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PROPAGATION OF AVOCADOS BY TISSUE CULTURE: DEVELOPMENT OF A CULTURE MEDIUM FOR MULTIPLICATION OF SHOOTS

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OPSOMMING

Groeipunte van die Duke 7 cultivar is asepties met behulp van weefselkultuur metodes gekweek om groei en vermeerdering van lote te bevorder. Binne 9 weke het groeipunte op 'n gewysigde Dixon en Fuller-medium met sitokinien en geen ouksien aanleiding gegee tot die ontwikkeling van 6.1 nuwe lote per eksplant. Die nuwe lote het ontstaan uitoksel-knoppe van die oorspronklike eksplant sowelas okselknoppe van die nuwe lote.

SUMMARY

Duke 7 shoot meristems have been cultured aseptically to promote growth and proliferation of shoots. Apices grown in a modified Dixon and Fuller medium with cytokinin but in the absence of auxin, supported the development of 6.1 new shoots per culture in 9 weeks. The new shoots arose from axillary buds of the original shoot explants and axillary buds of new developing shoots.

INTRODUCTION

Three distinct stages were described by Murashige (1974) for rapid plant propagation through tissue culture. Stage I involves establishing an aseptic tissue culture from shoot tips or other plant organs. During Stage II, conditions were altered to cause a rapid increase of shoots or other organs that will ultimately produce complete plants. Stage III involves changing cultural conditions to promote root development on the shoots and to harden these plants in preparation for successful transfer to soil.

The development of a tissue culture method for vegetatively propagating avocados has commercial value in reducing the time and space required to increase stock plants of valuable clones. Nel, Kotzé and Snyman(1983) have reported the successful propagation of *Persea indica* through tissue culture. Their attempts at proliferation of Duke 7 were, however, unsuccessful.

Preliminary experiments on establishing Duke 7 in culture indicated that the medium of Murashige and Skoog (MS) (1962) contained substances toxic to Duke 7. Explants became necrotic and died within a weak of being cultured. This paper is concerned with the establishment of Stage I and II culture conditions for rapid multiplication of Duke 7 shoots.

MATERIALS AND METHODS

Shoot tips were obtained from 6 week old greenhouse-grown Duke 7 plants. Apices with between 4 and 6 attached leaf primordia were surface sterilized for 15 minutes in a 1% sodium hypochlorite solution, with a small amount of Tween 20 (polyoxyethylene sorbitan) added. The shoot tips were then rinsed several times in sterile distilled water before being aseptically placed on the medium.

The basal nutrient medium consisted of the following constituents and concentrations:

Constituent	Concentration mg/1
Inorganic salts	Dixon and Fuller (DF) (1976)
Thiamine HCl	5
Nicotinic acid	5
Pyridoxine HCl	0.5
Myo-inositol	1 000
Sucrose	30 000
Difco Bacto-agar	8 000

pH adjusted to 5,75 with 1N KOH

Cultures were incubated at 27ºC under white fluorescent lamps with a 16h light: 8h dark, light regime.

RESULTS

Varying the concentrations of NH_4 and NO_3 or Fe EDTA concentrations in the Murashige and Skoog formula had no effect on tissue necrosis. A modification of the MS formula as suggested by Dixon and Fuller (1976) proved to be a better medium, although there was still evidence of explant chlorosis. Chlorosis appears to be a .common problem associated with tissue culture of fruit crops (Skirvin, 1981). Researchers have assumed that the problem is due to a high concentration of nitrogen-containing compounds or to an iron deficiency. The concentration of Fe was doubled using the procedure described by Murashige and Skoog (1962). When it was increased, the browning and early culture death symptoms were eliminated.

For the best Stage II culture conditions, the determination of optimum combinations and concentrations of auxins and cytokinins are critical. Initial experiments indicated that there was no absolute requirement for an auxin source. Comparing kinetin, 6-benzyladenine (BA) and iso-pentenyladenine (2iP) showed that kinetin was more effective than either BA or 2iP (Table 1). BA and 2iP seem to be quite detrimental to avocado tissue. The most effective concentration of kinetin for multiple shoot induction was 10 mg/ℓ. At this optimum concentration shoots are 10 to 15cm in length after 2 weeks in culture (Fig. 1) and 6.1 shoots a re obtained after 9 weeks in culture (Fig. 2).

Although good shoot growth was obtained, it must be noted that leaf expansion was very poor. It is generally agreed that reduced forms of nitrogen have value for certain plant tissue cultures. In particular, L-glutamine and L-arginine have been found to stimulate leaf expansion (Wang and Hu, 1983). Following the work of these authors 40 mg/ℓ of each were added to the culture medium. Leaf expansions were much enhanced and shoot growth greatly improved.

DISCUSSION

In the present research, rapid Duke 7 shoot multiplication arising from axillary buds has been demonstrated. A one-step procedure was satisfactory for both explant establishment and multiple bud induction (Stages I and II). This significantly reduced costs. Shoot proliferation with kinetin proved to be superior to either 2iP or BA. These results are contrary to the findings of Lundergan and Janick (1980) and Hutchinson (1984), who showed the highest rate of proliferation using BA with little and no proliferation using kinetin and 2ÍP respectively.

The essential compounds required for healthy shoot development in the basal medium were 40 mg/ℓ of L-glutamine, 40 mg/ℓ of L-arginine, 55,7 mg/ℓ of $FeSO_4.7H_2O$ mg/ℓ of kinetin. This combination of compounds resulted in an average of 6.1 shoots per-culture in 9 weeks. Since the shoots can be proliferated *in vitro* at a geometric rate, thousands of shoots could be obtained within a year. This

technique could be used in the rapid cloning of Duke 7 rootstocks once the *in vitro* root induction (Stage III) problem has been solved.

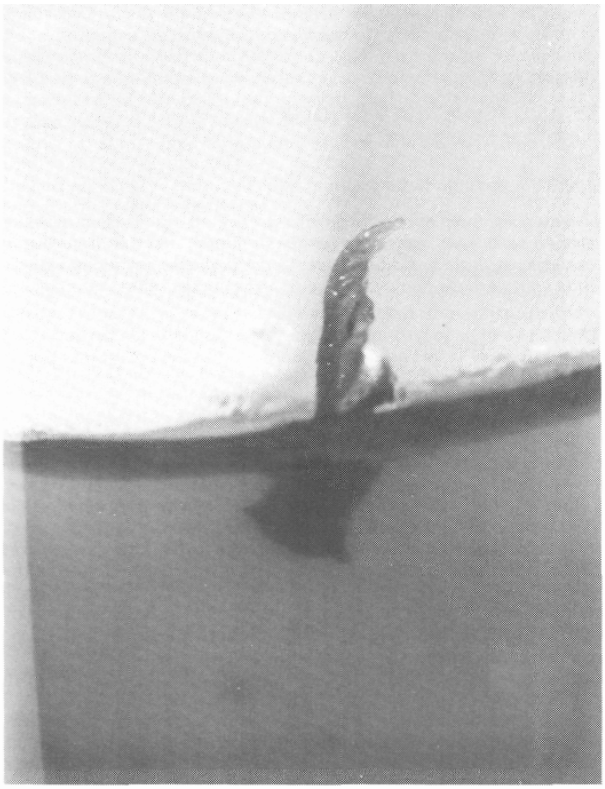

Figure 1: Duke 7 shoot apex after 2 weeks in culture.

Figure 2: Duke 7 shoots developed from single shoot apex cultured for 9 weeks on medium containing 10 mg/ℓ kinetin.

TABLE 1 The effects of kinetin, BA and 2iP concentrations on the survival rate of Duke 7 explants and the numbers of shoots after 9 weeks in culture

Cytokinin	mg/ℓ	Survival %	No. of Shoots/culture
	0	10	1.0 ± 0.0[3]
Kinetin	5	60	1.0 ± 0.0
Kinetin	10	90	6.1 ± 0.4
Kinetin	30	50	1.4 ± 0.2
Kinetin	60	30	1.0 ± 0.0
BA[1]	5	60	1.0 ± 0.0
BA	10	50	1.0 ± 0.0
BA	30	70	1.0 ± 0.0
BA	60	0	0
2iP[2]	5	60	1.0 ± 0.0
2iP	10	40	1.0 ± 0.0
2iP	30	0	0
2iP	60	0	0

[1] N_6-benzyladenine
[2] Isopentenyladenine
[3] Standard error of the mean

TABLE 2 The composition of Stage I and II Duke 7 medium

Mineral salts	mg/ℓ
KNO_3	2520
$MgSO_4.7H_2O$	360
$NH_4H_2PO_4$	290
$CaCl_2 2H_2O$	220
$MnSO_4.7H_2O$	13.2
H_3BO_3	5
$ZnSO_4.7H_2O$	1
KI	1
$CuSO_4.5H_2O$	0.2
$Na_2MoO_4.2H_2O$	0.1
$CoCl_2.6H_2O$	0.1
$FeSO_4.7H_2O$	55.7
Na_2 EDTA	74.5
Organic constituents	
Thiamine HCl	5
Nicotinic acid	5
Pyridoxin HCl	0.5
Myo-inositol	1000
Sucrose	30000
Difco Bacto-agar	8000
L-glutamine	40
L-arginine	40
Kinetin	10

REFERENCES

DIXON, RA. & FULLER, KW., 1976. Effects of synthetic auxin levels on phaseolina production and phenylalanine ammomalyase (PAL) activity in tissue cultures of *Phaseolus vulgaris L. Physiol. Plant Path.* 11: 287 - 292.

HUTCHINSON, JF., 1984. Factors affecting shoot proliferation and root initiation in organ cultures of the apple 'Northern Spy'. *Sci. Hort.* 22: 347 - 358.

LUNDERGAN, CA. & JANICK, J., 1980. Regulation of apple shoot proliferation and growth *in vitro. Hortic. Res.* 20: 19 - 24.

MURASHIGE, T. 1974. Plant propagation through tissue culture. *Ann. Rev. Plant Physiol.* 25: 135 - 166.

MURASHIGE, T. & SKOOG, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15: 473 - 497

NEL. DO., KOTZÉ, JM. & SNYMAN, CP., 1983. Progress in tissue culture of avocado. *S. Afr. Avocado Growers' Assoc. Yearbook* 5: 90 - 91.

SKIRVIN, RM., 1981. The tissue culture of fruit crops. In: *Cloning Agricultural Plants via in vitro*

techniques (ed. CONGER B.V.). Chemical Rubber Company Press, Inc., Boca Raton, Florida, USA. Pp 51 - 139.

WANG, P. & HU, C., 1983. *In vitro* cloning of the deciduous timber tree *Sassafras randaiense. Z. Pflanz.* 113: 331 - 335.