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

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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 run-off; 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.

Objective

The objective of our study is to generate and select for resistance to *Phytophthora cinnamomi* by utilization of the plant tissue culture technique of somaclonal variation. 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, and to then introduce it into *in vitro* culture as a callus, and subsequently regenerate plants from the callus. In this way, 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 2.5-years project was planned in three stages:

1. Initiation of Callus Cultures:

Germplasm from up to three sources, such as 'Duke 7', 'Toro Canyon', or 'Thomas', which demonstrate some degree of root rot tolerance, would be evaluated for callus induction from various explants, such as young leaves, petioles, and vegetative buds.

2 . Plant Regeneration from callus:

Once procedures to induce callus growth were defined, methods to induce plant regeneration from the callus would be defined. Depending on the response obtained, plant regeneration could occur by two different routes: 1) organogenesis, or the production of vegetative buds which can be excised and rooted to produce complete plants; or 2) somatic embryogenesis, or the production of somatic asexual embryos, similar to zygotic embryos from the callus mass.

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

Up to 100 somaclones (plants) would be produced for each genotype which successfully regenerates plants *in vitro.* Each plant or somaclone would be increased by cuttings to yield up to five replicates for root rot resistance evaluation. Concurrently, a resistance screening procedure would be designed to accurately assess the disease reaction of the somaclones. Known susceptible and tolerant genotypes would be included as controls. Test plants, which are rooted in soil, would be inoculated with a *P. cinnamomi* zoospore suspension. Zoospores would be induced from cultures of the avocado isolate of *P. cinnamomi* by the method of Dolan and Coffey (1986). Resistance to *P. cinnamomi* would be evaluated by the number of surviving plants, number of root lesions, and lesion size. Plants displaying a disease-resistant reaction could then be propagated by cuttings for further study and evaluation.

Progress and Accomplishments Made During 1988-89

The first stage of the 2.5-years project was to initiate explants from up to three genotypes, such as 'Thomas', Toro Canyon', or 'Duke 7', and produce callus cultures *in vitro.* Initially, Thomas' and Toro Canyon' were used for sterilization studies. Explants such as stem sections, petiole sections, leaf disks, immature flowers, shoot tips, and immature fruit were taken from actively growing trees and treated with or without 90% ethanol and varying concentrations of sodium hypochlorite for various treatment times in the laboratory. After rinsing with sterile water, the explants were further dissected and placed on sterile nutrient media containing a range of auxins at various concentrations. These auxins were used to promote callus growth. In addition, shoot tips were placed on media containing low concentrations of cytokinins for shoot growth.

Results of this first experiment were somewhat disappointing. The ethanol quick-dip caused death of all explants regardless of sodium hypochlorite or time treatment. Non-ethanol treated cultures died from either over-sterilization or fungal contamination. Immature flowers were very sensitive to the sterilization process.

Upon evaluation of the initial sterilization results, another set of experiments was performed using 'Thomas' and 'Toro Canyon'. The sterilization procedure used during this set of experiments produced much better results. The omission of ethanol reduced losses (Figure 1). After three weeks in culture, stem and petiole explants of both

genotypes began developing a white, compact callus. Callus development failed to occur on explants other than stem and petiole sections. Subsequently, cultures of 'Duke 7' were initiated using the protocols developed earlier with similar results being observed. Cultures on media containing the auxin 2,4-D and charcoal produced a more compact callus than did media containing the auxin IBA. In further callus development work, the auxin NAA proved to be as good, if not better for callus production, than did 2,4D when using stem and petiole sections. At this time, it appears that the auxins 2,4D and NAA produce good white, compact callus which is of a type most desirable for somatic embryogenesis (Table 1). Shoot tips of 'Thomas', 'Toro Canyon', and 'Duke 7' grew out on a low-cytokinin medium, but died upon transfer to fresh media.

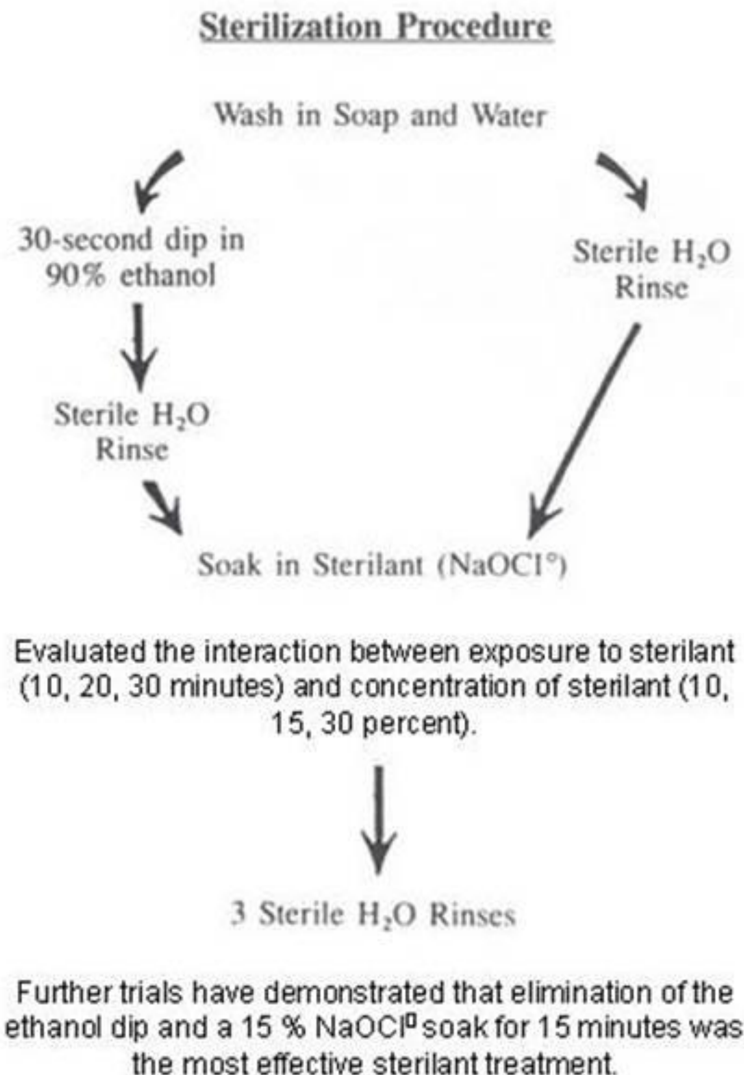

Figure 1. *Sterilization procedure for determining optimum concentration and duration for sterilizing explants.*

Stage 2 of this project addresses the regeneration of plantlets from callus cultures. We are currently in this stage of the project. The established callus cultures are being exposed to media containing reduced auxin (IBA, NAA, or 2,4-D) concentrations and/or organic compounds such as glutamine, casein hydrolysate, and activated charcoal. Cultures, maintained in petri dishes, were either wrapped in aluminum foil (to provide darkness) or were left unwrapped and exposed to fluorescent light (approximately 300 footcandles). Cultures which were placed on a medium containing a low concentration (5.0 μM) of 2,4-D with no charcoal, glutamine, or casein hydrolysate turned brown within two weeks, whether in the light or darkness. Cultures exposed to the same concentration of 2,4-D with charcoal added to the medium have remained viable. The charcoal is apparently reducing the toxic effects of the 2,4-D. Cultures on media containing either glutamine or casein hydrolysate have also remained viable, but at this time no organogenesis or somatic embryogenesis has occurred. Cultures in which callus induction occurred on a medium containing NAA appear to be the most viable over time, remaining white or green and compact on regeneration media longer than did cultures transferred to regeneration media from callus induction media containing IBA or 2,4-D. The presence of light did promote the greening of vitrified callus. The callus also appears sensitive to high temperatures, as there was some loss of callus at a temperature of 30°C, compared to our normal operating temperature of 24°C.

The initiation of further plant material will be continued for callus induction to be used in future regeneration experiments. A large pool of freshly initiated callus cultures is needed, since there is usually a loss of regeneration potential with older callus cultures. Further examination of explant type and growth regulators will continue regarding callus induction. Over the next year, the major research effort will be directed toward the second stage (regeneration) of the project, with a greater range of auxin, cytokinin, and organic compounds being examined for the production of somatic embryos or shoot development via organogenesis. The genotypes of 'Thomas', 'Duke 7', 'Toro Canyon', and 'Barr Duke' will be emphasized.

Table 1. Morphogenic Response of 'Thomas' and 'Toro Canyon' to the Growth Regulators 2,4-D, NAA, and Picloram.

	Conc. 2,4-D (μm)		
Explant	25.0	50.0	100.0
Stem	+	+ +	+ + +
Leaf	o	+	o
Petiole	+ +	+ +	+ + +
Recept.	o	o	o

o No callus growth
\+ Slight callus growth
\+ + Moderate callus growth
\+ + + Heavy callus growth

	Conc. Picloram (μm)		
Explant	0.1	0.5	1.0
Stem	o	o	+
Leaf	o	o	o
Petiole	o	+	o
Recept.	o	o	o

o No callus growth
\+ Slight callus growth

	Conc. NAA (μm)	
Explant	25.0	50.0
Stem	+ +	+ + +
Leaf	o	+
Petiole	+ +	+ + +

o No callus growth
\+ Slight callus growth
\+ + Moderate callus growth
\+ + + Heavy callus growth

With the possible availability of a selfed 'Thomas' and/or a cross between 'Thomas' and Toro Canyon' produced by University of California/Riverside researchers, there is a

good potential that immature zygotic embryos could be cultured to produce callus from which somatic embryos could be produced. Mooney and Van Staden (1987) and Pliego-Alfaro and Murashige (1988) have had success in producing somatic embryos from callus derived from cultured immature zygotic embryos. However, their work did not include these particular varieties. We feel that the potential of producing somatic embryos for resistance screening by using immature zygotic embryos could be very important to the avocado industry. Thus, we will begin research in this additional area as valuable materials become available from breeders.

Practical Applications and Summary

The development of rootstocks with increased tolerance or complete resistance to *Phytophthora cinnamomi* would be of long-term benefit to the avocado industry. The increasing cost of chemical control could be reduced by production of more tolerant or resistant rootstocks. Acreage which in the past may have been deemed unsuitable for avocado culture due to poor draining soils or existing *Phytophthora* infestations could potentially be brought into production. Site selection and preparation costs could also be potentially reduced in that the incorporation of surface and subsurface drains could be scaled down in quantity and design. Increased tolerance or resistance would increase yield in *Phytophthora-infested* orchards, in that trees could be productive over a longer period of time. Reduced chemical controls and replanting costs would also be important associated benefits.

Valuable germplasms of rootstocks with some degree of root rot tolerance have been identified to date. Various explant types, tissue sterilization procedures, and callus induction media have been, and are still being, evaluated as research continues. At this time, regenerated shoots and/or somatic embryos have not been produced *in vitro.* Continued explant initiation for callus induction is ongoing, while a greater range of shoot regeneration and/or somatic embryogenesis media is planned. Recently, a system for somatic embryogenesis in walnut has been developed (McGranahan *et al.,* 1988). We will examine this system as it applies to avocado. We feel that losses in the avocado industry due to root rot warrant the additional research concerning the culture of immature zygotic embryos of selfed or crossed genotypes with some degree of root rot tolerance. We will attempt to produce callus and then somatic embryos for the screening of potential somaclonal variants for their root rot resistance.

Literature Cited

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