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Organ Regeneration in Cultures of Vegetative Explants of an Antidiabetic Species *Scoparia dulcis* (Scrophulariaceae)

SANTOSH GARG and N S RANGASWAMY
Department of Botany, University of Delhi, Delhi 110007

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Vegetative explants obtained from aseptically grown plants of the so-claimed antidiabetic species *Scoparia dulcis* (Scrophulariaceae) were cultured on a modified Nitsch's medium. The effects of casein hydrolysate, 2,4-D, and NAA were studied on the regeneration ability of the explants. All explants differentiated roots, or shoot buds, or both roots and shoot buds/leafy shoots. The hypocotyl showed maximal and the apical leaf segment minimal regenerative ability. On 4 out of the 10 media tested, the leaf segments and hypocotyl produced a slight callus. Embryoid differentiation was never expressed by any of the explants in any of the treatments. The results are discussed with reference to *in vitro* studies on Scrophulariaceae. The importance of organ regeneration without the intervention of a callus in studies on propagation through tissue culture is emphasized.

Key Words: *Scoparia*, **Antidiabetic species, Vegetative explants, Regeneration *in vitro***

Introduction

Fresh foliage of *Scoparia dulcis* (Scrophulariaceae), a South-American weed naturalized in India, has been claimed to contain an antidiabetic principle named 'amellin'; unlike insulin, amellin is reported not to cause blood sugar drop below the normal level (Nath 1943, Nath & Chowdhury 1945, Nath et al. 1945, Mukerji 1957) and, of the 16 taxa of Scrophulariaceae on which organ and tissue culture studies have been conducted so far, somatic embryony has been induced only in two taxa. Owing to the claimed medicinal value and the negligible incidence of somatic embryony in Scrophulariaceae, a study was undertaken to determine whether or not *S. dulcis* is amenable for organ regeneration *in vitro*, and whether or not somatic embryony can be induced in cultures of vegetative explants of this species.

Material and Methods

Seeds of *Scoparia dulcis* Linn. were obtained from Tiruchirapalli, Tamil Nadu and stored up to 9 months in screwcap glass vials in laboratory cupboard. The stored seeds were treated with nascent chlorine water

for 20 min, washed thrice with sterile water, and sown l-seed-thick on 0.8% sterilised agar medium in culture tubes. From 7-day-old cultures many seedlings bearing the cotyledonary node were transferred to Nitsch's medium modified by Vatsala, BM for short (Vatsala 1976). In 10 days from transfer the seedlings produced 1 or 2 plumular leaves. From the plants grown *in vitro*, aseptic cultures of 13 types of explants were raised on BM, and BM supplemented with casein hydrolysate (CH), or 2, 4–D, or NAA (table 1). Before autoclaving, the *p*H of all media was adjusted to 5.8. Each culture received one explant only. Whole leaf and leaf segments were implanted with their abaxial surface in contact with the agarified culture medium; shoot system and root system were implanted with their basal end in the culture medium. The remaining types of explants were implanted horizontally on the medium. All cultures were maintained at 25±2°C under continuous light provided by cool-white fluorescent tubes (ca 2500 lux).

Results

Culture of Leaves and Leaf Segments

On BM entire leaves excised from 17-day-old seedlings produced a root each, while on BM+CH 500 ppm they produced either only roots or both roots and shoot buds from the petiolar region (figure 1A, table 2). In neither medium there was any callusing nor differentiation of embryoids.

To study the effect of age of the leaf on its organ regenerative ability, leaves excised from 104-day-old saplings were cultured on BM, and BM+CH 500 ppm. In 1 week from culture up to 40% cultures differentiated one root a leaf but no shoot buds. The percentage response increased with increasing age of cultures, but not beyond 3 weeks (table 2). The root invariably differentiated from the petiolar region of leaf.

In 4 of the 10 treatments (i.e. BM, BM+CH 500 or 600 ppm and BM+NAA 10^{-7}M) the apical segment of leaf invariably necrosed. On BM+2,4-D 10^{-5}M the leaf segment (inclusive of leaf apex) produced a profuse nodular pale-yellow callus (figure 1B). In 8-week-old callus cultures transferred to 2,4-D-free medium (i.e. BM), numerous roots (too copious to make an accurate count) differentiated within a week from transfer. By the third week from transfer of callus, shoot buds also differentiated, and by the end of seven weeks each culture bore 3 or 4 shoot buds. Nevertheless, no embryoids were formed in any of the cultures.

On the remaining five media, namely BM+CH 400 ppm, BM+2,4-D 10^{-7}M or 10^{-6}M, and BM+NAA 10^{-6}M or 10^{-5}M, the apical segment of leaf issued 1-10 roots usually near the cut edge, in 3–5 weeks from culture; the leaf apex lay quiescent. The percentage of responsive cultures in the five media was 25, 25, 50, 100 and 100, respectively. The roots originated from the surface of the leaf segment in contact with the agarified medium (BM+CH 400 ppm, BM+2,4-D 10^{-7}M or 10^{-6}M; figure 1G), or from both surfaces of the explant (BM+NAA 10^{-6}M or 10^{-5}M). On the two NAA-supplemented media, root morphogenesis was different. On BM+NAA 10^{-6}M, of the 10 roots produced, up to 7 were short and robust, and the remaining thin and elongate (figure 1C). On BM+NAA 10^{-5}M, not only the roots were shorter and more robust but also they differentiated intra-marginally on both surfaces of the lamina. During the fifth week the remnant of lamina started callusing, and by the seventh week a slight friable callus was formed. Irrespective of their length and site of origin, all the roots grew geotropically.

Rhizogenic cultures were transferred from NAA-supplemented media to NAA-free medium (BM). By 1 week from transfer the robust roots produced secondary roots and

Table 1 *In vitro culture of vegetative explants*

Explant	Source of explant	Culture medium	No. of cultures raised
Whole leaf (length 4–6 mm)	Seedlings formed in 17-day-old seed cultures on BM	BM	30
		BM + CH 500 ppm	30
Apical transverse-half, Basal transverse-half (from 1st pair of plumular leaves)		BM	12
		BM + CH 400, 500, 600 ppm, or 2, 4-D or NAA 10^{-7}, 10^{-6}, 10^{-5}M	12 each
Hypocotyl		BM	24
		BM + CH 400, 500, 600 ppm, or 2, 4-D or NAA 10^{-7}, 10^{-6}, 10^{-5}M	23 or 12 cultures each
Whole root system	42-to 49-day-old seedlings raised on BM	BM	20
Root tip segment (2–3 mm long)		BM	15
Middle segment of root (2–3 mm long)		BM	15
Basal segment of root (2-3 mm long, i.e. stub of hypocotyl + 1–2 mm basal region of primary root + basal regions of 1st, 2nd or 3rd secondary roots at the level of primary root)		BM	15
Whole shoot system		BM	20
Nodal segment (2–3 mm long) comprising stubs of petioles of both leaves + a portion of each sandwitching internode (from nodes/internodes 1, 2 from base of stem)		BM	30
Internodal segment (2–3 mm long)		BM	30
Shoot bud (i.e. uppermost visible pair of leaves + a part of subjacent internode)		BM	15
Whole leaf (length 4–6 mm)		BM	30
		BM + CH 500 ppm	30
Hypocotyl segment (i.e. middle 2–3 mm portion of 4–5 mm long hypocotyl)	104-day-old saplings raised on BM	BM	12
		BM + CH 500 ppm or 2, 4-D or NAA 10^{-7}M	12 cultures each

Table 2 *Organ differentiation in whole leaf cultures*[a]

Source material	Medium, and No. of cultures observed	Culture period (weeks) 1[b]	2[b]	3		4		5	
		R	R	R	R + S	R	R + S	R	R + S
17-day-old seedlings	BM (control), 29	3.4	38.0	55.1	0	55.1	0	Cultures not observed	
	BM+CH 500 ppm, 27	14.8	44.4	37.0	7.4	37.0	7.4	Cultures not observed	
104-day-old saplings	BM (control), 10	30.0	70.0	70.0	0	70.0	0	70	0
	BM+CH 500 ppm, 10	40.0	40.0	50.0	0	50.0	0	50	0

[a] Percentage of responsive cultures

[b] No other response was elicited up to 2 weeks from culture

R = Formation of roots R + S = Formation of both roots and shoot buds

shoot buds inside the agar medium. By 7 weeks from transfer the percent of responsive cultures reached 100, and the number of shoot buds ranged from 3 to 20/culture. However, there was no formation of embryoids.

Unlike the apical segment, the basal segment of leaf cultured on BM produced shoot buds, or first roots and then shoot buds in the same period of culture, but no embryoids. In the three treatments with CH the leaf segment produced 1–3 roots and one shoot bud opposite the roots. Higher concentrations of CH suppressed elongation of both roots and shoot buds (figure 1D–F).

On BM+2, 4-D 10^{-7}M, the basal segment of leaf produced roots only, or both roots and shoot buds sequentially, and in opposite loci. On BM+2, 4-D 10^{-6}M, all the cultures produced 1–3 roots in 2–3 weeks followed by callusing of the petiole as well as of a part of the lamina in 5–7 weeks from culture; 1 or 2 roots showed annular growth near their base (figure 1G). Both calli (from petiole and from lamina) were yellowish. In 3-4 weeks from transfer of such rhizogenic and callused cultures of the basal segment of leaf to 2,4-D-free medium (BM), 2 or 3 short zones behind the root tip turned light-green. From these zones as well as from the callus numerous roots and a few shoot buds differentiated at opposite loci. Thus, in 6–8 weeks from transfer to auxin-free medium the culture presented a conglomeration of innumerable roots and 1-5 shoot buds.

On BM+2, 4-D 10^{-5}M the basal segment of leaf formed, like the apical segment, a nodular callus in 5–7 weeks from culture (figure 1B). In 3 weeks from transfer to auxin-free medium, the callus produced as many as eight roots in close proximity from its surface in contact with the agarified medium, and one shoot bud at a location seemingly opposite the roots. In another 4 weeks the number of roots increased up to 12, and that of shoot buds up to three. However, embryoids failed to differentiate.

When cultured on BM+NAA 10^{-7}M, all the basal segments of leaf produced 1–3 roots and 1 or 2 shoot buds near the cut end of petiole (figure 1H). When such cultures were grown on NAA-free medium for 6 weeks, the roots elongated ca 4 cm and the shoot buds developed into 4–6 cm tall leafy shoots. Dissection under stereomicroscope showed the roots and the shoots to form continuous axes of regenerated plantlets.

On BM+NAA 10^{-6}M and BM+NAA

10^{-5}M, the basal segment of leaf elicited responses similar to those of apical segments cultured on these two media. However, the roots originated usually from the shortened petiole, and occasionally from the mid-region of adaxial surface of the lamina (figure 1C).

Responses of Hypocotyl and Hypocotyl Segments

On BM 33% of the cultures of hypocotyl, excised from 17-day-old seedlings, produced 1–5 roots followed by 1 or 2 shoot buds, but no embryoids. This response increased to 50% on BM+CH 400 ppm, and to 100% on BM+CH 500 ppm, BM+2,4-D 10^{-7}M or NAA 10^{-7}M. The roots and shoot buds differentiated from near the respective morphological poles, and occasionally from middle region of the hypocotyl. Roots were formed in 2–3 weeks, and shoot buds in a maximal period of 5 weeks from culture. The shoot buds bore 2–4 pairs of fully expanded decussate green leaves.

On BM+CH 600 ppm 25% of the hypocotyl cultures produced 1 or 2 shoot buds in 3–5 weeks, but no roots. In 2–3 weeks treatment with 2, 4-D 10^{-6}M the explants invariably produced 1 or 2 roots near the cut end, and sometimes from the middle region. By the fourth week from culture, both the cut ends callused slightly, and by the fifth week the two calli over-arched the explant as a single smooth and pale-yellow proliferation. When the rooted and callused cultures were 7-week-old (figure 1J), they were transferred to 2,4-D-free medium. In 7 weeks from transfer, each culture produced numerous roots (longer than those formed prior to transfer) and up to 13 leafy shoots, but no embryoids. While the roots differentiated from the surface of callus in contact with the culture medium, most of the shoots arose from the free surface of callus and 2 or 3 from the roots that were present at the time of transfer.

The shoots bore long internodes, and small light-green leaves. On BM+2,4-D 10^{-5}M the entire hypocotyl produced a nodular callus which rendered the explant unidentifiable. In 7 weeks from transfer to auxin-free medium, only 1 or 2 roots (but no shoot buds) differentiated from the callus. This is in contrast to the hypocotyl cultures that were induced to callus on BM+2,4-D 10^{-6}M and likewise transferred to auxin-free medium; however, in this instance also embryoids did not differentiate.

In all the hypocotyl cultures on BM+NAA 10^{-6}M, 3–5 club-shaped roots differentiated adjacent to the cut end of the explant, in 3 weeks. In another 2 weeks 50% of the rhizogenic cultures produced a slight callus from the cut ends. One week from transfer of the rooted and callused cultures to NAA-free medium, the club-shaped roots elongated into normal-looking roots. In 3 weeks from transfer 2–6 shoot buds differentiated from the attenuated zone of 2 or 3 roots. On BM+NAA 10^{-5}M the hypocotyl produced 4 or 5 stumpy roots by 3 weeks from culture. In another 2 weeks the non-rhizogenic areas of the hypocotyl callused. The callus was friable and its mass more than that obtained on BM+NAA 10^{-6}M. When 7-week-old, the cultures were transferred to auxin-free medium. By 3 weeks from transfer the stumpy roots as well as the callus differentiated many roots and leafy shoots, but no embryoids. By 7 weeks from transfer numerous thin and elongate roots and 20 shoots—14 from the stumpy roots and 6 from the callus—differentiated. The leafy shoots were tall and slender, and bore small light-green leaves.

In nearly 73% of the cultures on BM, the hypocotyl segment regenerated both root and shoot buds. In 1 week from culture 1 or 2 roots differentiated near the morphological root-end of the segment. In another 1–2 weeks usually one and rarely two shoot buds differentiated at the opposite polar region of the

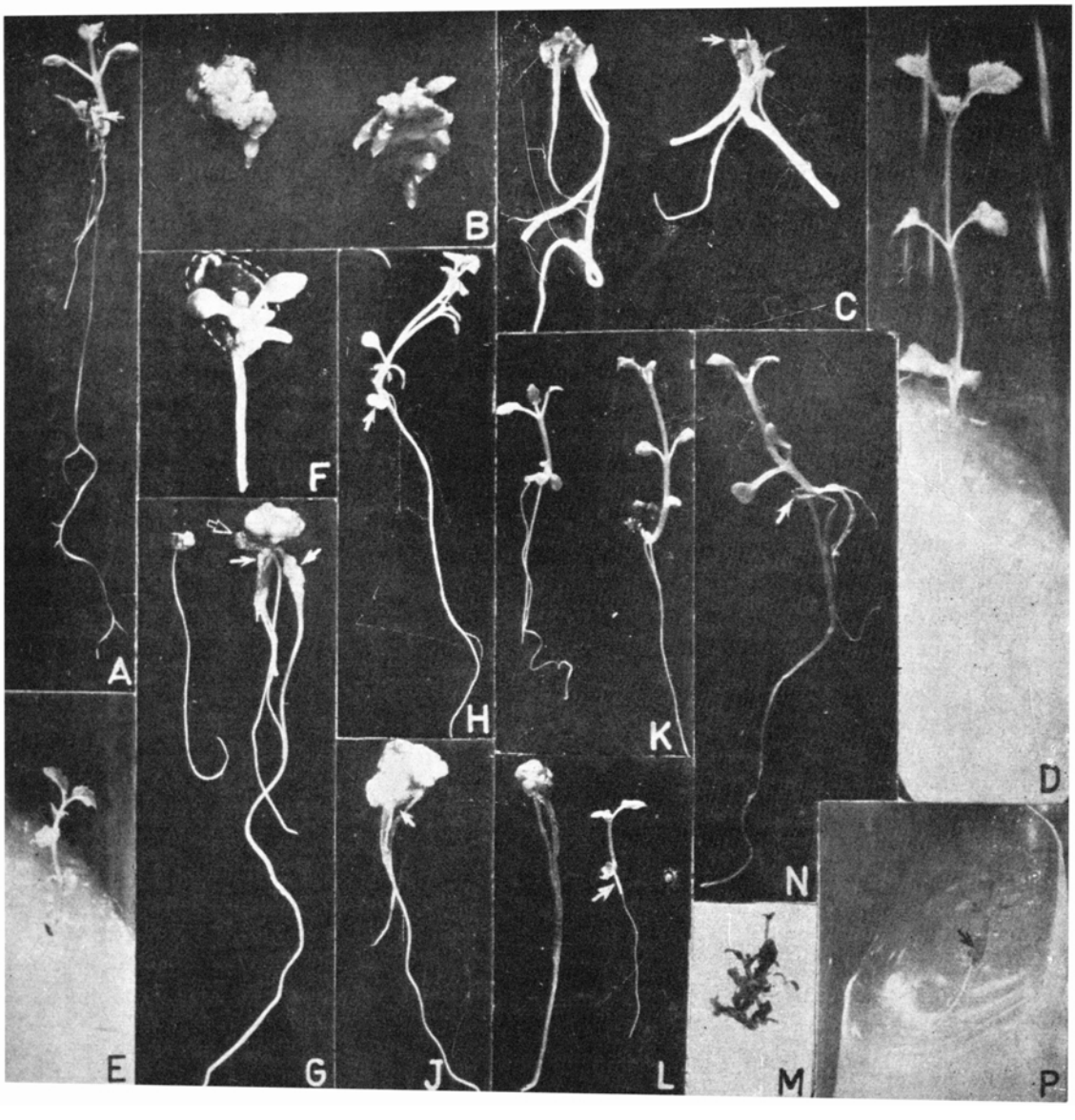

Figure 1A–P Organ regeneration in vegetative explants. **A–H** leaf and leaf segment cultures: **A**, 4-week-old culture of leaf excised from 17-day-old seedling and cultured on BM + CH 500 ppm; 3 roots and 2 shoot buds differentiated from petiole; arrow points to lamina of explant (×1.48); **B**, Nodular callus formed by apical segment (*left*) and basal segment (*right*) of leaf in 7 weeks on BM + 2, 4-D 10^{-6}M. Some nodules, especially from the basal segment, are root-like (×3.0); **C**, Leaf segments from 7-week-old cultures on BM + NAA 10^{-6}M. *Right*: apical segment has produced 2 robust roots from adaxial surface, and 2 thinner roots from abaxial surface. Arrow points to leaf apex. *Left*: basal segment of leaf produced 2 robust roots from swollen petiole and 1 thin root from lamina (×1.48); **D–F**, 7-week-old cultures of basal leaf segments on BM + CH 400, 500 and 600 ppm, respectively. **D, E** (both ×1.11) show regeneration of a solitary leafy shoot each; the root system embedded in the agar medium is rather delicate. In **E** the elongation of both roots and shoot buds is suppressed; **F** shows further suppression of elongation of the root-shoot axis; note 3 roots

(*contd*)

segment. Each shoot bud produced 1 or 2 pairs of leaves. As on BM, on BM+CH 500 ppm also the hypocotyl segment regenerated both roots and shoot buds forming plantlets (figure 1K). But the response occurred in only 33% of the cultures. On BM+ 2,4-D 10^{-7}M also the hypocotyl segment produced 1 or 2 roots and then a shoot bud at opposite pole, thus giving rise to a plantlet (40% response, figure 1L). In other details the cultures resembled those on BM or BM+CH 500 ppm. On BM+NAA 10^{-7}M, however, the hypocotyl segment differentiated roots only (92% cultures). Usually in 3 weeks from culture, 2 or 3 robust roots differentiated from its root end and a brownish-yellow callus almost all over the explant (figure 1L).

Complementary Regeneration

Whether or not segments from the root-shoot axis express complementary regeneration *in vitro* was also studied. The types of segments cultured on BM are listed in table 1.

Root tip segments usually produced 1–4 leafy shoots. In the first week the responsive segments developed 1 or 2 longitudinally lenticular swellings away from the tip. In the next 2 weeks a leafy shoot originated from the mid-region of each lenticular swelling. No roots differentiated even 4 weeks from culture. In cultures of the middle segment of root also, only leafy shoots differentiated (figure 1M). In gross morphology the formation of leafy shoots resembled that described for cultures of the root tip segment; but the response was 100% and 1–6 leafy shoots/culture were produced. The basal segment of root produced, in 82% of the cultures, both roots and shoot buds at opposite loci near the hypocotyl end (figure 1N).

Nodal segments cultured on BM differentiated both roots and shoot buds (58–84% cultures), or shoot buds only (16–42% cultures). All but one culture of internodal segments differentiated both roots and shoot buds (figure 1P) either sequentially (segment from internode 1—roots in 1 week and shoot buds in 2–3 weeks from culture), or simultaneously (segment from internode 2 — both roots and shoot buds in 2 weeks from culture).

Excised shoot buds cultured on BM elongated and produced a second pair of leaves

(*contd*)

(2 stumpy ones at *right*) and top view of the telescoped shoot bearing 2 pairs of decussate leaves; laminar part of explant is marked by broken line. **D, E** (×1.11); **F** (×5.19); **G**, Apical segment (*left*) and basal segment (*right*) of leaf as seen 7 weeks from culture on BM + 2, 4-D 10^{-6}M. Both segments show rooting. In the basal segment note the annular growth (solid arrows) near base of some roots and the massive callus derived partly from lamina and partly from petiole; owing to callus formation the remnant of lamina (hollow arrow) has occupied a lateral position. In the apical segment the quiescent apex is out of focus (× 1.48); **H**, Basal segment of leaf taken out from 7-week-old culture on BM + NAA 10^{-7}M. Note the formation of roots and twin leafy shoots from petiole; arrow points to remnant of lamina (× 1.48); **J–L** cultures of hypocotyl and hypocotyl segments. **J**, Hypocotyl as seen 7 weeks from culture on BM + 2, 4-D 10^{-6}M showing rooting and callusing from both cut ends. By a tilt the explant (arrow) has become partially visible (× 1.48); **K**, Plantlets regenerated from hypocotyl segments, in 6 weeks from culture, on BM (*left*) and BM + CH 500 ppm (*right*) (× 1.48); **L**, Hypocotyl segments removed from 8-week-old cultures on BM + NAA 10^{-7}M (*left*) and BM + 2, 4-D 10^{-7}M (*right*). The former shows rooting and callusing, and the latter plantlet regeneration. The explant (arrow) is identifiable in one but not in the callused culture (× 1.48); **M–P**, cultures of segments from root and stem. **M, N**, 3-week-old cultures, respectively, of the middle and the basal segments of root on BM; **M** shows differentiation of 5 leafy shoots, and **N** of both leafy shoots and roots. Arrow in **N** points to hypocotyl end; **M** (×1.48); **N** (×2.22); **P**, 3-week-old culture of internodal segment 2 showing simultaneous differentiation of a leafy shoot and a root from the respective polar ends of explant (arrow) (× 1.48).

in 2 weeks from culture; also, they issued 1–3 roots near the cut end. In another 2 weeks the third pair of leaves appeared and the roots grew into the agarified medium.

From 6- to 7-week-old saplings grown *in vitro*, the root system and the shoot system were severed and raised as separate cultures on BM. The cultures of root system regenerated one of the following: (*a*) 1 or 2 leafy shoots subjacent to the cut end of hypocotyl stub (45% cultures), (*b*) one leafy shoot from the cut end (25% cultures), or (*c*) shoot buds from some of the lateral roots (10% cultures). Usually 1 and, rarely 2 or 3 shoot buds or leafy shoots differentiated per culture; also, the leafy shoots were dwarf and bore only 1 or 2 nodes. The severed shoot system invariably regenerated 1–3 roots near the clipped end in 1–2 weeks from culture on BM. One of the cultures produced a shoot bud also from one of the regenerated roots. Embryoids did not differentiate in cultures of any of the segments from the root-shoot axis.

Discussion

In organ regenerative ability the four major parts of the plant body (from which the various explants were obtained) formed, in decreasing order of response, the series: hypocotyl, leaf, root and stem. However, when all the 13 types of vegetative explants are considered, the apical segment of leaf was the least regenerative. That hypocotyl is usually more responsive than other explants is a general lesson from contemporary literature on regeneration *in vitro*. For example, Gunay and Rao (1980) have reported that in hybrid tomato the hypocotyl was more responsive than cotyledons (of the same age) to the various treatments given; in certain treatments, only the hypocotyl regenerated a shoot. Similarly, in the tropical pasture legume *Stylosanthes humilis* while 100% of the hypocotyl-derived calli could form shoots, only 78% of the leaf-derived calli could do so and that too in a longer period (Meijer 1982). That organ regeneration could be achieved even on the basal medium (without employing any phytohormones) makes *Scoparia dulcis* a rewarding material for studies on regulation of morphogenesis.

The complimentary regeneration in *S. dulcis*, i.e. the ability to regenerate the missing part of the root-shoot axis by the segments from root or stem, is an expression of the high degree of morphogenic plasticity of this species. A similar observation has also been reported for another species of Scrophulariaceae, viz. *Kickxia ramosissima* (Vatsala 1976).

Todate organ and tissue culture studies on Scrophulariaceae, including our experimental species *S. dulcis*, are known for 16 species (table 3). In 14 species organ differentiation occurred directly from the cultured explants, and not through the intervention of a callus. This phenomenon coupled with the morphogenic plasticity has a greater relevance to clonal propagation of elite individuals of economic value. For example, regeneration of plantlets in cultures of seedling shoot tips of the officinal species *Digitalis lanata* (also of Scrophulariaceae) has been reported (Erdei et al. 1981). In our studies the plantlets were frequently obtained directly from the cultured leaves (the officinal part), hypocotyl and root segments. This finding should prove valuable when the antidiabetic property of *S. dulcis* is unequivocally established.

Callus production is often accompanied by cytologic aberrations leading to genetic variability. While this is less welcome in micro-propagation studies, we observed that up to 20 shoot buds could differentiate from even a slight mass of callus obtained in cultures of leaf segments and hypocotyl.

Table 3 *Tissue and organ culture studies on Scrophulariaceae**

Species	Explant	Reports formation of	Reference
1. *Alectra* sp.	Seed	Seedlings	Okonkwo 1975
2. *Antirrhinum majus*	Mesophyll	Embryoids	Poirier-Hamon et al. 1974
	Stem	Embryoids, plantlets	Sangwan & Harada 1975
	Ovule	Callus	White 1932 (see Rangan 1982)
3. *Bacopa monnieri*	Internodal segment	Roots, shoot buds	Thakur 1975
4. *Digitalis lanata*	Seedling shoot tip	Plantlets	Erdei et al. 1981
	Mesophyll protoplast	Plantlets	Li 1981
5. *D. purpurea*	Haploid callus	Callus, plantlets	Corduan & Spix 1975
	Leaf protoplast	Leaves, roots	Hu 1982
6. *Kickxia ramosissima*	Entire leaf	Roots, shoot buds	Vatsala 1976
	Trans- & longi-segments of leaf		
	Depetiolated leaf		
	Internodal segment		
	Root segment		
	Petiole	Shoot buds	Vatsala 1976
	Excised epidermal & sub-epidermal tissue from internode	Shoot buds & callus, shoot buds	
	Flower bud	Shoot buds	
	Calyx, corolla segment	Floral buds	
	Ovary segment	Carpelloids, stigmatoids	
7. *Limnophila chinensis*	Internodal segment	Shoots	Sangwan et al. 1976
8. *Linaria vulgaris*	Seedling root	Shoots	Charlton 1965
9. *Mazus pumilus*	Internodal segment	Shoot buds	Raste & Ganapathy 1970
	Inflorescence axis		Raste 1971
10. *Paulownia taiwaniana*	Internodal segment	Plantlets	Fu & Hu 1976 (cited in Thorpe 1981)
11. *P. tomentosa*	Ovule	Embryos, plantlets	Radojević 1979
12. *Rehmannia glutinoza*	Anther	Plantlets	Hu 1982
13. *Scoparia dulcis*	Leaf	Roots, shoots, plantlets, callus	Present work
	Leaf segment		
	Hypocotyl		
	Hypocotyl segment		
	Nodal segment	Shoots, plantlets	
	Internodal segment		
	Root segment	Shoots	
	Root system		
	Shoot tip	Roots	
	Shoot system		

(*contd*)

Table 3 (*contd*)

Species	Explant	Reports formation of	Reference
14. *Striga angustifolia*	Embryo from dry seed	Seedlings	Rangaswamy & Rangan 1969
15. *Torenia fournieri*	Leaf	Shoot buds, plantlets	Bajaj 1972
	Stem segment / Leaf segment	Vegetative and floral buds	Chlyah 1973
	Internode	Callus, roots	Kamada & Harada 1979
16. *Verbascum thapsus*	Stem	Shoots	Caruso 1971

*The listing is alphabetical and not chronological

This is significant because it could provide a large gene pool for selection.

Yet another observation which calls for an explanation is the absence of formation of embryoids in any of the explants cultured. Although explants of diverse origin belonging to 16 species of Scrophulariaceae (including *Scoparia dulcis*) have been cultured (table 3), somatic embryogenesis has been reported for two species only. This general inability of Scrophulariaceae (including *S. dulcis*) to form embryoids *in vitro* can be perhaps attributed to the occurrence of the triterpenoids, namely cucurbitacins in the family (Lavie & Glotter 1971, Guha & Sen 1975), which have been demonstrated to be antiembryogenic in tissue cultures (Rangaswamy 1982, Tewari & Rangaswamy 1983). Phytochemical studies on *S. dulcis* are limited (Ali & Rahman 1966, Chen & Chen 1976, Ramesh et al. 1979, Mahato et al. 1981), and whether or not cucurbitacins occur in the species must await further studies.

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