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Propagation In Vitro of Rootstocks of Avocado

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Introduction

Propagation in vitro has opened up new possibilities for the rapid cultivation of clonal tissue cultures, selected for their resistance under limited conditions. The qualities which make them suitable for fruit growing are maintained. The purpose of this work is to develop a method for the micropropagation of two types of avocado: the 'Colin V-33', which is characterized by its dwarf size, and a selection from the West Indian strain of avocado, which is adapted to alkaline soils.

Materials and Methods

Before placing the two strains of avocado in vitro, they were first set in an etiolation chamber for a period of 30 days. Once etiolated, they were then established in vitro.

The etiolated buds were cut and taken to the laboratory where they were disinfected, rinsed, and soaked for 10 minutes in cloralex with 10% of active ingredient. The buds were placed in a variegated flow chamber where they were rinsed again in water which had been both distilled and sterilized so that none of the disinfectant residues remained.

The axilar explants were placed in separate test tubes under the basic conditions required for propagation as suggested by Murashige (1962): 100 mg/1 of myoinositol are added to half of the substratum and the remainder is made up of 3 % sucrose and 4% thiamine. A reducing agent, potassium methasulfate, was added as a form of pretreatment which also enhances the nutritive value of the substratum. This pretreatment involved a concentration of 5 % potassium methasulfate in a solution of distilled water. This solution was sterilized in an autoclave. The explants remained in the sterilized solution until they were transferred to the correct environment for propagation.

The tested nutrients benciladenine (BA) and gibberellic acid (GA_3) were added to the previously sterilized substratum by means of a millipore filter, with a membrane diameter of 0.22 mm. The substratum had now the form of solid gel. To obtain this consistency, 2 gr/1 of gelrite had been added and the pH balance had been increased to 5.5. Once the shoots were planted, these conditions were then preserved in an incubator under a controlled environment for a photoperiod of sixteen hours of light and eight hours of darkness. Light intensity was at 3000 lux and temperature at 25 °C.

Results and Discussion

A total control over oxidation was gained through the application of potassium methasulfate which was used as a pretreatment.

In regard to budding, there was a visible growth among the explants throughout the experiment. However, when the nutrients benciladenine (BA) and gibberellic acid (GA_3) were added, by means of a millipore filter, a substantial increase in the growth rate of the explants was noticed. The optimum ratio was 2 mg/l with equal amounts of benciladenine and gibberellic acid (Figure 1).

Figure 1. *Effect of autoclave and millipore filter sterilization.*

	Colin V-33		Selection	
	Autoclave	Millipore	Autoclave	Millipore
Oxidation percentage	0	0	0	0
Budding percentage	75	75	100	100
Average of shoots by explant	1	3	1	4
Average LOF longitude of shoot (cm)	4	4	2	2

These results agree with those recorded by Wang and Hu (1983) who favored kinetine 10 mg/l as a more effective substance than BA and the 2ip. However, Lundergan and Janick (1980) together with Hutchinson (1984) concluded that better results were obtained with the nutrient BA than with 2ip and kinetine.

It has been observed that the nature of the conditions in which the mother plant has been cultivated affect the propagation period of the new bud. Best results were obtained among buds taken from plants, kept under greenhouse conditions (Harty, 1985), and also among those kept under total darkness. This is in agreement with the observations made by Schroeder (1979). They show that plant tissue under complete darkness has a capacity for improving this type of clonal propagation when grown "in vitro".

By adding periodic sprinkling of agrimicin at a ratio of 2 g/1 to explants taken from greenhouse conditions, the control over bacterial diseases was improved.

The darkening of the tissue culture, especially among those most recently isolated, occurs most frequently in tropical strains. Harty (1985) recorded the presence of oxidation among avocado explants when placed in an M. S. environment. This caused break-down of the plant tissue. In this investigation of two types of avocado, the 'Colin V-33' and the "West Indian" strain, oxidation control was found to be of notable importance. It is considered a decisive factor in regard to successful propagation among explants from the moment they are cut to the moment they are placed in the correct growing environment. By using a reducing agent as a form of pretreatment, the rate of oxidation in the explants was reduced to zero. In regard to budding, there was a higher growth rate in the West Indian strain of avocado. 25% of the Colin V-33 explants died due to the presence of a bacterium; this is still being investigated.

Summary

After a month, the multiplication coefficient had been established based on the number of shoots in each of the tests.

A higher multiplication coefficient occurred in those media which had been previously sterilized in an autoclave and later treated with nutrients by means of a millipore filter.

The multiplication coefficient was higher in the West Indian strain of avocado.

These results indicate a break-down in nutrients when the substratum is subject to high temperatures of sterilization.

For this reason, the filtering of various substances, in this case GA_3 and BA, by means of a millipore filter, is strongly advisable.

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