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In Vitro Propagation of Avocado *(Persea americana* Mill.) Abstract

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A method with high success frequency for propagation of avocado is described. Embryonic axes of mature avocado seeds from six Floridian cultivars were excised and cultured in Murashige and Skoog (MS) medium containing growth regulators. Two types of cytokinins were used: benzyladenine (BA) and thidiazuron (TDZ). Explants were incubated in the dark for 7-10 days to reduce browning, then transferred to 18 h photoperiod. Multiple shoots were subcultured for further multiplication. Shoots were rooted in MS medium with 2 mg/1 indolebutyric acid (IBA). This procedure could have application in improving the cloning of avocado varieties and rootstocks having desirable characteritics.

Introduction

Avocado is a New World crop which has been consumed for centuries, now cultivated in many countries worldwide under different climates (Ahmed and Barmore, 1980). Thousands of cultivars of avocado resulting from breeding and selection were locally developed (International Board For Plant Genetic Resources, 1986), but few are useful for commercial purposes. The application of biotechnology could enhance and improve avocado characteristics and productivity. Avocado tissue culture, however, is still in early development, and more research is needed to establish a successful protocol for regeneration and propagation of avocado. Skene and Barlass (1983), Mooney and Van Staden (1987) and Pliego-Alfaro and Murashige (1988) reported a system for the rescue of immature embryo *in vitro.* Shoot multiplication from shoot tip and axillary bud has been reported (Schroeder, 1980; Young, 1983; Schall, 1987; Skene and Barlass, 1983), but with low success frequency and limited numbers of shoots. The need for a rapid method for plant regeneration with high success frequency is prerequisite for the improvement of avocado. A method with high success frequency of multiple shoot formation from embryonic axes of mature embryo from six Floridian cultivars is described herein.

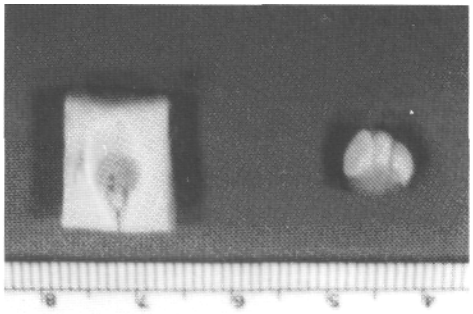

Figure 1. Typical explants used for plant regeneration from mature seed of avocado.

Materials and Methods

Seeds from mature avocado fruits of cultivars of Dade, Maxima, Cataloina, Tower 2, Waldin, and Choquette were used. Seed coats were removed and seeds were washed with detergent, then incubated in 20% chlorox for 20 minutes. Seeds were taken under the hood, and with a wedge the two cotyledons were separated. The embryonic axes were carefully separated from the cotyledons *(Fig. 1)* and incubated in 5% chlorox for 15 minutes. In some instances, about a 5 mm square from the cotyledon, where the embryo was connected, was excised; incubated in 5% chlorox for 15 minutes; and used as explant as well *(Fig. 1).* All explants were rinsed three times in sterile distilled water and incubated in culture media. The medium used was Murashige and Skoog (1962) medium (MS) supplemented with 100 mg/l myo-inositol, 30 g/l sucrose; 8 g/l agar without growth regulators or with different combination of benzyladenine (BA) 1, 2, 3 mg/l BA or thidiazuron (TDZ); 0.2, 1, 2, mg/l TDZ and 0.1 mg/l nephthaleneacetic acid (NAA). Explants were kept in the dark for 7-10 days after incubation in culture media to reduce browning, then transferred to an 18 hr photoperiod. Shoots were formed within 4-5 weeks in BA containing medium which were transferred for rooting. Explants cultured in TDZ formed multiple buds, which were transferred to BA containing medium to induce shoot formation. For rooting, shoots were separated and cultured in MS medium containing 2 mg/l indolebutyric acid (IBA).

Results and Discussion

Embryonic axes explants formed multiple shoots after four weeks from transfer to light. On the other hand, cotyledonary tissue explants produced few buds with low frequency, were more susceptible to browning, and subsequently were not used any further. Culture incubation in the dark for 7-10 days reduced browning, compared to those kept under light (Mohamed-Yasseen *et al.,* 1992); and similar observations were reported with eucalyptus (DurandCresswel and Nitsch, 1977). Light may stimulate enzymatic and chemical (nonenzymatic) oxidation of phenolic compounds present in avocado through the production of free radicals. Embryonic axes respond to the addition of BA, and shoot numbers increased with BA concentration producing up to eight shoots per explant. When MS medium was used alone, without growth regulators, one single shoot was

formed and small lateral buds were present but depressed by the apical dominance of the main shoot. Avocado seeds have been noted to contain more than one shoot, a phenomenon called multisprouting which had been observed early by Traub and Auchter (1933 a, b). Multi-sprouting *(Fig.* 2) is race-dependent and is more frequent in the Guatemalan and West Indian races, which produce more sprouts per seed than are produced in the Mexican race (Salazar-Garcia and Borys, 1983). Explants cultured in a TDZ-containing medium produced multiple buds *(Fig. 3),* and few short shoots were only observed at low concentration (0.1 mg/l). The number of buds increased with the increase of TDZ concentration. Explants with multiple buds were transferred to a BA-containing medium to induce shoot formation. TDZ is a new cytokinin which is more potent than BA (Mohamed-Yasseen and Splittstoesser, 1990), and is cost effective. It has a high degree of cytokinin activity for shoot regeneration (Mohamed-Yasseen and Splittstoesser, 1991) and shoots proliferation (Mohamed-Yasseen and Splittstoesser, 1990; Mohamed-Yasseen *et al,* 1992).

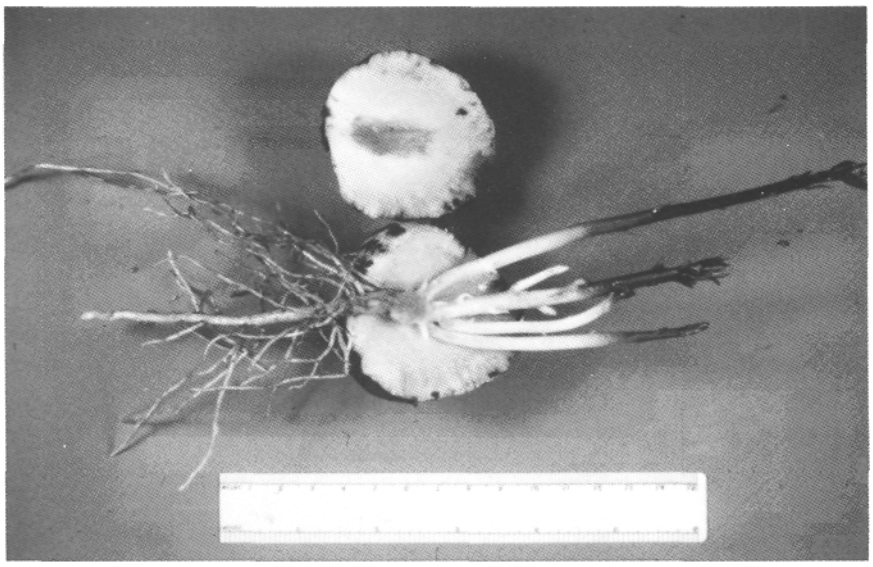

Figure 2. Typical multi-sprouting observed in Waldin seedling.

Shoots, formed in MS alone or supplemented with BA, were transferred to a fresh medium containing BA for further proliferation; and each shoot produced about four shoots. All shoots were transferred for root formation in MS containing 2 mg/l IBA. Two types of roots were observed: one type with adventitious shoots, which was common *(Fig. 4),* the other type with one single thick root.

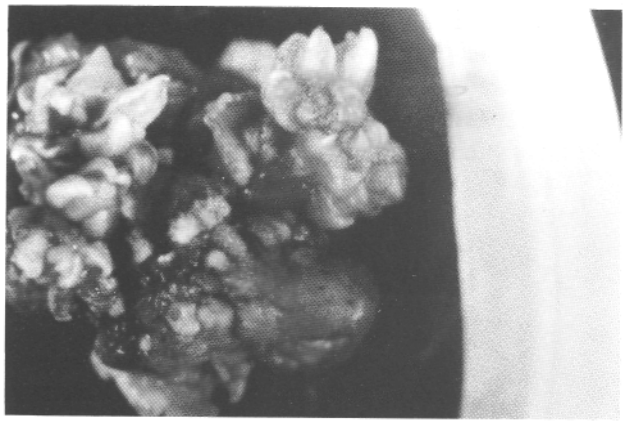

Figure 3.
Multiple buds and callus formation in a medium containing Thidiazuron.

Figure 4.
Rooted shoot in MS containing IBA before transfer to soil.

This procedure showed that embryonic axes of mature avocado respond to the increase of cytokinins and produce multiple shoots with high frequency. More improvement of the percentage of rooted shoots and recovered plants in soil would make it possible to accomplish rootstock cloning and advance avocado breeding.

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