TISSUE CULTURE TECHNIQUES APPLIED TO NATIVE AMERICAN CROPS: MICRO-PROPAGATION OF OCA (*Oxalis tuberosa* Moll.) AN ANDEAN TUBER-BEARING SPECIES[1] /

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Resumen

Este es el primer informe sobre resultados positivos en la propagación in vitro *de oca empleando ápices caulinares como explantos iniciales para el cultivo. El mayor tamaño de estos explantos luego de 30 días de cultivo se logró en el medio de Murashige and Skoog (MS) suplementado con 0.1 mg/l ANA (ácido 1-naftalen acético) y 5.0 mg/l Kin (6-furfuril aminopurina). El medio de MS, líquido, con 0.5 mg/l GA_3 (ácido giberélico) como la única hormona agregada, y en cultivo con agitación (120 excursiones por minuto), resultó ser el mejor, tanto para la proliferación de yemas como para el enraizamiento de los brotes obtenidos.*

Introduction

At present, we are engaged in a research project on the *in vitro* growth requirements of several species and hybrids of the genus Oxalis (2), as a tool for studying the cytogenetics and evolution in the family Oxalidaceae.

Recently it was recommended that major efforts be made for the propagation and/or conservation of minor crops of great regional importance, such as Andean tubers, medicinal plants, tropical fruit tree species, nuts and woody plants for reforestation (3).

Among the minor tuber crops in America, oca (*Oxalis tuberosa* Moll.) stands second after potato in several Andean regions (5). Since oca is always propagated vegetatively and almost never forms seeds, it was of interest to develop a tissue culture technique for large scale propagation (9).

To date only a few authors have studied the most suitable conditions for *in vitro* propagation and/or culture in the genus *Oxalis* (2, 8). Moreover, only one report on the successful micropropagation of one of the minor American tubers has been published so far (7).

The purpose of the experiments described in this paper was to determine the most suitable media and conditions for large-scale propagation of oca through shoot tip culture.

Materials and methods

Explants were obtained from tuber-grown plants kept in a greenhouse at 20 ± 5°C under a 16 h/8 h (light/dark) photoperiod, with Lucalox lamps (400 W) to supplement daylight.

Stem sections (2 cm long) bearing either one axillary or the apical bud were harvested and disinfected in a NaClO solution (1.2% Cl_2) + 0.1% Tween-20 for 10 min., followed by several sterile-water rinses.

The initial explants for culture were 1 mm shoot tips comprising the meristematic dome and as much as 3 leaf primordia.

The basal medium (BM) was Murshige and Skoog's (1), modified by the addition of (mg/l): thiamine-HCl, 0.2; pyridoxine-HCl, 1.0; nicotinic acid, 1.0; glycine, 2.0; myo-inositol, 100; sucrose, 30 000 and with 0.0 or 0.8 g/l agar.

This BM was supplemented with either 0.1 mg/l auxins combined with 0.5, 1.0, or 5.0 mg/l cytokinins, or 0.5 mg/l GA_3 (gibberellic acid) only. The growth regulators tested were 4-(indole-3yl)butyric acid (IBA) or 1-naphthalene acetic acid (NAA) and

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6-benzylaminopurine (BAP) or 6-furfurylaminopurine (Kin). The pH was adjusted with 1 N KOH to 5.8 and then the culture media were autoclaved for 20 min. at 1 013 x 10^5 Pa

The culture vessels used were 20 x 120 mm tubes with 10 ml medium each for the establishment of explants (Stage I) and for the rooting of shoots (Stage III), and either 250 ml glass flasks with 30 ml semisolid medium were used for bud proliferation (Stage II), or 150 ml Erlenmeyer flasks with 15 ml liquid medium were used for both bud proliferation and rooting (Stages II and III).

Whenever liquid media were tested, the cultures were agitated on a reciprocal shaker (120 excursions per min.), as recommended elsewhere (6) Culture conditions were 24 ± 1°C and a 16 h light photoperiod given from cool white fluorescent tubes (1.8 W/m^2)

Each medium and culture condition was replicated 20 times, and the experiments were repeated twice

The growth (mm) of the initial explants by the end of Stage I, the multiplication rate (newly formed buds/subcultured bud) at Stage II, and the percentage of rooted shoots at Stage III were recorded.

Results and discussion

Ten percent of the initial explants were found to be dead or contaminated after 7 days in culture

By the end of Stage I (30 days *in vitro*), a differential effect of the growth regulators on the responses of explants was observed. The largest explants ($\bar{x}$ = 30 mm) were detected on BM supplemented with 1 0 mg/l NAA and 5.0 mg/l Kik by the end of Stage I

Explants obtained with IBA instead of NAA were always smaller ($\bar{x}$ = 5 mm), regardless of the type and concentration of the cytokinin added to the culture medium. This result contrasts with our previous findings in *O. erosa* shoot tip cultures (2). Moreover, when the best media for *O. erosa* shoot tips were used for oca cultures, either the percentage of mortality was high, or explant size increased only slightly ($\bar{x}$ = 5 mm).

Stage I was considered completed when a rosette of several expanded leaflets with some axillary buds began to appear.

Stage II media contained 0.1 mg/l NAA combined with 1 0 or 5.0 mg/l of either BAP or Kin. The best responses were obtanied on BM supplemented with 0 1 mg/l NAA and 5.0 mg/l Kin, as for Stage I. Multiplication of both axillary and adventitious buds was greatly enhanced ($\bar{x}$ = 15 buds per initial explant) on this medium, but no leaf expansion or internode elongation was detected. Fifty percent of explants had formed roots after 20 days culture on this medium (i.e. 50 days *in vitro*). None of these rooted rosettes survived transfer to the soil

In order to obtain phenotypically normal plants, shoots grown on the multiplication medium (BM + 0 1 mg/l NAA + 5.0 mg/l Kin) were subcultured onto either liquid or semisolid BM with 0 5 mg/l GA_3 as the sole hormonal supplement. The benefits of GA_3 on internode elongation, multiplication, phenotypic normality and the further rooting of shoots have already been reported for both potato (6) and *O. erosa* (2).

A high multiplication rate and the elongation of internodes in the shoots were observed after 15 days in the liquid medium. Furthermore, a sporadic tuberization of shoots occurred spontaneously

When similar shoots were transferred to a similar agar-containing medium, both the medium and the explants showed browning, an indication of phenolic oxidation, and eventual death

Individual nodes were then cut from the shoots that had been grown and were subcultured again onto the liquid multiplication medium, for further large-scale propagation. About 8.1 shoots/node were produced every 15 days

Two different methods were tested for rooting shoots (Stage III): a) nodes were subcultured on the multiplication medium as described above, but the culture period was extended to 30 days; and b) complete shoots were harvested from the multiplication medium and subcultured for 30 days on a half-strength, semisolid, hormone-free BM (BM 1/2).

Both methods yielded 100% rooting after 30 days in culture. The liquid medium produced rooting of initial nodes as well as multiplication and rooting on the newly formed shoots (Fig 1). A similar result has been reported in potato cultures (6). On the other hand, when the BM 1/2 was tested, only subcultured shoots rooted and no further multiplication and rooting took place (Fig 2).

The final goal of every micropropagation assay is to obtain a large quantity of true-to-type plants quickly, in a small area, and at a reasonable price (4). Hence, a two-step procedure using BM supplemented

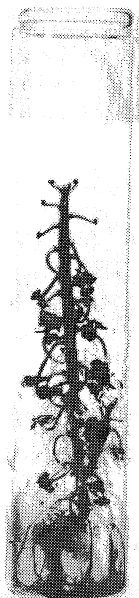

Fig. 1. Effects of ·n agar-containing medium on the multiplication anu rooting of shoots.

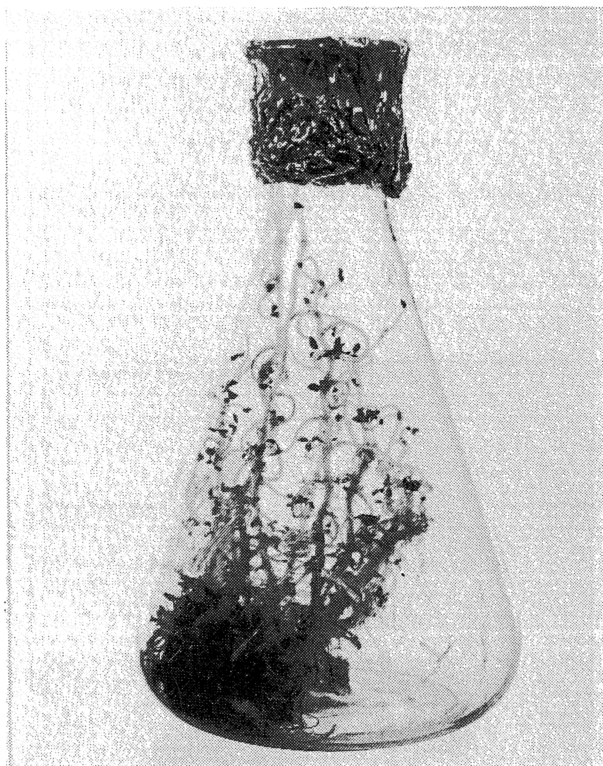

Fig. 2. Results obtained with shaken culture on the BM with 0.5 mg/l GA_3 added see simultaneous multiplication and rooting.

with 0.1 mg/l NAA and 5.0 mg/l Kin for establishing explants (Stage I), and BM with only 0.5 mg/l GA_3, omitting agar and using shaken culture for both multiplication (Stage II) and for the rooting of shoots (Stage III) might be recommended for micropropating oca through shoot tip culture.

The plants obtained in this way were transferred to pots containing soil:peat (3:1), and after 10 days under high humidity conditions were able to survive in the open air. At present, comparative experiments are underway with plants propagated conventionally.

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Reseña de libros

PETER SMITH. Agricultural project management: monitoring and control of implementation. Elsevier Applied Science Publishers, London, 1984. 190 p.

Cuando hace un tiempo se nos pidió que reseñáramos el libro en cuestión, lo hicimos con el temor de que las experiencias poco exitosas que profesionalmente hemos tenido en la aplicación del Método del Camino Crítico en proyectos de desarrollo pesará demasiado en nuestro espíritu. Esperamos que no haya sido así.

El libro consta de nueve capítulos, de los cuales el 1, 2 y 9 realmente poco añadieron a lo ya conocido. El noveno intenta incursionar en el área de aplicación en el uso de computadoras. Creemos que las sugerencias del colega, aunque interesante, son marginales para ser útiles.

Los Capítulos 3 y 4 son los mejores. El material y las ideas brindadas, en cuanto a recolección de información y programación, son muy buenas, dado que intentan cerrar la brecha entre la teoría y la práctica. Decididamente nos impresionaron.

El Capítulo 5 plantea formas de reprogramación de tiempos; realmente no nos agradó y creemos que se quedó corto en un área importante, dado que dio poco tratamiento a la calendarización.

El Capítulo 6 detalla los problemas de estimación de la probabilidad de ocurrencia de los eventos incluidos en una red CPM. Los ejemplos usados hacen énfasis en obras civiles. Señala pero no aclara los problemas normales del cálculo de probabilidad en proyectos de desarrollo. Desde luego que esos problemas no los ha aclarado nadie.

Los Capítulos 7 y 8 nos encantaron; hacen dos resúmenes brillantes de los aspectos organizativos y de uso práctico de las redes. Creemos que sólo esos dos justifican el libro.

El libro es bueno, quizás con un título demasiado ambicioso, del cual el lector puede llegar a esperar demasiado. Vale la pena leerlo y quizás una próxima versión debería excluir la poca teoría que tiene y concentrarse en la aplicación práctica; experiencia que el colega sin duda posee.

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