DIFFERENTIAL REQUIREMENTS AMONG TISSUE SOURCE IN *Solanum tuberosum* L. ssp. *andigena* CALLUS CULTURES[1]

*S.J. OCHATT**
*O.H. CASO**

Resumen

En este trabajo se discuten las diferencias en los requerimientos nutritivos y hormonales tanto para la inducción y proliferación de callo, como para la diferenciación y regeneración de plantas completas de Solanum tuberosum *L. spp.* andigena *cv. "Runa" a partir de tejidos de tubérculo, hoja y médula del tallo. También se detectaron diferencias en los requerimientos de intensidad luminica para los tres tejidos. En tanto que los tejidos de tubérculo requirieron alta intensidad de luz, los de hoja crecieron mejor en oscuridad, y los de la médula del tallo cuando la intensidad luminica empleada fue baja. El ácido alfa-naftalen acético (ANA), a razón de 2.0 mg/l resultó ser lo mejor para tejidos de tubérculo y hoja, mientras que el ácido 4-(indol-3yl)acético) (AIA) permitió mejores respuestas con cultivos de médula del tallo. El callo de tubérculo requirió 6-bencil aminopurina (BAP); sin embargo, los otros dos tejidos crecieron mejor con 6-furfuril aminopurina (Kin). Las mejores respuestas de regeneración se obtuvieron en una secuencia de dos pasos, i.e. el medio de Murashige y Skoog (MS) + 0.1 mg/l ácido 4-(indol-3yl)butírico) (IBA) + 1.0 mg/l BAP + 0.1 mg/l ácido giberélico (GA_3), seguido de un subcultivo más en MS + 0.5 mg/l GA_3, líquido y con agitación, independientemente del tejido originalmente cultivado.*

Introduction

Solanaceous plants, and particularly the potato, have proved to be amenable to tissue culture techniques (1, 4, 5, 6, 9, 14, 15, 18). However, only a few papers have compared cultures from different sources in the same species (8, 17), or similar tissues from different species in a single genus (3).

This paper describes experiments comparing the hormonal and nutritional requirements of cloned lines of tuber, leaf and stem-pith tissues of *Solanum tuberosum* L. spp. *andigena* in culture.

The induction of differentiation from the cultures, and the further regeneration of complete plants in this species are also reported.

Material and methods

Solanum tuberosum L. spp. *andigena* cv. "Runa" plant, grown in a chamber with a temperature of 25 ± 1°C and a photoperiod of 14 h light from HPL-N lamps (400 W) were used as the source of explants.

The initial explants for culture were: a) tuber discs 7 mm in diameter and 3 mm thick; b) 25 mm^2 internerval leaf sections; and c) 0.125 cm^3 stem-pith prisms.

The various explants were cultured on different basal media (BM):

a) Tuber explants were cultured on BM containing Murashige and Skoog's (MS) mineral salts (11) at 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4-fold during preliminary assays. The MS 1.2-fold was found best for callus induction, regardless of other addenda. However, for proliferation, better growth was achieved on BM where the mineral salt concentration was reduced to MS 1.0-fold. Each one of these salt concentrations was supplemented with either MS, Nitsch and Nitsch (12) or Gamborg (2) organics. Growth regulators were added as auxins:

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* Centro de Ecofisiología Vegetal (CONICET-FECIC-FUND. M. LILLO). Serrano 665. 1414 Capital Federal. Argentina.

cytokinins: gibberellins (in mg/l): 0.4:8:0.4 or 2.0:0.5:0.0.

b) Leaf explants were grown on BM containing MS or Miller (10) mineral and organic components, and supplements of 1.0, 2.0 or 5.0 mg/l auxins combined with 0.5 mg/l cytokinins.

c) Stem-pith explants were cultured on MS or Miller formulae, supplemented with 2.0 mg/l auxins and 0.5 mg/l cytokinins.

The growth regulators tested were NAA (l-naphthalene acetic acid), IBA (4-indole-3yl)butyricacid), IAA (4-indole-3yl)acetic acid) or 2.4-D (2.4-dichlorophenoxyacetic acid) as auxins, BAP (6-benzylaminopurine) or Kin (6-furfurylaminopurine) as cytokinins, and gibberellic acid (GA_3).

Media were autoclaved for 20 min at 1.013 x 10^5 Pa, after adjusting the pH to 5.8 with 1 N KOH.

The culture vessels were 20 x 120 mm tubes with 10 ml medium each for callus induction and proliferation, and either 250 ml flasks with 30 ml semisolid medium each or 150 ml Erlenmeyer flasks with 15 ml medium each for differentiation and regeneration assays. Cultures in liquid media were agitated at 90 excursions per min on a reciprocal shaker, as recommended by Roca *et al.* (14).

The cultures were grown at a constant temperature of 24 ± 1°C, and under three different light regimes: a high light intensity (irradiance of 1.8 W/m^2), a low light intensity (irradiance of 0.095 W/m^2), both with a 16 h light/8 h dark photoperiod from cool white fluorescent tubes, and in the dark.

Each medium and culture condition tested included at least 5 replications, and experiments were repeated twice. Cultures were transferred every 30 days.

The results obtained in callus growth were assessed through a subjective scale ranging from 0 (without response) to 4 (massive callus proliferation). The regeneration tests were evaluated by the phenotypic normality of regenerated plants.

Results and discussion

a. Tuber callus

A differential requirement for organic compounds was found. In fact, the tissues proliferated best on media with Nitsch's organics. With Gamborg's organics, only a minute callus was formed at the cut surface, whereas MS yielded intermediate responses. The importance of organics on **in vitro** responses of potato tissues was raised by Jarret *et al* (6), and Nitsch's organics had previously been reported as suitable for tuber cultures (7, 16).

The best results were observed on BM supplemented with 2.0 mg/l NAA and 0.5 mg/l BAP. Despite previous reports (7, 16), in our experiments, when the BM was supplemented with 0.4 mg/l auxins + 0.8 mg/l cytokinins + 0.4 mg/l GA_3, no callus tissues suitable for further proliferation after subculture were obtained. Furthermore, when any auxin other than NAA was tested, no callus proliferation occurred. This result contrasts data by Wang and Huang (17), where 2.4-D was the best auxin to support callus growth.

Regarding cytokinin effects, whenever Kin was added to the BM, even with 2.0 mg/l NAA, only localized callus proliferation was observed.

The best results were obtained under high light intensities, whereas low light intensity or dark conditions always resulted in poor callus proliferation.

After the third subculture, several dark green, nodular portions appeared in the cultures; however, no bud or root differentiation occurred when these calli were subcultured on the same medium. Media containing 0.1 mg/l GA_3, with auxin concentrations were reduced or omitted and cytokinin augmented, were tested for regeneration.

Runa calli differentiated buds and roots on media supplemented with (mg/l) 0.1 IBA, 1.0 BAP and 0.1 GA_3, thirty days after transfer (Fig. 1). These structures were capable of regeneration upon transfer to a similar fresh medium. No further addition was needed for organogenesis; however, GA_3 was found to be essential, and BAP stimulatory. These results support data by Jarret *et al* (5, 6) with *S. tuberosum* L. spp. *tuberosum* cultures. Tuberization was also detected on this medium (Fig. 2).

The regenerated plants always had short internodes and small leaves, with one or more irregularly growing stolons at each node (Fig. 3), as previously reported elsewhere (7, 16). Although these plants were able to survive and resume a normal phenotype upon transfer to soil, a new experiment performed to test agar and GA_3 effects on internode elongation and leaf expansion.

When 1 cm long nodes from those abnormal regenerants were transferred to 15 ml liquid BM with 0.5 mg/l GA_3, leaf expansion and a normal internode

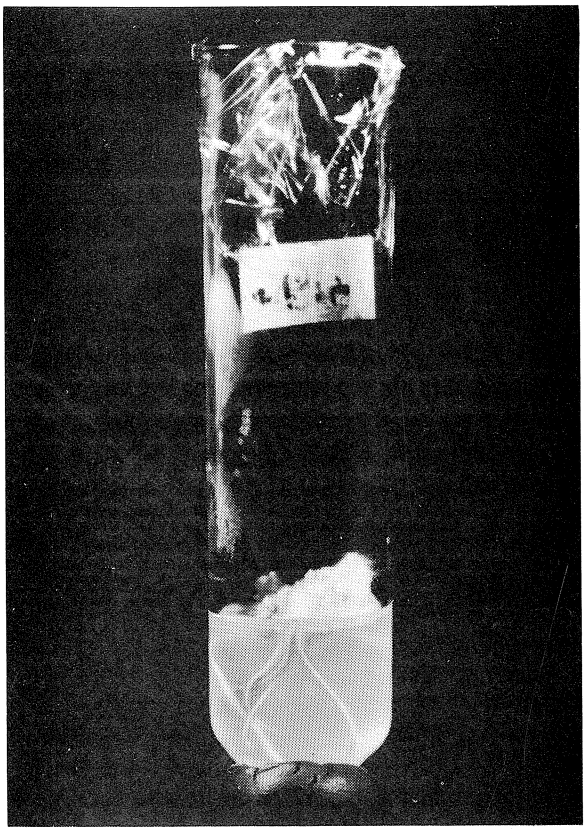

Fig. 1. Runa tuber callus showing regeneration of roots and buds.

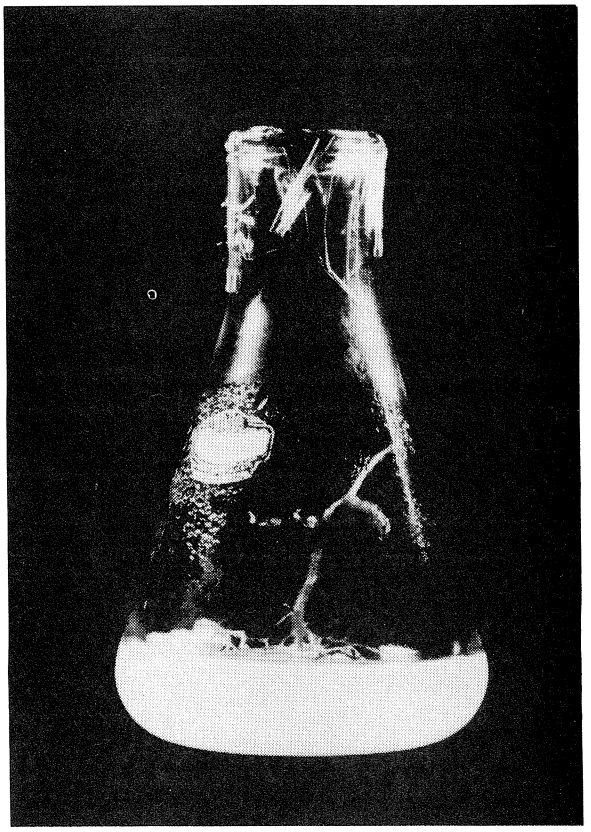

Fig. 2. Spontaneous tuberization of stolons regenerated from tuber callus.

length were obtained after 10 days, thus supporting data for potato (14) and oca (13) cultures.

b. Leaf callus

NAA at 2.0 mg/l was the best type and concentration of auxin (Table 1), as had been the case with tuber tissues. This results contrasts previous reports where both *S. tuberosum* L. spp. *tuberosum* and *S. acaule* leaf callus were obtained on media with 2.4-D (3, 17). The best responses were detected on BM with MS salts and 0.5 mg/l Kin, supporting data by Meins and Lutz (8), who found cloned lines of leaf, stem-cortex and pith tissues of tobacco to exhibit different cytokinin requirements. The cultures grew best under dark conditions, a result similar to those of Gavinlertvatana and Li (3).

In contrast to our results with tuber tissues, neither of the media tested for callus induction and proliferation permitted any differentiation after three subcultures. Therefore, as alternative media, the BM was supplemented with 0.0, 0.1, 0.5 or 1.0 mg/l NAA combined with 0.5, 1.0 or 2.0 mg/l Kin.

After 30 days culture under dark conditions on the BM without auxins and with 0.5 mg/l Kin added, differentiation of many roots and meristematic nodules was detected.

Further increases in the concentration of Kin up to 2.0 mg/l resulted only in tissue browning without any growth of the differentiated structures. However, when media were supplemented with 5.0 mg/l Kin and cultures transferred from dark to high light intensity conditions, plants were successfully regenerated. The effects of light intensity on organogenesis were also described elsewhere (17).

The media and culture conditions described for tuber regenerants proved suitable for obtaining normal plants from leaf calli.

c. Stem-pith callus

Differential requirements among tissue sources were apparent after 30 days in culture (Table 2). The best BM for callus induction and proliferation from Runa stem-pith explants contained MS formula

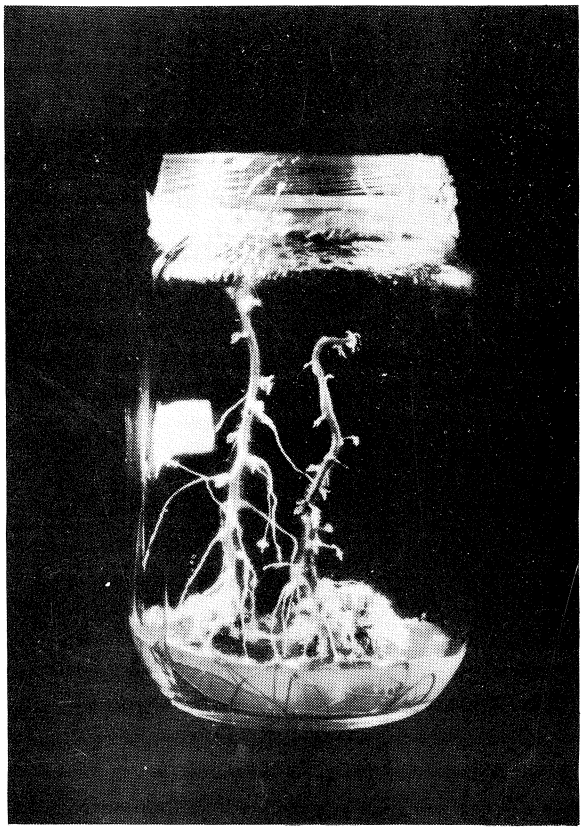

Fig. 3. Phenotypic appearance of a plant regenerated from tuber callus on a medium with Murashige and Skoog salts, Nitsch organics, and (mg/l) = 0.1 IBA; 1.0 BAP and 0.1 GA_3.

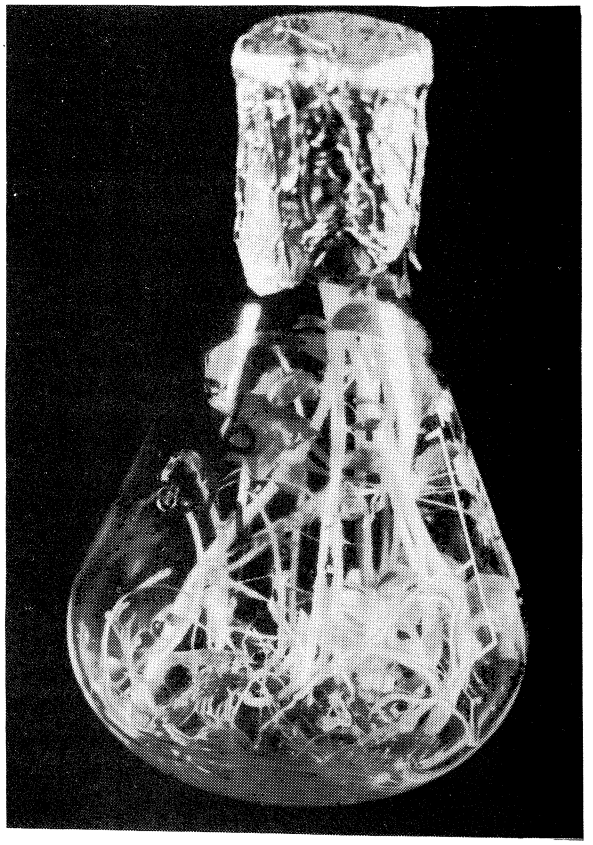

Fig. 4. Node plants regenerated from stem-pith callus on a liquid Murashige and Skoog medium with 0.5 mg/l GA_3, after 10 days in shaken culture.

Table 1. *Solanum tuberosum* **L. spp** *andigena* **c.v. "Runa" leaf callus proliferation on different culture media.**

Type	Auxins concentration (mg/l)	Cytokinins (0.5 mg/l) and mineral salts*			
		BAP		Kin	
		M	MS	M	MS
NAA	1.0	1**	2	1	2
	2.0	3	3	3	4
	5.0	1	0	0	0
IBA	1.0	0	1	0	1
	2.0	1	1	1	2
	5.0	1	2	1	2
IAA	1.0	0	1	0	1
	2.0	1	2	2	3
	5.0	1	1	1	1
2.4-D	1.0	0	1	0	1
	2.0	2	2	2	3
	5.0	0	1	1	2

* MS: Murashige and Skoog; M; Miller.
** The growth was assessed from 0 (no growth) to 4 (massive growth).

Table 2. Runa stem-pith callus proliferation on media containing Murashige and Skoog (MS) or Miller (M) mineral salts supplemented with 2.0 mg/l either auxin and 0.5 mg/l cytokinin.

Auxins	Cytokinin			
	BAP		Kin	
	M	MS	M	MS
NAA	0*	1	1	2
IBA	0	1	1	2
IAA	2	2	3	4
2 4 D	1	1	1	2

* Growth was assessed from 0 (no growth) to 4 (massive growth)

supplemented with IAA and Kin. When Miller salts and any auxin other than IAA were tested, results ranged from localized to zero callus formation, thus contrasting data by Wang and Huang (17). The best culture condition for stem-pith tissues was a low light intensity, with dark as the worst; high light intensity gave an intermediate response on all the hormonal combinations tested. After several subcultures on this medium, roots and meristematic nodules spontaneously differentiated from the callus tissue.

Both regeneration and normality were obtained on the media and conditions described for tuber and leaf cultures, i.e. MS salts with supplements of (mg/l) 0.1 IBA, 1.0 BAP and 0.1 GA_3 as the only hormone in shaken culture (Fig. 4). Additionally, as was the case with leaf cultures, an increase in light intensity (transfer of cultures from low to high light intensity) promoted organogenesis.

To date, no phenotypic variations were detected in the regenerated plants after transfer to soil, irrespective of the tissue source. Nevertheless, experiments are under way to determine if any genetic alteration occurred.

Summary

The differences in nutritional and hormonal requirements both for callus induction and proliferation, and for differentiation and regeneration of complete *Solanum tuberosum* L. spp. *andigena* cv "Runa" plants from tuber, leaf and stem-pith tissues are discussed. Differences in light intensity requirements for the three tissue sources were also detected. Whereas tuber tissues needed a high light intensity, leaf tissues grew best in the dark, and stem-pith tissues liked with low light intensity. NAA (1-naphthalene acetic acid) at 2.0 mg/l proved best for tuber and leaf tissues, while IAA 4-(indole-3yl) acetic acid yielded better results with stem-pith cultures. Tuber calluses needed BAP (6-benzylaminopurine), whereas the other two sources grew better with Kin (6-furfuryl aminopurine). A two-stepped sequence on Murashige and Skoog's formula (MS) + 0.1 mg/l IBA 4-(indole-3yl) butyric acid + 1.0 mg/l BAP + 0.1 mg/l GA_3 (gibberellic acid), followed by MS + 0.5 mg/l GA_3 in liquid shaken culture yielded the best regeneration responses, regardless of the tissue source.

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