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THERMOTHERAPY, SHOOT TIP CULTURE, AXILLARY BUD PROLIFERATION AND PLANT REGENERATION IN YAM (DIOSCOREA TRIFIDA L.)

(Thermothérapie, culture d'apex, prolifération de bourgeon axillaire et régénération de plantes chez l'igname Dioscorea trifida L.)

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SUMMARY

Axillary bud proliferation in *Dioscorea trifida* L. (CATIE introduction n° 10715) was achieved when axillary bud shoot tips with 4-6 leaf primordia were cultured on semi-solid Murashige and Skoog (MS) medium supplemented with several concentrations of N^6- isopentyladenine (2-iP) in combination with napthaleneacetic acid (NAA). The emerging buds were excised and individually recultured onto semi-solid MS medium devoid of growth regulators in order to promote root development and recovery of complete plants.

RESUME

La prolifération de bourgeon axillaire chez Dioscorea trifida L. (introduction n° 10715 du CATIE) a été réussie à partir d'apex ayant 4-6 primordia foliaire cultivé sur un milieu semi-solide de MURASHIGE et SKOOG (MS) complété par diverses concentrations de n-isopentyladenine (2-iP) en combinaison avec l'acide naphtalène acétique (ANA = NAA). Les bourgeons apparus ont été excisés et repiqués individuellement sur milieu MS semi-solide régulateur de croissance pour favoriser le développement racinaire et parvenir à des plantes complètes.

INTRODUCTION

Yams (*Dioscorea* spp.) are an important source of medicinal compounds such as steroids and are also a carbohydrate staple of subsistence farmers in several countries of the Caribbean, Central America, Africa and Asia. With the recent immigration of some of these peoples to the United States and Europe these countries have initiated importation of yams from the West Indies (4) and Costa Rica.

Yams are vegetatively propagated from tuber pieces, vine cuttings, or setts (6). These methods are inneffective for maintaining pathogen-free stock plants and increase the risk of dissemination of disease or pest infected planting materials. Maintenance of yam germplasm involves the use of field collections that are costly to maintain and may result in the loss of valuable genotypes due to natural disaster, human error, or attack by pathogens.

Tissue culture techniques for the propagation of yams include the culture of meristems for virus elimination in *D. alata* (4), and propagation utilizing cultured nodal segments in *D. alata* and *D. rotundata* (3) or from bulbil explants from *D. bulbifera* and *D. alata* (2). In *D. trifida* it has been difficult to induce roots from cultured nodal segments (1). Culture of meristems, shoot tips or axillary buds has not been reported. In our laboratory meristem and shoot tip culture are being utilized for virus elimination, propagation, and germplasm maintenance of *D. trifida* and several other tropical root and tuber crops.

This research was conducted to investigate the feasibility of inducing axillary bud development from cultured shoot tips of *D. trifida* as an alternative means of rapid clonal propagation of disease-free material. For investigators involved in yam production this may represent a viable method facilitating germplasm conservation or exchange and possibly its genetic improvement. It may also facilitate more accurate evaluation of yam germplasm.

MATERIALS AND METHODS

Yam tuber pieces of about 250 g were taken from the head of the main tuber of CATIE introduction n° 10715 2 months after harvest. These pieces were washed, soaked for 15 minutes in a solution of commercial fungicide (1 per cent, w/v Benomyl), planted in 85 x 125 mm pots containing sterilized soil and either grown in a greenhouse or incubated in a thermotherepy chamber. Thermotherapy treatment consisted of a hot air treatment of 39 $\pm$ 1°C for 6 weeks under 3.5 klux illumination provided by white fluorescent lamps on a 16 hr (day) photoperiod. Plants were irrigated daily and maintained under 60-80 per cent relative humidity.

When plants reached 30 cm in height, four nodes about 1.0 cm in length were taken from the apical portion of the shoots and sterilized in 10 per cent (v/v) commercial laundry bleach with the surfactant Tween 80 (2 drops/100 ml) for 15 minutes with agitation.

In an aseptic environment, and with the aid of a dissecting microscope, small leaves were removed to expose the axillary bud shoot tips. Shoot tips, 0.1 to 0.5 mm in length and possessing 4 to 6 leaf primordia, served as primary explants. These explants were rinsed in sterile water and

individually transferred to 25 x 150 mm culture tubes containing 10 ml of test media. All cultures were initiated on basal medium containing MURASHIGE and SKOOG MS salts (5) and the following in mg/liter : thiamine HCl, 0.1 ; pyridoxine HCl, 0.5 ; nicotinic acid, 0.5 ; glycine, 2.0 ; myo-inositol, 100 ; sucrose, 30,000 ; Difco Bacto agar, 7,000 ; and supplemented with 2-iP at 0, 0.5, 1.0, 2.0, and 3.0 in combination with NAA at 0, 0.1, 0.2, 0.3, 0.4, and 0.5, in a factorial experimental design with 3 replications per treatment.

The pH of all media was adjusted to 5.7 with KOH or HCl and sterilized at a constant 121°C at 15 psi for 15 min. All experiments were replicated 3 times.

Cultures were maintained at a constant 28°C and 16 hr photoperiod under 3 klux illumination. Cultures were scored visually for the occurence of callus and axillary bud proliferation at two week intervals.

RESULTS AND DISCUSSION

No problems with culture contamination or explant oxidation were encountered. Thermotherpy treated tuber pieces sprouted and plants developed more quickly from them than from those planted in the green house. Each tuber piece produced 8 to 12 shoots which provided about 40 shoot tips for use as explants in a six week period.

One week after culture initiation shoot tips had become chlorophyllous, however no callus or further shoot development was observed. Five weeks later many small green axillary buds (usually more than 5/culture) were evident in cultures containing a 2-iP concentration of 0.5 mg/l and NAA at 0.2 mg/l. Other auxin to cytokinin ratios were also effective, but less so. (Table 1). Medium devoid of growth regulators also stimulated axillary bud development. By ten weeks after culture initiation shoot development had occurred and axillary shoots with well developed leaves, but without roots, were see. In control cultures shoot elongation proceeded normally and individual shoots reached about 1.0 cm in height (Fig. 1). Shoot elongation was most rapid (about 3 cm after 10 weeks) in cultures containing 2.0. mg/l 2-iP and 0.5 mg/l NAA (Fig. 2).

In order to further stimulate shoot elongation and root formation entire proliferating shoot tips were transferred to basal medium. In these cultures rhizogenesis was commomly observed after 8 to 12 days. However, roots were sparse and did not elongate readily (Fig. 3). In contrast, when individual axillary buds or developing shoots were excised and transferred individually to basal medium rapid root formation and elongation was observed. This treatment also stimulated the development of secondary roots and root hairs. Subsequent shoot and leaf growth was rapid and leafy shoots were obtained after 6 weeks on basal medium. For

Table 1. Effect of the growth regulators on the promotion of multiple axillary buds sprouting on cultured axillary shoot tips of *Dioscorea trifida*.

	2iP (mg/liter)				
NAA (mg/liter)	0.0	0.5	1.0	2.0	3.0
0.0	M B	M B	M B[z]	M B	-
0.1	S B	M B	M B	-	-
0.2	S B	M B	M B	-	-
0.3	S B	M B	M B	S B	-
0.4	S B	M B	M B	S B	M B[z]
0. 5	S B	M B	M B[y]	-	-

SB = Single Bud

MB = Multiple Buds

z = more than 10 buds/treatment

y = between 5-10 buds/treatment

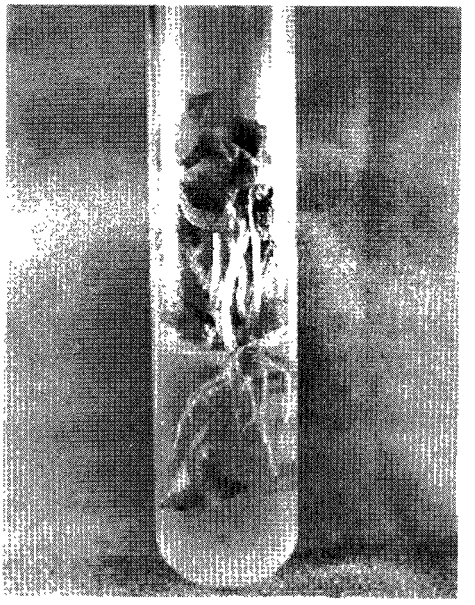

Figure 1. Many small leaves emerging from one single shoot in the control media devoid of growth regulators.

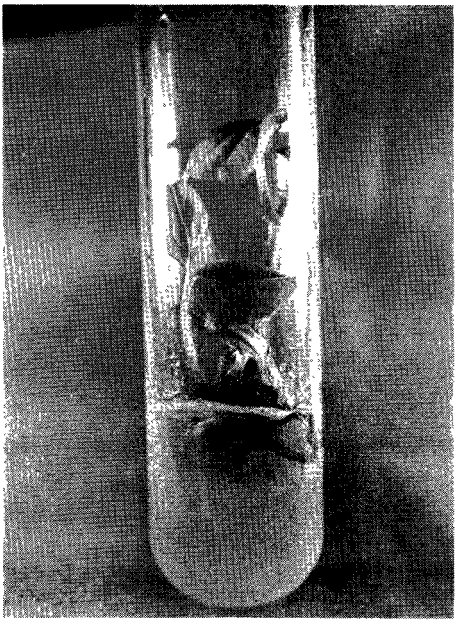

Figure 2. Shoot elongation of *V. trifida* from multiple buds with few roots.

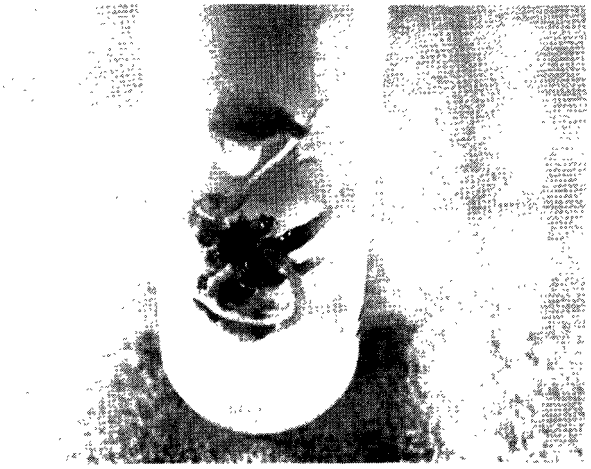

Figure 3. Multiple buds sprouting in cultured axillary bud shoot tip culture of *D. trifida*.

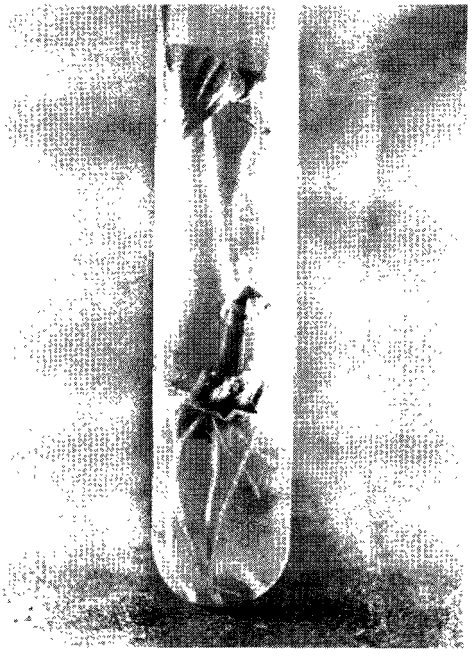

Figure 4. Complete plant of *D. trifida* resulting from single bud cultured in MS semi-solid medium devoid of growth factors.

continued propagation, isolated buds were excised and recultured to basal medium supplemented with 3.0 mg/l of 2-iP and 0.4 mg/l NAA. This procedure promoted additional axillary bud development.

CONCLUSION

Multiple bud sprouting was induced in *D. trifida* by culturing axillary shoot tips in MS semi-solid medium containing several concentrations of 2iP and NAA in a factorial experiment. The results of our experiment indicate that it is possible to obtain a great number of plants from axillary shoot tips using tissue culture techniques.

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