IN VITRO REGENERATION OF SEVERAL ACCESSIONS OF *SOLANUM TOPIRO* HUMB & BONPL. AND *S. SESSILIFLORUM* DUN. (CÚBIO)[1]

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ABSTRACT - Leaf and hypocotyl explants of twelve accessions of *Solanum topiro* and one accession of *S. sessiliflorum* ("cúbio") were tested for their morphogenetic response by culturing them in Murashige & Skoog (1962) medium supplemented with combinations of naphthaleneacetic acid (NAA) and benzylaminopurine (BA). Hypocotyls were the best explants for shoot regeneration. The maximum number of shoots was obtained in a medium supplemented with $5x10^{-7}$M NAA and $1x10^{-6}$M BA.

Index terms: plant morphogenetic capacity, tissue culture, naphthaleneacetic acid, benzylaminopurine.

REGENERAÇÃO *IN VITRO* DE VÁRIOS ACESSOS DE *SOLANUM TOPIRO* HUMB. & BLONP. E *S. SESSILIFLORUM* DUN (CÚBIO)

RESUMO - Explantes de folha e hipocótilo de 12 acessos de *Solanum topiro* e um acesso de *S. sessiliflorum* (cúbio) foram testados quanto às suas respostas morfogenéticas, cultivando-as em meio de Murashige & Skoog (1962), adicionado com combinações de ácido naftalenoacético (NAA) e benzilaminopurina (BA). Os hipocótilos foram os melhores explantes para regeneração de brotos. O número máximo de brotos foi obtido no meio adicionado com $5x10^{-7}$M NAA e $1x10^{-6}$M BA.

Termos para indexação: capacidade morfogenética em planta, cultura de tecido, ácido naftaleno acético, benzilaminopurina.

INTRODUCTION

Efficient *in vitro* plant regeneration is the requisite for the improvement of crop plants through the use of somaclonal variation, somatic cell hybridization or transformation with exogenous DNA. Very promising techniques have been limited by the lack of suitable methods to regenerate whole plants from cultured cells.

Solanum topiro has great vegetative development, and an erect stem with strong leaves (Fig. 1). Its fruits, that can be used as food, vary in size, color and shape, suggesting a great genetic variability. *S. sessiliflorum* is essentially similar to *S. topiro* although having leaf spines (both are known as "cúbio"). Several varieties of these plants are being cultivated in the Amazonian region (Peru). They have susceptibility to diseases and pests (virus, fungi, nematodes, white flies and acarids). Preliminary observations suggest these plants to be resistant to *Pseudomonas solanacearum*. An initial evaluation of agronomic characteristics of several accessions of both species described by Leal & Cordeiro (1987) suggests that this material might be a promissing crop plant.

This paper describes our success in establishing growing calli, roots and shoots and regenerating full grown plants hypocotyl and leaves of different accessions of *S. topiro*, calli and roots with one accession of *S. sessiliflorum*.

MATERIALS AND METHODS

The *S. topiro* accessions used are: Acari, Andirá, Arara, Belém, Estirão, Eurunepé, Iquitos, Itapiram-

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pa, Letícia, Manicoré, Umariaçu, Yurimáguas and the *S. sessiliflorum* accession is San Fernández.

The seeds were germinated asseptically to establish a source of seedling explants. The seeds were immersed for ten minutes in a solution containing 2% sodium hypochlorite. They were rinsed three times with autoclaved distilled water and transferred to germination flasks. These flasks were provided with a cotton platform. Half strength Murashige & Skoog salts were poured into each flask, which were capped with aluminium foil and autoclaved for 15 minutes. Germination was allowed to occur under 13-hour daily exposure to 1000 lux illumination and a constant 25°C. Explants from young actively growing plants nearly 10 cm high were used. Leaves were cut in approximately 5 mm squares and hypocotyls at nearly 5 mm length. Six explants were cultured per bottle and a minimum of 18 explants were used to test each medium. The cultures were incubated at the same conditions for seed germination. The nutrient basal medium was composed of MS salts (Murashige & Skoog 1962) 3% sucrose and in mg/l: myoinositol, 100; thiamine HCl, 1,0; pyridoxine HCl, 0,5; nicotinic acid, 0,5; glicine, 2,0 and agar 8.000. This medium was supplemented with α-naphthaleneacetic acid (NAA) and 6-benzylamino purine (BA). A total of four hormonal combinations have been tested for each cultivar: the concentrations were $5x10^{-7}$M NAA + $1x10^{-6}$M BA (M1), $5x10^{-6}$M NAA + $1x10^{-6}$M BA (M2), $5x10^{-7}$M NAA + $1x10^{-5}$M BA (M3), and $5x10^{-6}$ NAA + $1x10^{-5}$M BA (M4).

Evaluation of cultures were made at the 10th, 20th and 35th days. Tables 1 to 3 show the mean morphogenetic response and was based on relative growth of calli and/or roots per total number of explant used in each experiment. As the shoots appeared individually on each explant, total number per treatment was scored. After evaluation, some shoots were rooted in MS medium with 0,1 mg/l of indole-3-acetic acid (IAA) (Fig. 2). Some shoots were placed in MS medium supplemented with 1,5 mg/l of kinetine (KIN) for shoot proliferation. Plantlets were potted in vials and maintained in greenhouse.

RESULTS AND DISCUSSION

The morphogenetic response of hypocotyl and leaf explants of cúbio to various concentrations of NAA and BA added to MS medium is summarized in Tables 1 to 3. Although individual accessions showed marked differences in response, there were many overall similarities in the morphogenetic patterns observed in both species.

Callus cultures were established in each strain with reasonable efficiency and good

FIG. 1. Plant of *S. topiro* growing in greenhouse.

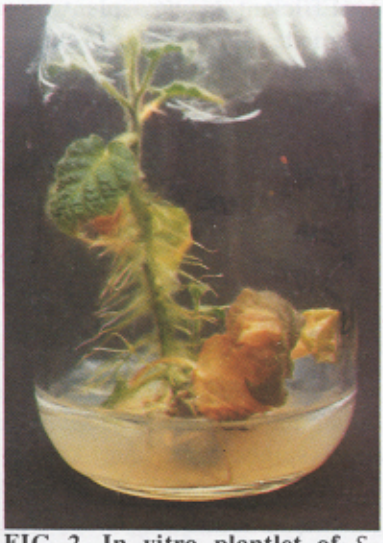

FIG. 2. In vitro plantlet of *S. topiro* with nearly two months, in MS + 0,1 mg/l IAA.

TABLE 1. Effects of NAA concentration in combination with BA on morphogenetic response of hypocotyls of *S. topiro*.

Medium / Strain		5×10^{-7}M 1×10^{-6}M	M1 NAA BA	5×10^{-6}M 1×10^{-6}M	M2 NAA BA	5×10^{-7}M 1×10^{-5}M	M3 NAA BA	5×10^{-6}M 1×10^{-5}M	M4 NAA BA
Acari	C	(18)	$0{,}28^{++}$	(18)	$0{,}67^{++}$	(21)	$1{,}00^{+++}$	(20)	$0{,}50^{++}$
	R		$0{,}17^{++}$		$0{,}22^{++}$		$0{,}43^{++}$		$0{,}30^{+}$
	S		0,56		0,00		0,29		0,15
Andirá	C	(37)	$1{,}00^{++}$	(18)	$0{,}89^{++}$	(19)	$1{,}00^{++}$	(32)	$0{,}94^{++}$
	R		$0{,}41^{++}$		$0{,}56^{++}$		$0{,}68^{++}$		0,00
	S		0,16		0,00		0,37		0,00
Arara	C	(18)	$0{,}78^{+++}$			(18)	$0{,}50^{+++}$	(18)	$0{,}56^{++}$
	R		$0{,}72^{+++}$				0,00		0,00
	S		0,11				0,11		0,00
Belém	C	(18)	$1{,}00^{+++}$	(18)	$1{,}00^{+++}$	(18)	$0{,}83^{++}$	(20)	$0{,}85^{+++}$
	R		$1{,}00^{+++}$		$1{,}00^{+++}$		$0{,}28^{+}$		0,00
	S		0,06		0,00		0,00		0,00
Estirão	C	(18)	$0{,}67^{++}$	(37)	$0{,}78^{++}$	(29)	$0{,}69^{++}$	(28)	$0{,}64^{+++}$
	R		$0{,}67^{+++}$		$0{,}24^{++}$		$0{,}28^{++}$		$0{,}18^{+}$
	S		0,17		0,05		0,10		0,11
Eurunepé	C	(24)	$0{,}83^{+++}$	(18)	$0{,}89^{++}$	(18)	$0{,}78^{++}$	(30)	$0{,}93^{++}$
	R		$0{,}29^{++}$		0,00		$0{,}89^{++}$		0,00
	S		0,04		0,00		0,33		0,00
Iquitos	C	(18)	$0{,}83^{++}$	(18)	$0{,}89^{+++}$	(19)	$0{,}79^{+++}$	(18)	$0{,}89^{++}$
	R		$0{,}39^{+++}$		0,00		$0{,}16^{++}$		0,00
	S		0,11		0,11		0,21		0,00
Itapirampa	C							(104)	$0{,}69^{++}$
	R								$0{,}04^{+}$
	S								0,00
Manicoré	C	(18)	$0{,}50^{++}$	(18)	$0{,}89^{++}$	(30)	$0{,}83^{++}$	(18)	$0{,}89^{++}$
	R		$0{,}28^{++}$		0,00		$0{,}07^{+}$		0,00
	S		0,00		0,00		0,03		0,00
Umariaçu	C	(50)	$0{,}50^{++}$	(27)	$0{,}22^{++}$	(26)	$0{,}35^{+}$	(18)	$0{,}78^{++}$
	R		$0{,}26^{++}$		$0{,}26^{++}$		$0{,}08^{++}$		$0{,}17^{+}$
	S		0,22		0,33		0,19		0,11
Yurimáguas	C	(29)	$0{,}76^{+++}$	(18)	$0{,}67^{+++}$	(18)	$0{,}83^{++}$	(24)	$0{,}63^{+++}$
	R		$0{,}66^{+++}$		0,00		0,00		$0{,}04^{+}$
	S		0,07		0,00		0,00		0,00

C^{+}, C^{++}, C^{+++} = percentage of calli relative growth.
R^{+}, R^{++}, R^{+++} = percentage of roots relative growth.
S = percentage of shoot formations
( ) = number of explants.
Obs.: percentage in decimal base (total = 1,00).

degree of reliability. Other workers have shown a pronounced effect of explants source on initiation of calli cultures in Solanaceae species (Alicchio et al. 1982, Locy 1983, Tewes et al. 1984). Freshly excised explants of cúbio growing rapidly regenerated callus within two weeks on culture media. Explants showing callus proliferation were characteristically spongy and ranged from pale white to green in color. Sometimes, especially leaf explants, turned dark and died. With hypocotyls translucid calli were noted in some cultures. Hypocotyls also showed the highest calli growth in M1 and M2 media. (Table 1).

TABLE 2. Effects of NAA concentration, in combination with BA on morphogenetic response of leaf discs of *S. topiro*.

Medium / Strain		5×10^{-7}M 1×10^{-6}M	M1 NAA BA	5×10^{-6}M 1×10^{-6}M	M2 NAA BA	5×10^{-7}M 1×10^{-5}M	M3 NAA BA	5×10^{-6}M 1×10^{-5}M	M4 NAA BA
	C			(19)	$0{,}63^{+}$	(19)	$0{,}84^{+}$	(18)	$0{,}17^{+}$
Acari	R				$0{,}95^{+++}$		$0{,}05^{++}$		$0{,}78^{+++}$
	C	(23)	$1{,}00^{+}$	(19)	$0{,}58^{++}$	(18)	$1{,}00^{+}$	(18)	$0{,}78^{++}$
Andirá	R		$1{,}00^{+++}$		$0{,}63^{++}$		$0{,}89^{++}$		$0{,}33^{++}$
	S		0,00		0,00		0,06		0,00
	C	(18)	$1{,}00^{++}$					(18)	$0{,}83^{+++}$
Arara	R		$1{,}00^{+++}$						$0{,}56^{++}$
	C	(18)		(18)	$1{,}00^{+++}$	(18)	$0{,}89^{+}$	(20)	$0{,}65^{+++}$
Belém	R		$1{,}00^{+++}$		$1{,}00^{+++}$		$0{,}22^{+}$		$0{,}60^{++}$
	C	(18)	$1{,}00^{+}$	(18)	$0{,}83^{+++}$	(19)	$0{,}63^{+++}$	(22)	$0{,}77^{++}$
Estirão	R		$1{,}00^{+++}$		$0{,}89^{++}$		$0{,}32^{++}$		$0{,}55^{++}$
	S		0,00		0,06		0,00		0,05
	C	(18)	$0{,}44^{+++}$	(18)	$0{,}83^{+++}$	(18)	$0{,}67^{++}$	(24)	$0{,}92^{+++}$
Eurunepé	R		$0{,}61^{+++}$		$0{,}22^{+++}$		$0{,}94^{+++}$		$0{,}38^{++}$
	C	(18)	$0{,}83^{++}$	(18)	$0{,}89^{+++}$	(22)	$0{,}73^{+++}$	(24)	$0{,}71^{+++}$
Iquitos	R		$0{,}94^{+++}$		$0{,}94^{+++}$		$0{,}95^{+++}$		$0{,}64^{++}$
	C	(35)	$0{,}23^{+}$						
Letícia	R								
	C	(21)	$0{,}29^{+}$	(18)	$0{,}72^{++}$	(22)	$0{,}32^{+}$	(18)	$0{,}56^{++}$
Manicoré	R		$0{,}67^{+++}$		$0{,}78^{+++}$		$0{,}45^{++}$		$1{,}00^{+++}$
	C	(37)	$0{,}11^{+}$	(25)	$0{,}12^{+}$	(24)	$0{,}17^{+}$	(24)	$0{,}83^{++}$
Umariaçu	R		$0{,}46^{+++}$						$0{,}71^{+++}$
	C	(42)	$0{,}43^{+}$	(19)	$0{,}89^{+++}$	(23)	$0{,}52^{+}$	(18)	$0{,}67^{+++}$
Yurimáguas	R		$0{,}62^{+++}$		$1{,}00^{+++}$		$0{,}35^{+++}$		$0{,}44^{+}$
	S		0,05		0,00		0,00		0,00

C^{+}, C^{++}, C^{+++} = percentage of calli relative growth.
R^{+}, R^{++}, R^{+++} = percentage of roots relative growth.
S = percentage of shoot formations
( ) = number of explants.
Obs.: percentage in decimal base (total = 1,00).

The experiments suggest that plant leaf discs and hypocotyls possess a total dependence on external stimuli such as organic and inorganic nutrients, auxins and cytokinins for shoot and root formation. Bisides this, distinct differences in *in vitro* response were observed between explants from the two different organs tested. This was also observed in other wild Solanum species (Gleddie et al. 1985 and Mattos & Cordeiro 1989). For example, when cúbio young leaves discs are cultured with NAA, their rooting capacity is similar in two different combinations despite the concentration of BA. However, with hypocotyl it was observed that more roots were produced when the medium was lower in BA and root growth was inhibited in the M4 (Table 1).

The data also show that lower concentrations of NAA (M1 and M3) favored the induction of leaflets which formed within 20 and 30 days from hypocotyl explants. It was observed that the calli size was not positively associated with the production of shoots, as can be observed in M1, where Acari hypocotyl explants produced small calli and many shoots (10/18) and Eurunepé showed big calli and only one shoot out of 24 explants.

Leaf explants showed low shoot formation irrespectively of the media used, and in M1 some leaves increased much in size. Complete organogenesis has been obtained by Hendrix et al. (1987) using 0-0,89 μM BA and 0-1.1 μM NAA with *S. sessiliflorum*. That study, done with *S. candidum* Lindl., *S. quitoense* Lam. and *S. sessiliflorum* Dun. also showed a better production of shoots with leaf explants using various concentrations of kinetin and indoleacetic acid, than in the present work.

The failure of the authors of the present work to regenerate plants from *S. sessiliflorum* leave explants might be due to differences in the growth factors balance or to accession origin, or both.

One hundred and three plantlets were obtained from 1.056 hypocotyl explants and five plantlets from 879 leaf explants. Regenerated shoots were cut off and transferred on rooting medium (MS + 0,1 mg/l IAA). Roots were formed after 10-12 days of culture. Shoot tips and axillary buds subcultured in MS + 1,5 mg/l KIN grew into whole plantlets in two months.

CONCLUSIONS

1. The regenerated plants grown in greenhouse showed a normal morphology, and no difference (somaclonal variation) from

TABLE 3. Effects of NAA concentration, in combination with BA on morphogenetic response of hypocotyls and leaf discs of *S. sessiliflorum*.

Medium / Strain		5×10^{-7}M 1×10^{-6}M	M1 NAA BA	5×10^{-6}M 1×10^{-6}M	M2 NAA BA	5×10^{-7}M 1×10^{-5}M	M3 NAA BA	5×10^{-6}M 1×10^{-5}M	M4 NAA BA
Hypocotyls	C	(18)	$1,00^{+++}$	(18)	$1,00^{+++}$	(18)	$0,61^{+++}$	(18)	$0,89^{++}$
	R		$1,00^{++}$				$0,33^{+}$		$0,22^{+}$
Leaf discs	C	(18)	$1,00^{+++}$	(18)	$1,00^{+++}$	(18)	$1,00^{+}$	(18)	$0,78^{++}$
	R		$1,00^{+++}$		$1,00^{+}$		$1,00^{++}$		$1,00^{++}$

C^{+}, C^{++}, C^{+++} = percentage of calli relative growth.
R^{+}, R^{++}, R^{+++} = percentage of roots relative growth.
S = percentage of shoot formations
( ) = number of explants.
Obs.: percentage in decimal base (total = 1,00).

plants grown directly from seedling explants (control) was observed up to the present time. Some of them developed into flowering plants but no fruits have set. This is due to the narrow climatic tolerance of cúbio, which produced no fruits in an extensive culture by seeds in a field near Rio. They are being used as sources to provide material for further *in vitro* regenerations aiming at producing somaclonal variation.

2. Due to relative ease in obtaining plant regeneration, *S. topiro* appears to be suitable for protoplast isolation and regeneration.

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