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IN VITRO SHOOT PROLIFERATION IN AVOCADO (*Persea americana* Mill.) INDUCED BY CPPU.

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Abstract

Efficient methods of clonal propagation are highly required by the avocado industry, in order to increase the use of rootstocks with salinity and *Phytophthora* resistance. Tissue culture techniques in avocado are difficult because the tissues have the tendency to produce browning and later necrosis. It is scarce the rooting potential *in vivo* and *in vitro* as well.

Active growing shoots from summer, fall and spring vegetative flushes, were excised and kept in a solution of 500 mg/l ascorbic and citric acids, with 50% v/v of ice. WPM medium was used, in assays for types of explant; season; surface sterilization; antioxidants and growth regulators, with the cultivars 'Lula' and 'Velvick'.

The enhanced protocol consist of using axillary buds, surface sterilized with 95% ethanol for 5 seconds, followed by 0. 5% sodium hypochlorite plus 0.1 ml/l Tween 20 and five rinses with antioxidant solution. Once inoculated, cultures were kept, first in darkness and then, under increasing light intensities (500, 1500 and 3500 lux) for equal periods of seven days each.

Stage I and II were carried out in the same medium (using 0.2% agar, 0.2% gelrite, 60 mg/l ascorbic acid). The optimal condition for 'Lula' and 'Velvick' were 0.5 and 0.1 mg/1 CPPU [N-(2-chloro-4-pyridfl)-N-phenylureal, obtaining up to 92% of establishment rate and 1.4 shoots per explant. Lower levels of browning were detected when using CPPU, in comparison to TDZ [1-phenyl-3-(1,2,3-thiadiazol-5-yl) urea] and BA. No seasonal effect was detected, as long as the mother plant presented vegetative growth.

1. Introduction

Current clonal propagation in avocado is carried out by the method of double grafting (Brokaw, 1987), in an expensive and very time consuming process. Limited success has been obtained in cutting propagation and tissue culture. Juvenile tissues have been used *in vitro,* obtaining roots from microcuttings (Pliego-Alfaro et al., 1987). Use of juvenile material has the disadvantage of genetic variation by the previously required seed propagation of stock plants. An alternative method can be used, by grafting adult material on seedling stocks *in vitro,* which may increase the rooting potential of the scion (Pliego-Alfaro, 1988). This is the first report in regeneration with adult material in 'Lula' and 'Velvick'.

2. Material and Methods

Active growing terminal shoots (5-7 cm) were selected from 4-year-old trees of 'Velvick', and 8-year-old 'Lula'. Vegetative material from summer, fall and spring flushes were used. Explants were kept in 500 mg/l ascorbic acid and citric acid, plus 50% v/v ice. Axiltary buds were excised under the same acids. WPM medium (Lloyd and McCown, 1981) was used, with (mg/1) 0.4 thiamine, 500 casein hydrolysate, 80 glutarnine, 100 myo-inositol, 1000 polyvinylpolypyrrolidone, 60 ascorbic acid, and 30.000 sucrose, solidified with 0.2% agar + 0.2% gelrite. Thirty replicates were used in each experiment. Cultures were kept in the dark for 7 days, and progressive illumination (500, 1500, 3500 lux), every 7 days, at 25±2ºC and 16 h of fight. Explants were surface sterilized with 95% ethanol for 5 sec, and 0.25% sodium hypochlorite + 0.01 mg/l tween 20 for 20 min. Different concentrations of BA, IBA, GA3, CPPU [N-(2-chloro-4-pyiidil)-N-phenylurea] and TDZ [1-phenyl-3-(1,2,3- thiadiazol-5-yl) urea] were used (tables 1 and 2), using methods reported by Dalsaso and Guevara (1989), Phego-Alfaro et al. (1987) and Pliego-Alfaro (1988). Results were obtained after 90 days in culture, subculturing afterwards in the same media.

3. Result

Different response was obtained between the cultivars. Highest shoot formation was observed with 0.65 mg/I BA in 'Lula!, while 'Velvick' presented a recalcitrant performance in all the treatments (table 1). Nevertheless, highest proliferation rate was produced by 0. 1 mg/l CPPU in both cultivars. Best treatments for each cultivar were repeated (table 2), to compare with TDZ and CPPU.

In the second experiment, the number of shoots per explant was always one. Differences were observed in the necrosis rate (table 2). In 'Lula', CPPU produced the highest rate of shoot formation and the lowest necrosis.

4. Discussion

4. 1. Experiment 1

Regenerative response was variable, obtaining from 0 to 7 shoots per explant, even when the explants were uniform in size and developmental stage. This response may be due to the species performance *in vitro,* reported as recalcitrant (Solorzano, 1989) (table 1).

Table 1 - Effect of growth regulators on shoot production. Shoot formation proportion (SFP) in % of total tubes inoculated. Average number of shoots per explant (NSE). Concentration of growth regulators in mg/l.

	`Velvick'		`Lula'	
Treatment	SFP	NSE	SFP	NSE
Control	0	0	50	1
2 BA + 0.5 IBA	25	1.4	10	1
0.65 BA	5	1	70	1
2 BA + 2 GA_3	0	0	0	0
0.1 CPPU	10	1.5	10	1.5
0.01 CPPU	5	1	5	1

4.2. Experiment 2

In 'Velvick', the effect of BA on shoot formation was isolated from IBA, but a different rate of necrosis was observed, which may indicate that the auxin is required for normal development, when BA is present.

In addition to an increased proliferation, the effect of CPPU was to prevent browning. Many explants of 'Velvicle and 'Lula! were growing for 4-6 weeks and then shifted to necrosis. This effect may be produced by increasing the sink activity in the tissue, or by inhibiting the activity of polyphenoloxidases. CPPU and TDZ induced adventitious shoots, originated from epidermal tissue at the distal portion. Shoots produced in BA treatments arose from pre-formed terminal or lateral buds. Subcultured shoots presented necrosis and defoliation; survival rate were 20 to 45%, even when using low temperature, antioxidants and darkness to prevent browning. No difference was observed in the survival rate between shoots from different treatments.

Table 2 - Effect of growth regulators on necrosis and shoot formation. SFP and necrosis proportion (NP) in % of total tubes inoculated. Concentration of growth regulators in mg/l.

	`Velvick'		`Lula'	
Treatment	SFP	NP	SFP	NP
Control	48	48	32	32
2 BA + 0.5 IBA	92	20	-	-
2 BA	88	48	-	-
0.65 BA	-	-	36	8
0.1 CPPU	96	28	72	8
0.5 CPPU	44	40	92	4
0.1 TDZ	44	44	32	48
0.01 TDZ	60	20	56	12

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