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University of California, Riverside

GENERATION AND SELECTION OF PHYTOPHTHORA CINNAMOMI RESISTANT AVOCADO ROOTSTOCKS THROUGH SOMACLONAL VARIATION

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I. INTRODUCTION

A. Project Objectives

The objective of this research is to generate and select avocado plantlets resistant to Phytophthora cinnamomi through the plant tissue culture technique of somaclonal variation. The most efficient approach to applying the technique of somaclonal variation is by utilizing existing genotypes exhibiting as many commercially desirable characteristics as possible.

B. Description of Project - Plan and Procedures

This technique will involve three stages. The first stage requires the production of callus tissue from vegetative explants such as stems, petioles, and leaves. It has been found with many species of plants that during this phase, variation among individual cells or groups of cells can occur. In the second stage, the callus produced from the above explants is placed on a culture medium to stimulate the production of adventitious shoots and/or somatic embryos. These are allowed to grow out *in vitro* and are then acclimated in the greenhouse. In the third stage, the acclimated plantlets are screened for resistance to Phytophthora cinnamomi.

II. PROGRESS AND ACCOMPLISHMENTS MADE NOVEMBER 1, 1989-FEBRUARY 23, 1990

During this project period, further initiation was carried out using the genotype 'Thomas'. As with previous work, all material was collected from the Brokaw Sexton Canyon nursery. In one initiation experiment, two surface sterilization techniques were compared for their effects on controlling contamination. The first technique involved surface sterilization of explants for 15 minutes in a 15% bleach solution. The contamination rate of explants treated in this fashion was 33.6%. The second technique involved surface sterilization of explants for 15 minutes in a 15% bleach solution which had been pH adjusted to 6.0. The contamination rate of explants handled this way was 10.0%. Clearly, pH-adjusting the surface sterilization solution had a profound effect on reducing contamination rates.

In another initiation experiment, a different stem explant cutting procedure was examined in an attempt to expose more of the cut explant surface to the callus induction medium, thereby increasing callus yields. It was observed that splitting stem sections in

half resulted in callus production comparable to that observed from stem discs; however, callus was more easily removed from split stem sections. This should facilitate a step subsequent to callus initiation in which callus that has been removed from explants is grown and multiplied.

A range of growth regulators, wider than what has been examined previously, are currently being evaluated for their effects on improving callus yield and quality. The results of an initiation experiment examining the effects of 2,4-D and kinetin concentrations on initiation of callus, both from split stem sections and petiole sections, are presented in Tables 1 and 2. Overall, callus yield was higher from split stem sections than from petioles. Essentially no callus was produced from either tissue type on media containing 10 or 30 µM 2,4-D, or 100 µM kinetin. On other media, three different types of callus were observed. One type of callus was brown, tough, and nodular. It is possible that this was not callus at all, but rather expanded vascular tissue. This was most prevalent on media containing low concentrations of growth regulators. A second type of callus that was observed was loose and white and often grew along lenticels. This callus type was observed in most treatments tested. The third type of callus observed was off-white in color and compact. No relationship was observed between its presence and medium composition. Callus from each treatment of this experiment has recently been removed from explants, smashed, and placed back onto fresh, medium identical to that from which it came. This was done in order to multiply the callus. However, to date, most of that callus has turned brown and does not appear to be growing.

A more uniform callus has been developing from many of the treatments being tested in a separate growth regulator experiment investigating the effects of NAA and kinetin concentrations on initiation of callus from split stem sections. The type of callus most frequently observed is off-white in color and compact. Soon this * callus will also be removed from explants and multiplied on fresh media.

III. FUTURE PROJECT OBJECTIVES

The initiation experiments currently in progress will be continued. Callus will be multiplied and then placed on a separate culture medium to stimulate the production of adventitious shoots and/or somatic embryos. New initiation experiments will also be started in order to continue comparisons between different growth regulators as well as different avocado genotypes. Callus will then be screened for somatic embryo and/or adventitious shoot formation (regeneration) on a separate medium. The components of this medium will be manipulated as necessary to maximize shoot/embryo yields.

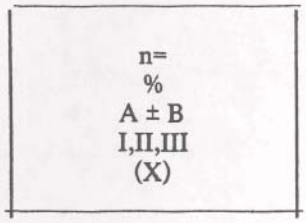
It may prove necessary to add an extra step to the tissue culture protocol in order to induce adventitious shoots and/or somatic embryos from callus. This could involve a growth regulator pulse or a growth regulator wash out just prior to regeneration.

Finally, arrangements are currently being made with University of California Riverside researchers for the acquisition of immature fruit of selfed 'Thomas', 'Barr Duke', and/or crosses between 'Thomas' and 'Toro Canyon'. There is strong potential that immature zygotic embryos could be cultured to produce callus from which somatic embryos could be produced. Research into this method of immature zygotic embryo culture may be very important towards the goal of producing plantlets for resistance screening. As long

as mild weather prevails, immature fruit should be available in late March and early April of 1990.

KEY FOR TABLES 1 AND 2

NOTE: Each treatment started out with 5 replicates of 5 stem sections each (for a total of 25 stem explants per treatment) and 5 replicates of 7-10 petiole sections each (for a total of 35-43 petiole explants per treatment). However, due to contamination, some of the starting material had to be discarded.



n=: Total number of explants evaluated
%: Percentage of explants which formed callus
A ± B: Callus yield (in grams) per explant (Mean ± Standard Deviation)
I,II,III: Callus Type
- I: This type of callus was brown, tough, and nodular.
- II: This type of callus was white and loose and often formed on lenticels.
- III: This type of callus was off-white in color and compact.

(X): Comments
- (1): All explants died and/or were inactive.
- (2): Most explants died and/or were inactive. Only trace amounts of dead callus were observed..
- (3): Most explants died and/or were inactive. Only trace amounts of active callus were observed.

Table 1. The Effects of Varying 2,4-D and Kinetin Concentrations on Initiation of 'Thomas' Avocado Callus from Split Stem Sections

KINETIN in µM	2,4-D in µM: 0.3	1.0	3.0	10	30
0	n=20 100% 0.355 ± 0.074 I,II,III; mostly I	n=25 100% 0.277 ± 0.089 I,II; mostly II	n=15 53.3% 0.030 ± 0.007 I,III; mostly I	n=15 0% 0.0 ± 0.0 None (1)	n=15 0% 0.0 ± 0.0 None (1)
0.1	n=20 100% 0.246 ± 0.068 I,II,III; mostly I	n=10 100% 0.227 ± 0.161 I,II,III; mostly I	n=20 35% NT II (2)	n=20 5.0% NT III (3)	n=18 5.6% NT III (2)
1.0	n=20 100% 0.264 ± 0.134 I,II,III	n=10 100% 0.257 ± 0.074 I,II; mostly II	n=20 45.0% 0.030 ± 0.026 I,II,III (3)	n=10 0% 0.0 ± 0.0 None (1)	n=15 0% 0.0 ± 0.0 None (1)
10	n=15 93.3% 0.188 ± 0.051 I,II,III	n=20 100% 0.236 ± 0.112 I,II,III; mostly II and III	n=10 90.0% 0.128 ± 0.163 II,III; mostly II	n=19 0% 0.0 ± 0.0 None (1)	n=10 0% 0.0 ± 0.0 None (1)
100	n=20 0% 0.0 ± 0.0 None (1)	n=25 5.0% NT II (3)	n=15 0% 0.0 ± 0.0 None (1)	n=20 0% 0.0 ± 0.0 None (1)	n=15 0% 0.0 ± 0.0 None (1)

Table 2. The Effects of Varying 2,4-D and Kinetin Concentrations on Initiation of 'Thomas' Avocado Callus from Petiole Sections

KINETIN in µM	2,4-D in µM: 0.3	1.0	3.0	10	30
0	n=31 96.8% 0.053 ± 0.013 I,II; mostly I	n=32 93.8% 0.084 ± 0.009 I,II	n=41 9.8% 0.010 ± 0.006 II (3)	n=18 0% 0.0 ± 0.0 None (1)	n=42 0% 0.0 ± 0.0 None (1)
0.1	n=34 94.1% 0.060 ± 0.009 I,II	n=30 96.7% 0.106 ± 0.059 I,II; mostly II	n=34 5.9% NT I,II (3)	n=32 0% 0.0 ± 0.0 None (1)	n=42 0% 0.0 ± 0.0 None (1)
1.0	n=42 88.1% 0.071 ± 0.043 I,II; mostly I	n=41 92.7% 0.151 ± 0.139 I,II,III; mostly III	n=43 46.5% 0.030 ± 0.012 I,II; mostly I (3)	n=40 0% 0.0 ± 0.0 None (1)	n=39 0% 0.0 ± 0.0 None (1)
10	n=25 36% 0.017 ± 0.009 I (3)	n=40 22.5% 0.017 ± 0.013 I (3)	n=40 77.5% 0.089 ± 0.039 I,II,III; mostly III	n=40 5.0% NT II (3)	n=30 0% 0.0 ± 0.0 None (1)
100	n=24 0% 0.0 ± 0.0 None (1)	n=33 0% 0.0 ± 0.0 None (1)	n=26 15.4% 0.006 ± 0.010 I (3)	n=42 0% 0.0 ± 0.0 None (1)	n=40 0% 0.0 ± 0.0 None (1)