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In Vitro Clonal Propagation of Avocado Rootstocks

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INTRODUCTION

Current commercial propagation methods of avocado rootstocks are slow and laborious, and improved methods need to be developed. Rapid *in vitro* clonal propagation as an alternative method could benefit the nursery industry considerably. Benefits would include clonal propagation of commercial rootstocks at low cost; rapid introduction of new selected or imported rootstocks; and, above all, the production of pathogen-free rootstocks.

MATERIALS & METHODS

General

Conventional tissue culture procedures were followed. Tissues undergoing growth and differentiation were kept in a growth room with a 16-hour photoperiod provided by cool white fluorescent tubes, with a light intensity of approximately 36 µmol/m^2/s at 28 °C. Sucrose was incorporated in the media at 30 g/ℓ as a carbon source. The medium was solidified with 2 g/ℓ Gellan gum (Gelrite™) after adjustment of the pH to 5,8, and autoclaved at 121 °C for 20 min.

Source of plant material

Shoot tips and lateral buds were obtained from virus-free Duke 7 mother plants. Established cultures were maintained by transferring shoot tips and lateral shoots to fresh medium every four to six weeks. Nodal cultures used in the shoot growth and differentiation reported here were cut from these mother cultures. Shoots 10-30 mm long grown *in vitro* were cut from mother cultures for rooting experiments.

Phase one: Development of techniques for the successful aseptic initiation of Duke 7 explants

Nodal explants were cut in 20 mm pieces and pre-treated with 5,0 g/ℓ Benlate (Benomil) for 30 min. Explants were subsequently dipped in 70 % ethanol for 2 min before further sterilization. Three different sterilizing agents were used: sodium hypochlorite, calcium hypochlorite and mercuric chloride in concentrations of 0,5 %; 1,5 % and 3,5 % for 15 min. Explants were then rinsed with sterile distilled water.

Culturing Duke 7 explants usually resulted in phenolic browning of the tissue which

inhibits any tissue growth. Where tissues are liable to browning, explants are often submerged in a solution of a reducing agent (antioxidant) immediately after excision (George & Sherrington). Various anti-browning agents were used as a pre-wash for Duke 7 explants: 1 g/ℓ PVP, 20 mg/ℓ L-cysteine, 100 mg/ℓ citric acid and 150 mg/ℓ ascorbic acid.

The most important item for successful establishment of organ cultures is an appropriate nutrient medium. It was decided therefore to test a range of media.

Plant material: Lateral buds of glasshouse-grown Duke 7 avocado rootstocks were washed with the anti-browning agents and sterilized as described. Lateral buds were subsequently inoculated on four different media.

Culture conditions: The basic media tested were as follow: Murashige & Skoog (1962), Dixon & Fuller (1976), Gamborg (1975) and Woody Plant medium (1980). Media are described in Hartmann & Kester (1975).

Results

Optimum sterilization was obtained by exposing plant tissue to 5 g/ℓ Benlate (Benomil) for 30 min, followed by 70 % ethanol for two minutes and 3,5 % sodium hypochlorite for 15 minutes.

Browning was overcome with 150 mg/ℓ ascorbic acid and 100 mg/ℓ citric acid as a pre-wash for 15 minutes before buds were inoculated in culture.

Murashige & Skoog (MS) medium was optimal for initiation of cultures. Due to leaf chlorosis $FeSO_4$-H_2O and Na_2EDTA were replaced by 40 ml/ℓ FeNaEDTA, as an iron source. Successful initiation of Duke 7 lateral buds was obtained after one week in culture (figure 1).

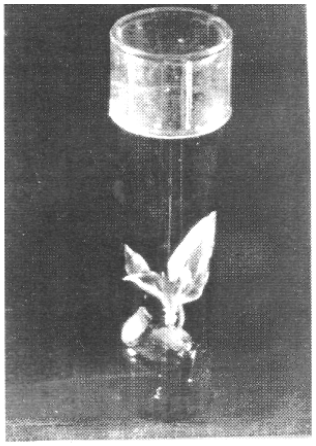

Figure 1
Initiation of Duke 7 lateral bud

Phase two: Development of successful bud multiplication *in vitro*

Benzyl amino purine (BAP) as a cytokinin source had the most pronounced effect on multiplication of cultures. Benzyl amino purine (BAP) and índole butyric acid (IBA) were tested in sixteen combinations of various concentrations.

Plant material: Ten nodal cultures of *in vitro* grown Duke 7 avocado plants were used for each combination tested in this experiment.

Culture conditions: The basal nutrient MS-medium was used as previously described. Sucrose at 30 g/ℓ was added. Various combinations of growth regulators were tested.

Results

The optimum combination of growth regulators were: 1,0 mg/ℓ. BAP with 0,1 mg/ℓ IBA, plants produced 4,5 new leaves over an 8-week period (figure 2).

Phase three: Development of successful rooting of tissue cultured avocado microshoots

Efforts to root tissue-cultured avocado cuttings using various concentrations and combinations of auxins have met with little success in the previous research year. It was therefore decided to combine the auxin treatment with an etiolation treatment.

The basal areas of tissue-cultured Duke 7 shoots were etiolated. Controls were done. Reports in the literature indicated that IBA and NAA are most commonly used for rooting. George & Sherrington (1984) also found IBA superior to NAA where rooting was concerned.

Plant material: Fifty *in vitro* grown Duke 7 shoots 10-30 mm long were used for each treatment.

Culture conditions: Murashige & Skoog (1962) medium as previously described was used as the basal media. Rooting was tested with IBA and NAA at concentrations of 1,0; 3,0 and 5,0 mg/ℓ, each with or without 0,1 mg/ℓ BAP.

Results

Results were observed after 60 days in culture. Etiolation was essential for initiation of rooting. Plants not etiolated showed little or no rooting. Results showed that BAP inhibited rooting. Rooting did occur with BAP in the medium but at much lower rates than on media without BAP. The auxin a-naphthalene acetic acid (NAA) resulted in callus formation.

Etiolation in combination with 5 mg/ℓ IBA resulted in 20 % rooting (figure 3).

CONCLUSION

Successful *in vitro* initiation and multiplication of Duke 7 avocado plants was obtained. A combination of growth regulators (0,1 mg/ℓ IBA and 1,0 mg/ℓ BAP) gave an average multiplication rate of 4,5 new leaves with lateral buds. This multiplication rate compares very favourably with other commercially applied tissue culture systems. Rapid Duke 7 shoot multiplication arising from axillary buds has been demonstrated and could benefit the nursery industry considerably if it could be commercially applied.

Figure 2
In vitro multiplication of Duke 7 microshoots.
Plants produced 4,5 new leaves over an 8-week growth period

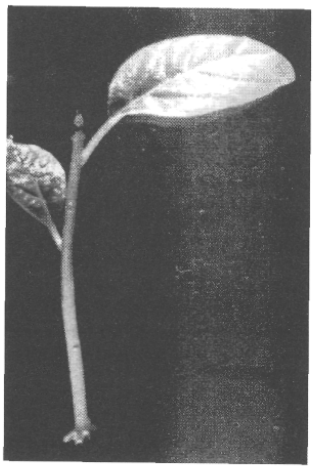

Figure 3
Rooted Duke 7 avocado tissue-cultured shoot

Initial rooting results show that roots can be initiated. Rooting of adult Duke 7 plants could be obtained but rooting was very inconsistent. This inconsistency probably resulted from physiological differences in the shoots at the start of the rooting experiments. Although the cultures as such are of the same age, all the buds did not sprout simultaneously, resulting in physiologically different ages within a culture. From the results presented, it was concluded that the cytokinin BAP inhibits rooting and that IBA at 5 mg/ℓ in combination with etiolation of the tissue cultured shoots gave the best rooting rate and root development of all the concentrations and auxins tested.

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