophthora cinnamomi* by utilization of the plant tissue culture technique of somaclonal variation. Somaclonal variation is defined as the phenotypic and genotypic variation observed in plants which have been regenerated from an undifferentiated mass of cells or callus (Larkin and Scowcroft, 1981). For this technique, alternate plant regeneration pathways are preferred, including adventitious shoot regeneration; i.e., the regeneration of shoots from a callus or even directly from explant sources, such as leaf or stem segments. In addition, adventives plants can be

obtained by the process of somatic embryogenesis, whereby asexual, nonzygotic embryos are obtained from callus or directly from the explants. It has been documented in the published literature that in addition to explant source and regeneration routes, other factors of importance in obtaining genetically variable plants include: the types and concentrations of growth regulators in the culture medium; the length of culture period. It is important to note that if genetically identical or clonal copies of a plant are desired, as is the case in the commercial micropropagation industry, then the explants of choice would be preformed shoot meristems, either apical shoot meristems or axillary shoot buds.

There have been numerous reports in the published literature of obtaining useful variation such as disease resistance, flower or fruit color and size, as well as plant habit for a wide range of plant species (Reisch, 1983; Evans and Sharp, 1986). In particular, potato germ plasm resistant to *Phytophthora infestans* has been obtained by application of the technique of somaclonal variation (Secor and Shepard, 1981). Plants were regenerated from a callus initiated from leaf cells and were screened and selected for their *Phytophthora* resistance. This resistance has remained stable after sexual hybridization. More recently, there have been documented cases describing the usefulness of somaclonal variation in the improvement of woody plants, specifically in regard to trees. This technique is particularly useful in tree improvement programs due to the following: 1) recovery of new genotypes more quickly compared to conventional breeding methods; 2) increased rate of mutation; 3) year-round selection is possible (Ahuja, 1987; Diner and Karnosky, 1987). Traits such as salt tolerance, oil composition, vegetative characteristics, herbicide resistance, and disease reaction have been selected for with tree species by application of the technique of somaclonal variation.

In the past year, important progress has been made regarding the responsiveness of avocado to tissue culture. Somatic embryos can be successfully regenerated from callus initiated from zygotic embryos (Mooney and Van Staden, 1987; Pliego Alfaro and Murashige, 1988). In addition, there has been a recent report concerning the successful regeneration of multiple shoots from cultured vegetative buds (Schall, 1987). This work, in combination with the regeneration of multiple shoots from zygotic embryos (Skene and Barlass, 1983), indicates that both shoot organogenic and embryogenic regeneration pathways are now possible for avocado tissue cultures.

Project Plan and Procedures:

The ultimate goal of this project is to develop root rot resistant avocado rootstocks. The most efficient approach to applying the technique of somaclonal variation is the utilization of an existing genotype exhibiting as many commercially desirable characteristics as possible to introduce into culture as a callus and to then regenerate plants from this callus. By this approach, there is a good probability that some of the plants regenerated from the callus will exhibit disease resistance and retain many or all of the desirable characteristics of the original genotype.

The entire 2½ year project can be broken down into three stages as outlined below. The first twenty-two months of the project will concentrate on the development of the required adventitious plant regeneration systems, whether via organogenesis

(adventitious shoot regeneration) or somatic embryogenesis.

1. Initiation of Callus Cultures:

Germ plasm from up to three sources, such as 'Duke 7', 'Toro Canyon' or 'Thomas', which demonstrate some degree of root rot tolerance, will be evaluated for callus induction from various explants. Since zygotic embryos represent a heterozygous genetic constitution, and further introduce possible undesirable traits into the gene pool, explants will be obtained from somatic tissues, such as leaves, petioles, vegetative buds, flower receptacles, and immature, diploid fruit tissues which represent the true genetic constitution of the commercially acceptable genotype. Thus, these explant sources will maintain the desirable traits of a variety, as well as allow for the selection of additional desirable traits. (Estimated time required: four months.)

2. Plant Regeneration from Callus:

Once procedures to induce callus growth have been defined, methods to induce plant regeneration from the callus must be developed. Depending on the response obtained, plant regeneration could occur by two different routes: 1) organogenesis; i.e., the production of adventitious, vegetative shoots which can be excised and rooted to produce complete plants; or 2) somatic embryogenesis; i.e., the production of somatic, asexual embryos, similar to zygotic embryos found in seeds.

The established callus cultures will be exposed to a range of cytokinin and auxin concentrations and combinations to determine culture conditions conducive to organogenesis or somatic embryogenesis. Procedures to acclimate and establish the resulting plants will also be defined. (Estimated time required: eighteen months.)

3. Production of Somaclones and Screening for Resistance to *Phytophthora cinnamomi*:

Up to one hundred somaclones (plants) will be produced for each genotype which successfully regenerates plants *in vitro*. Each plant or somaclone will be increased by cuttings to yield up to five replicates for root rot resistance evaluation. Concurrently, a whole plant resistance screening procedure will be designed to accurately assess the disease reaction of the somaclones. Known susceptible and tolerant genotypes will be included as controls. Test plants, which are rooted in soil, will be inoculated with a *P. cinnamomi* zoospore suspension. Zoospores will be induced from cultures of the avocado isolate of *P. cinnamomi* by the method of Dolan and Coffey (1986). Resistance to *P. cinnamomi* will be evaluated by the number of surviving plants, number of root lesions, and lesion size. Plants displaying a disease resistant reaction can then be propagated by cuttings for further study and evaluation. (Estimated time required: eight months.)

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