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Short communication

In vitro organogenesis and plant regeneration from leaves of *Solanum candidum* Lindl., *S. quitoense* Lam. (naranjilla) and *S. sessiliflorum* Dunal

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Abstract. Adventitious shoots and roots were regenerated from leaf segments of 3 *Solanum* species: *S. candidum* Lindl., *S. quitoense* Lam. and *S. sessiliflorum* Dunal. Leaf explants differentiated shoots on modified MS medium supplemented with 23–163 μM kinetin and 0–5.7 μM indoleacetic acid (IAA). Excised shoots were induced to form roots by transfer to media with benzyladenine (BA) and naphthaleneacetic acid (NAA) at 0.09 and 0.11 μM respectively for *S. quitoense* and 0.01 μM NAA for *S. candidum* and *S. sessiliflorum*. Adventitious roots were produced directly from leaf explants with 0–140 μM kinetin and 0–5.7 μM IAA in combination. Rooted plants were successfully established in the greenhouse.

Introduction

The naranjilla (*Solanum quitoense* Lam.) is a large, lignescent perennial that is cultivated for the juice of its fruit in Andean South America and in some Central American countries [13]. Its cultivation is severely limited by climatic requirements and susceptibility to diseases and pests, particularly the rootknot nematode [6]. Morphological variation in this species is essentially limited to the degree of occurrence of stem and leaf spines [12]. Whalen and Caruso [12] demonstrated that there was virtually no variation for 6 different isozymes in the species after examining 13 *S. quitoense* accessions from widely separated locations in Ecuador and Colombia. They suggested that the genetic base of naranjilla may be very narrow.

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Solanum sessiliflorum Dunal is a related species that is cultivated in northern South America and to a lesser extent in Central America [13]. Its fruit is used for cooking and for juice. It is grown at lower elevations and is tolerant of higher light intensity than the naranjilla. *Solanum candidum* Lindl. is a wild species with a distribution from Mexico through Central and South America. Because its fruit is covered with persistent hairs, it is not cultivated, but is important because it is the species most closely related to the naranjilla [7, 12, 13] and has a wide tolerance of elevation, temperature and soil types. Neither *S. candidum* nor *S. sessiliflorum* hybridizes readily with naranjilla [7]; therefore, conventional breeding strategies involving the transfer of disease and pest resistance or extending naranjilla cultivation to the lowland tropics have been mostly unsuccessful. This study was undertaken to describe in vitro techniques that could be used for the production of somaclonal variants and perhaps for regeneration of plants from transformed leaves and leaf protoplasts.

Materials and methods

Seeds of *S. candidum* were collected from fruit obtained at the Preston B. Bird and Mary Heinlein Fruit and Spice Park in Homestead, Florida. Seeds of *S. quitoense* were obtained from fruit in a public market in Cali, Colombia. *Solanum sessiliflorum* seeds were provided by the Centro Agronomica Tropical de Investigacion y Ensenanza (C.A.T.I.E.), Turrialba, Costa Rica. All seeds were germinated and grown in a commercial potting mixture and plants were maintained in a greenhouse that provided 50% shade. Ambient temperature was 10–30 °C.

In vitro-grown stock cultures of all 3 species were initiated from excised shoot tips from greenhouse plants placed on sterile culture medium contained in 100 × 80 mm pyrex storage dishes (1–4 explants/dish). Shoot tips were surface-sterilized with 20% (v/v) commercial bleach that contained 2–3 drops of Tween 80 per 100 ml followed by 5 successive rinses with sterile distilled water. Culture media consisted of Murashige and Skoog medium [10] modified by the addition of 30 g l^{-1} sucrose, 7 g l^{-1} Difco Bacto agar, and various combinations of BA (0–0.89 μM) and NAA (0–1.1 μM). The media were adjusted to pH 5.7 prior to autoclaving for 15 minutes at 1.1 kg cm^{-1} and 120 °C. Shoot tips and axillary buds were subcultured every 4–6 weeks.

Expanded leaves from in vitro-grown plants were used as explants for organogenesis experiments. Leaf segments (1 cm^2), including the midrib and sometimes part of the petiole, were placed adaxial side down on solidified

media in 100 × 20 mm petri dishes (1–5 explants/dish) or in 150 × 25 mm test tubes (1 explant/tube). The culture medium used was that described above except that various concentrations of kinetin (0, 23, 46, 93, 116, 140 and 163 μM) and IAA (0, 0.06, 0.57 and 5.7 μM) were substituted for BA and NAA. (The IAA was filter-sterilized and added after the media were autoclaved.) Filter-sterilized gibberellic acid (GA3), at 1.45 μM, was also added to some media after autoclaving. There were 3–9 replicates for each treatment. Adventitious shoots were subcultured onto stock culture media for root formation.

All cultures were incubated in a growth chamber at 21.5 °C with a 15 hr photoperiod provided by Agro Lite fluorescent tubes (50 μmol S^{-1} m^{-2}). Selected rooted plantlets were transferred to commercial potting mixture and hardened off under inverted plastic cups in a greenhouse for 10–14 days.

Cultured leaf pieces were fixed in Belling's modified Navashin fluid [8]. Material was washed overnight in water, dehydrated in a tertiary butanol series, and embedded in Tissue-prep (Fisher Scientific). Sections were cut at 10 μM on an American Optical 820 rotary microtome, stained in safranin and fast green [1] and mounted in Permount (Fisher Scientific).

Results

Shoot tip and axillary bud explants of the 3 *Solanum* species developed into rooted plants on basal medium or on media containing low concentrations of BA and/or NAA (Table 1). *Solanum quitoense* was routinely cultured on medium containing the optimum concentrations of 0.09 μM BA and 0.11 μM NAA, whereas *S. candidum* and *S. sessiliflorum* responded best on medium with 0–0.01 μM NAA alone. Plantlets grew to culture vessel capacity within 4–6 weeks. Axillary bud proliferation was not observed.

Cultured leaf explants from all 3 *Solanum* species formed small amounts of compact callus at cut edges, particularly near the midrib or major veins, but also from laminae. Distinctive green nodules developed from the callus (Fig. 1) which became fully differentiated as adventitious meristems (Fig. 2) 4–6 weeks after the cultures had been initiated. Adventitious meristems formed from leaf explants (Fig. 3) of all 3 *Solanum* species on media containing 46–163 μM kinetin alone or in combination with 0–5.7 μM IAA (Table 2). Meristem differentiation from leaves of *S. candidum* occurred over the widest range of phytohormone formulations. Regeneration from *S. quitoense* leaves was less dependent on high concentrations of kinetin. The efficiency of meristem regeneration from leaf callus was species-dependent. There was a higher frequency of regeneration from *S. candidum* explants in

Table 1. In vitro responses of shoot topis and axillary buds of *S. candidum*, *Solanum quitoense* and *S. sessiliforum*[a]

Species	BA (μM)	NAA (μM)			
		0	0.01	0.11	1.1
S. candidum	0	P	P	P	CX
	0.01	P	P	CX	CX
	0.09	P	P	CX	CX
	0.89	CX	CX	CX	CX
S. quitoense	0	P	P	P	CR
	0.01	P	P	P	CR
	0.09	P	P	P	CR
	0.89	X	X	X	CR
S. sessiliflorum	0	P	P	P	C
	0.01	P	P	P	C
	0.09	P	P	X	C
	0.89	X	X	X	CX

[a] Based on a minimum of 5 explants/treatment
C = Callus
R = Adventitous roots
P = Rooted, growing plantlets
X = Abnormal development (stunted growth, poor rooting)

comparison with the other 2 *Solanum* species. Leaf explants of *S. quitoense* were the least responsive.

When kinetin concentrations were below 116 μM, the adventitious buds on *S. quitoense* leaves failed to elongate and develop into plantlets. However, normally developed shoots were obtained if GA_3 (1.45 μM) was added to the organogenesis medium before inoculation of the explants. The other two *Solanum* species did not have this requirement for GA_3.

Excised shoots from cultured leaf explants of all 3 *Solanum* species rooted within 2–3 weeks on stock culture medium (Fig. 4). Rooted plantlets acclimated readily to greenhouse conditions (Fig. 5) after gradual hardening off.

Discussion

Previous studies have indicated that many *Solanum* species can be regenerated in vitro from various explants including leaves, stem pith, tubers, shoots and hypocotyls [5]. *Solanum* species that have been regenerated from leaves include *S. dulcamara* [14], *S. khasianum* [2], *S. laciniatum* [13], *S. nigrum* [2, 14] and *S. tuberosum* [11]. This study provides the first report of organogenesis from species in the Lasiocarpa section of *Solanum*. It confirms earlier observations with *S. laciniatum* and *S. khasianum* that shoot

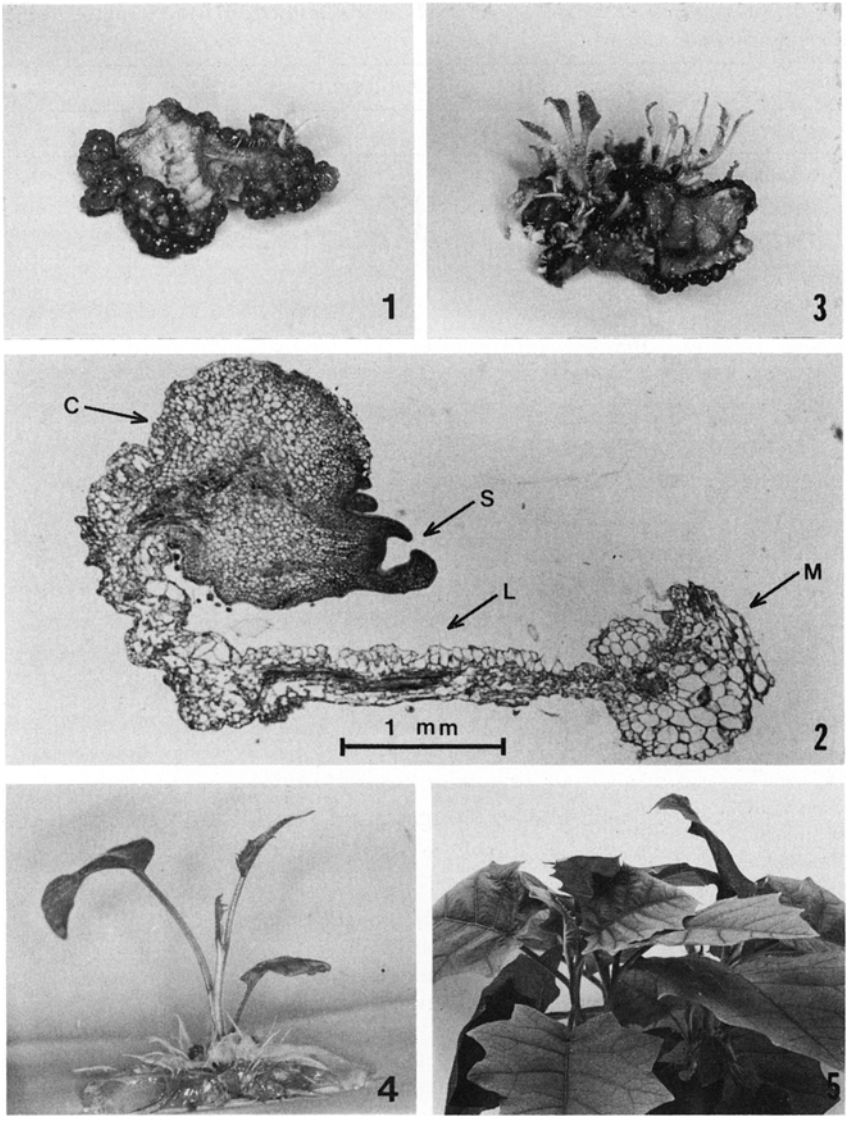

Fig. 1. Solanum quitoense leaf explant with nodular callus.

Fig. 2. Transverse section through *Solanum candidum* leaf explant showing midrib (M), lamina (L) nodular callus (C) and adventitous shoot (S).

Fig. 3. Solanum sessiliflorum leaf explant with adventitious shoots.

Fig. 4. Regenerated *Solanum quitoense* plantlet in vitro.

Fig. 5. Greenhouse-acclimated *Solanum quitoense* plant derived from cultured leaf tissue.

Table 2. Responses of leaf explants of 3 *Solanum* species to in vitro culture on media containing IAA and kinetin

Species	Kinetin (μM)	IAA (μM)			
		0	0.06	0.57	5.7
S. candidum[a]	0	0	0	R	R
	23	(R)	R	R	R
	46	(R)	(+)	+	+
	93	(+)	+++	+++	+++
	116	(+)	+++	+++	+++
	140	(+)	++	++	++
	163	(+)	+	+++	+++
S. quitoense[b]	0	(R)	R	R	R
	23	+R	+	+++	(+)R
	46	+	++	+R	++R
	93	0	+	+	+
	116	0	+	0	(+)
	140	(+)	+	+	0
	163	(+)	+	+	+
S. sessiliflorum[c]	0	0	R	R	R
	23	0	(+)	R	R
	46	+	+	+	R
	93	0	+++	++	R
	116	0	0	++	++R
	140	0	+	++	R
	163	0	+	+	+++

[a] 3–5 replicates/treatment
[b] 3–9 replicates/treatment
[c] 3–7 replicates/treatment
R = Adventitious roots
(+) = Abnormally formed or aborted adventitious shoots
+ = 50% of explants producing adventitious shoots
++ = 50–75% of explants producing adventitious shoots
+++ = 76–100% of explants producing adventitious shoots

regeneration is dependent on a high cytokinin:low auxin ratio [3, 9]. Regeneration can also occur with lower frequency in the absence of auxin, as was reported for *S. dulcamara* [2, 14] and *S. nigrum* [3, 14]. In particular, *Solanum candidum* and *S. sessiliflorum* required much higher cytokinin concentrations (46–143 μM) for shoot differentiation than has been reported elsewhere. The requirement by *S. quitoense* for GA_3 for internodal growth confirms similar GA_3-dependency reported by Kowalczyk et al. [9] with *S. khasianum* and by Roest and Bokelmann [11] with *S. tuberosum*.

The ability to regenerate plants from leaves of the naranjilla has interesting implications, because of the recalcitrance of this crop from a classical plant breeding perspective [7]. Passage of tissue through an in vitro cycle has

been demonstrated in many species to have a destabilizing effect, resulting in variant plant types [4]. The recovery of potentially useful somaclonal variants from naranjilla leaves could have a major impact on the direction of improvement for this crop. Moreover, it is probable that biotechnological approaches involving protoplast culture or transformation of cultured leaf segments with *Agrobacterium tumefaciens* as a vector will be facilitated.

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