Organogenesis in cultured petiole explants of *Begonia × erythrophylla*: the timing and specificity of the inductive stimuli

David J. Burritt¹ and David W.M. Leung

Plant and Microbial Science Department, University of Canterbury, Private Bag, Christchurch, New Zealand

Received 19 May 1995; Accepted 7 December 1995

Abstract

Caulogenesis and rhizogenesis were studied in cultured petiole explants of *Begonia × erythrophylla* in order to link the developmental stages of primordia initiation with the physiological requirements of the explant. Petiole sections excised from *B. × erythrophylla* plants grown in vitro, were highly organogenic, with shoots and roots arising directly from cells of epidermal origin. Epidermal cells associated with glandular hairs appeared to be most responsive to organogenic stimuli. The point of explant determination for each form of organogenesis was ascertained by media transfer experiments. Explants became determined for caulogenesis after 7 d exposure to shoot-inducing medium (SIM), while requiring 3 d on root-inducing medium (RIM) for determination. Explants were strongly canalized for caulogenesis once determined, but 5 d on RIM were required before becoming strongly canalized for rhizogenesis. No organ specific differentiation was observed at the point of determination for explants exposed to either shoot- or root-inducing conditions. Preculture on a basal medium containing no growth regulators resulted in a gradual loss of competence with time, but preculture for up to 2 d on SIM or RIM resulted in a reduction in the time for determination for both forms of organogenesis.

Key words: Organogenesis, *Begonia × erythrophylla*, tissue culture, epidermis, determination.

Introduction

The ability of organs such as shoots, roots and flowers to arise adventitiously is a widespread natural phenomenon, which is essentially being exploited in plant tissue culture (Flick *et al.*, 1983). Despite commercial application and significant research, organogenesis is still poorly understood.

Organogenesis has been studied histologically in many plant species (Banks, 1979; Christianson and Warnick, 1983; Wright *et al.*, 1986; Reynolds, 1989); organogenesis can occur either directly from cells of the original explant, or indirectly via callus formation. Often cells associated with the vascular tissues are the origins of meristemoids which give rise to organ primordia, but studies have also shown that even specialized cells like those of the epidermis can, if exposed to the right conditions, undergo organogenesis (Tran Thanh Van, 1973).

More recently the application of tissue-transfer experiments between various culture media has revealed a number of stages in the organogenic process. Using *Convolvulus arvensis* leaf explants, Christiansen and Warnick (1983) divided the process of organogenesis into three phases. In the first phase the explant gains the ability to respond to an organogenic induction, that is it acquires 'competence', which in *C. arvensis* involves the production of a small amount of callus along the cut edges of the explant. Christiansen and Warnick (1983) found that competence could be achieved on shoot- or root-callus-inducing media. The next phase is organogenic induction; through the influence of the growth regulator composition of the medium competent callus becomes determined to follow a particular developmental pathway. Once determined, the tissue can continue on this developmental pathway in the absence of growth regulators. Organogenesis in other tissue culture systems can also be divided into these phases (Flinn *et al.*, 1988; Attfield and Evans, 1991b).

¹ Present address and to whom correspondence should be sent: Department of Botany, Otago University, PO Box 56, Dunedin, New Zealand. Fax: +64 3 479 7583. E mail: david@phyton.otago.ac.nz

© Oxford University Press 1996
In this study, the developmental patterns of shoot and root formation in Begonia × erythrophylla petiole sections are outlined and these are correlated to phases in the initiation of both forms of organogenesis, as identified by various media transfer experiments.

**Materials and methods**

**Plant material**

All experiments were carried out using explants taken from Begonia × erythrophylla J. Neuman (Beefsteak begonia) plants established in vitro (DJ Burritt, unpublished results). B. × erythrophylla J. Neuman, also known as B. × feistii Hort. ex L. H. Bailey, is a hybrid begonia (B. manicata × B. hydrocotylifolia) probably of garden origin.

Micro-cuttings consisting of the shoot apex, two or three expanded leaves and numerous adventitious roots were subcultured every 8 weeks into 250 ml polycarbonate screw-capped tissue culture vessels, each containing approximately 50 ml of maintenance medium. Plants were maintained in a tissue culture room at 24 °C under continuous lighting, provided by cool-white fluorescent lamps supplemented with tungsten bulbs. The fluence rate was approximately 100 μmol m⁻² s⁻¹.

**Culture media and procedures**

Murashige and Skoog (1962) salts plus inositol 0.55 mM, nicotinic acid 40.6 μM, pyridoxine HCl 2.43 μM, thiamine HCl 1.48 μM, glycine 26.7 μM, folic acid 1.13 μM, biotin 0.2 μM, 3% sucrose, and 0.7% Davis agar was used as a basal medium (BM) for the culture of explants, with the addition of 0.54 μM naphthalene acetic acid (NAA) and 4.44 μM benzyladenine (BA) for shoot induction (SIM), and 5.4 μM NAA and 0.22 μM BA for root induction (RIM). Axenic plants were maintained in culture on a maintenance medium (MM) consisting of half-strength Murashige and Skoog salts and 0.7% agar, no organics were included in this medium. All media were adjusted to pH 5.8 prior to autoclaving.

Petiole explants were cut into 1-2 mm sections and fixed at room temperature for 6 weeks in culture. Leafy shoots were dissected from the explant and counted. Roots were easily counted without dissection. A treatment was considered to be organogenic if the mean value of organs per explant was greater than one.

**Histology**

Petiole explants were cut into 1-2 mm sections and fixed at room temperature for 3-4 h under water vacuum in 2.5% glutaraldehyde, in a 0.075 M sodium phosphate buffer pH 7.2. Tissues were dehydrated for 2 h in an ethanol series for 20 min in each of 10, 20, 40, 60, and 80%, with 2 × 30 min changes in absolute ethanol. Specimens were also re-evacuated at a lower surface tension, in 80% and 100% alcohol changes in order to remove any air adhering to hairs on the surface of the specimens. Tissues were infiltrated in mixtures of 25, 50, 75, and 100% LR White acrylic resin (London Resin Company) dissolved in ethanol, for 2 d each at room temperature. Polymerization of the resin was carried out at 4 °C using the LR White cold curing procedure. Flat-embedding caps were filled with LR White acrylic resin to which the cold-cure additive had been added (1 drop of cold cure additive to 12 ml of resin), tissue sections were then quickly positioned in the resin. The cap was then filled to overflowing and covered with a piece of parafilm taking care that no air was trapped under the parafilm. The cap was then covered with a small piece of heavy glass and placed in a refrigerator at 4 °C overnight.

Transverse sections (2 or 4 μm) of petiole explants were obtained with a Reichert rotocut 2000 EX equipped with glass knives. Thin sections were stained with Methylene blue-azure A (Warmke and Sheu-Ling, 1976) and mounted in immersion oil. Bright-field micrographs were recorded on Kodak Ektar 25 film using a Zeiss 1M35 photomicroscope.

**Results**

**Light microscopy of organ development**

Transverse sectioning of the petiole revealed anatomy typical of the Begoniaceae. Petioles consisted of a single-layered epidermis, with underlying corner-thickened col-
lenchyma cells, followed by a cortex, consisting of equal-sized, highly vacuolated cells, with interspersed discrete vascular bundles (Plate 1A). Epidermal, collenchyma and cortical cells all showed only slight cytoplasmic staining, indicating a low level of metabolic activity.

Development on basal medium: Culture on basal medium containing no growth regulators or on SIM without sucrose (SIM-) resulted in no sign of organogenesis. Explants remained green on BM for up to 14 d after which they rapidly turned chlorotic. Sectioning after 14 d

Plate 1. (A) Transverse section through the petiole at day 0. (B) Transverse section through a petiole explant after 3 d culture on SIM, showing an early epidermal division. (C) Transverse section through a petiole explant after 4 d culture on SIM, showing numerous divisions in the epidermis at the base of a glandular hair and the underlying collenchyma tissue. (D) Transverse section through a petiole explant after 5 d culture on SIM. Meristematic regions composed of small densely stained cells with prominent nuclei form most often beneath glandular hairs. (E) Meristematic dome at the base of a glandular hair (not visible in this section) after 7 d on SIM. Note the lack of differentiation and minimal disruption of surrounding tissues. Bars = 50 μm.
culture showed no primordia and only rarely were regions of meristematic activity observed. Explants cultured on (SIM—) remained green for longer than those cultured on BM, with many still green after 28 d in culture.

**Development on shoot-inducing medium:** The first cell divisions, usually observed after 3 d, were periclinal divisions in the epidermal and, sometimes, immediately underlying collenchyma cell layers (Plate 1B). These divisions seldom resulted in meristem formation. Meristem regions, composed of clusters of densely staining, small, mitotically active cells, with prominent nuclei, were first observed after 4 d in culture. These meristem regions were derived from anticlinal and periclinal divisions of epidermal cells in close proximity to glandular hairs and were distributed over the entire explant (Plate 1C). Only rarely were meristem regions not associated with glandular hairs.

Continued cell division in these regions resulted in the formation of small discrete zones of highly cytoplasmic rapidly dividing cells (Plate 1D), which after at least 7 d culture developed into meristem domes (Plate 1E). The domes appeared continuous with the epidermis with no epidermal rupture. By day 9 the domes had enlarged and were often enclosed by a distinct tunica (Plate 2A). Sometimes a vascular trace was associated with the developing domes.

Foliar primordia were observed after 14 d (Plate 2B) and, although cell divisions were observed in the cortical cells surrounding the vascular bundles, the bundles themselves showed no meristematic activity associated with caulogenesis. By day 18, numerous apical domes enclosed by developing leaves were present on the explant.

By day 24, the most advanced shoots had well organized apical meristems, with leaf primordia developing from the shoot apex. The apex had the oblique orientation typical of Begonia. Most shoots contained some vascular tissue but remained isolated from the vascular bundles of the initial explant.

**Development on root-inducing medium:** Histologically, the initial stages of rhizogenesis were similar to those of caulogenesis. Small meristem regions mostly, but not always, associated with glandular hairs were observed after 4 d in culture (Plate 2C). Unlike culture on SIM, these were confined to the lower half of the explant. Continued cell division in these regions gave rise to root primordia. Primordia only developed from the superficial layers of the explant (Plate 2D). No adventitious roots of perivascular origin were observed and no connection to the vascular tissue had taken place by day 24 of culture (Plate 2D).

In undamaged explants, little internal disruption of the original explant occurred although some cell division in the cortical and vascular tissues did take place with prolonged culture. Many explants did, however, show regions of random cell division. Most of these regions were devoid of meristematic structures and consisted of large, highly vacuolated, dividing cells, similar to wound callus. The epidermal and collenchyma layers above these regions often appeared disrupted, containing collapsed cells. This localized damage, or wounding of explants, probably occurred during the initial excision and subsequent handling of the petiole sections. Such regions were also observed in explants cultured on SIM.

**Media transfer experiments**

Reciprocal transfer experiments were carried out to ascertain the duration of exposure to an inductive medium required for determination.

**Length of exposure to SIM for determination:** Culture for 7 d on SIM, before transfer to BM, was the minimum requirement for shoot induction with most surviving explants producing at least one shoot (Fig. 2). Prolonging the period of culture increased the number of shoots per explant, up to a maximum after 18–21 d on SIM (Fig. 2). Further culture caused little change in the number of shoots subsequently produced. Transfer prior to day 7 resulted in only occasional shoot formation.

Experiments involving delayed exposure to SIM were also conducted by preculturing explants on BM prior to exposure to SIM. Culture for up to 3 d on BM, before transfer to SIM, did not affect the number of shoots produced per explant (Fig. 2). Further culture resulted in reduced shoot numbers with complete loss of competence after more than 7 d on BM (Fig. 2).

**Length of exposure to RIM for determination:** Culture for 3 d on RIM, before transfer to BM, was the minimum time required for determination (Fig. 3). A maximal response was observed after 10 d of culture on RIM, further culture did not result in a further increase in root number. Explants could be cultured on BM for up to 3 d, with no effect on root number, but prolonged culture caused a rapid decline with few roots produced from explants pre-cultured for 7 or more days on BM (Fig. 3).

**Effect of BA concentration on the time required for shoot determination:** To ascertain if BA concentration could influence the time required for determination, explants were exposed for various lengths of time to different concentrations of BA, before transfer to BM. Culture on a medium containing half the concentration of BA in SIM (BA0.5) resulted in few shoots, irrespective of the time of transfer (Fig. 4). Culture on a medium containing four times the concentration of BA in SIM (BA4) or eight times that in SIM (BA8) reduced the time required for minimal determination and increased the number of shoots determined by day 10 of culture (Fig. 4). Increasing the concentration of BA affected neither the
time required for a maximal shoot forming response, nor the number of shoots produced (Fig. 4).

Despite reducing the time for minimal determination, exposure to increased BA concentrations did not speed up the development of shoot primordia. Both in surface view, and histologically, no difference was observed between explants cultured on SIM or BA8 for the same period of time.

Effects of SIM to RIM transfer regimes: Although only requiring exposure to SIM for 7 d for determination, the question arose whether growth regulator-containing media could still influence the formation of shoots beyond this point. Media transfer experiments were conducted where explants were moved from either SIM to RIM, BA8 to RIM or RIM to SIM at various times over a 21 d period. After 22 d all explants were transferred to BM to allow organ development.

SIM to RIM: With this transfer regime a similar pattern of shoot formation was observed (Fig. 5) to that seen with transfer from SIM to BM. Transfer prior to day 7...
resulted in only an occasional shoot. Seven days on SIM was required for a mean value of greater than one shoot per explant. Culture for more than 7 d increased the mean number of shoots per explant (Fig. 5).

Root induction showed a reciprocal pattern. Culture for up to 7 d on SIM caused a slight reduction in root number compared to culture on RIM only. Transfer after day 7 caused a steady decline in root number. Unlike culture in the absence of growth regulators, culture on SIM did not appear to inhibit the ability of explants to respond to RIM.

Although a 5 d exposure to BA8 resulted in shoot production after transfer to BM, exposure to BA8 for less than 7 d resulted in few shoots when explants were transferred to RIM (Fig. 6). Transfer from BA8 to RIM produced a similar pattern of determination to that observed with transfer from SIM to RIM, or SIM to BM.

RIM to SIM: Root formation upon transfer from RIM to SIM was similar to that produced upon transfer to BM, except fewer roots were produced (Fig. 7). Transfer to SIM suppressed the development of roots that would have developed with transfer directly to BM. Three days on RIM, before transfer to SIM, induced few roots per explant. Five days were required to induce a mean number of roots similar to the value produced after only 3 d on RIM and transfer to BM. To give a near maximal response 18 d exposure were required.

Caulogenesis was dependent upon the time of exposure to SIM. Culture for up to 3 d on RIM did not affect shoot number, with a maximal response obtained. Further culture resulted in a rapid decline in shoot number.
Organogenesis in cultured Begonia petiole explants

Fig. 6. Mean numbers of shoots produced from explants exposed to SIM or BA8 and then transferred to basal medium or RIM. Mean ± SE. n = 30. Values with the same letter are not significantly different at P = 0.05 (30 explants per treatment).

Fig. 7. The number of shoots and roots produced after transfer from RIM to SIM. Mean ± SE. n = 30. Explants were all transferred to basal medium after 22 d. Organs were scored after a total of 6 weeks in culture.

Growth regulator requirements during the inductive period:
To ascertain if explants require exposure to SIM throughout the inductive period for shoot production, explants were precultured on BM or RIM prior to transfer to SIM for various lengths of time, and finally to BM. The same procedure was carried out for root production, but explants were exposed to BM or SIM before transfer to RIM.

BM to SIM/RIM to BM: Culture medium containing no growth regulators had no inductive effect on either rhizogenesis or caulogenesis. Preculture on BM did not reduce the time required on either inductive medium (data not presented).

SIM to RIM to BM: Irrespective of preculture regime, exposure to SIM for less than 5 d resulted in only an occasional shoot being produced (Fig. 8). Preculture on RIM for 24 or 48 h did, however, significantly reduce the time required on SIM for minimal determination, to 6 d and 5 d, respectively. Further preculture caused no further reduction in the time required for minimal determination, but resulted in a reduction in the number of shoots produced (Fig. 8). The promotive effects of preculture were still observed even with prolonged culture on SIM (Fig. 8).

RIM to SIM to BM: Irrespective of preculture regime, exposure to SIM for less than 5 d resulted in only an occasional shoot being produced (Fig. 8). Preculture on RIM for 24 or 48 h did, however, significantly reduce the time required on SIM for minimal determination, to 6 d and 5 d, respectively. Further preculture caused no further reduction in the time required for minimal determination, but resulted in a reduction in the number of shoots produced (Fig. 8). The promotive effects of preculture were still observed even with prolonged culture on SIM (Fig. 8).

SIM to RIM to BM: Preculture for up to 2 d on SIM significantly reduced the time required on RIM for minimal determination (Fig. 9). Preculture for 3 d caused no further reduction, just a decrease in the number of roots produced. Unlike the prolonged promotive influence of RIM on caulogenesis, the promotive influence of SIM on rhizogenesis became insignificant when explants were cultured for more than 5 d on RIM.

From these results it is apparent that both organogenic processes require exposure to growth regulators at the beginning of the inductive period, but the ratio of auxin to cytokinin only becomes critical at a later stage.

Discussion
Adventitious primordia develop from two general areas of the explant, either in close proximity to the vascular tissue (Sterling, 1951; Bonnett and Torrey, 1966; Reynolds, 1989) or from the epidermis or sub-epidermal layers (Tran Thanh Van, 1973; Chlyah, 1974; Von Arnold
and Gronroos, 1986). Often caulogenesis and rhizogenesis do not both occur directly in the same explant (Attfield and Evans, 1991a). Shoots and roots may develop from different cell types. Begonia species are interesting, for in B. × erythrophylla petiole sections, as reported here, and B. rex thin layers (Chlyah and Tran Thanh Van, 1984), both shoots and roots are formed directly from cells of epidermal origin.

The ability of epidermal cells to give rise to differentiated structures has been the subject of considerable research (Walker, 1978; Walker and Bruck, 1985; Bruck and Walker, 1985, 1986). Walker and Bruck suggested that only under special circumstances, may epidermal cells become less differentiated and give rise to differentiated structures (Walker and Bruck, 1985). However, Tran Thanh Van (1973) has demonstrated the totipotent nature
of the epidermis of *Nicotiana tabacum* L. by its ability to produce floral buds. Chlyah and Tran Thanh Van (1984) have also clearly shown that epidermal cells in thin layers of *B. rex* are capable of organogenesis. In *B. x erythrophylla*, the pattern of both shoot and root formation is similar to that seen in *B. rex* thin layers (Chlyah and Tran Thanh Van, 1984).

The initial stages of both types of organogenesis also appear similar (Plates 1D, 2C), with meristematic regions of epidermal origin developing into primordia with minimal epidermal disruption. It can not be definitely stated that these meristematic regions are derived from a single epidermal cell, for often many cells in the same epidermal region divide. Therefore, meristematic regions and hence shoot primordia may be derived from the division of one or more epidermal cells.

The vast majority of epidermal cells responding to either organogenic medium were associated with glandular hairs. This specificity has also been shown in several cultivars of *B. rex* (Prevot, 1948; Bigot and Chlyah, 1970). A few primordia showed no association with glandular hairs; these may have evolved from epidermal cells about to develop into glandular hairs.

Attfield and Evans (1991a) proposed that the state of differentiation of individual cell populations within the explant may influence their response to growth regulators and that some cells within an explant may be considered to be less differentiated than others. In other words, certain cell types may be more responsive than others to an inductive stimulus.

When leaf fragments of *B. rex* are cultured on a root-inducing medium, roots originate from the inner tissues of the explant and form only from epidermal cells using the thin layer system (Chlyah and Tran Thanh Van, 1984). Either growth regulators do not reach the epidermal cells, or the breakdown of tissue correlations is required to induce roots of epidermal origin. In *B. x erythrophylla* petiole sections, obtained from plants grown in vitro, there is no predisposition of cells of the inner tissues for root formation as seen in *B. rex* leaf fragments (Chlyah and Tran Thanh Van, 1984). The epidermal layer appears equally capable of giving rise to roots or shoots providing the explant is given the correct stimulus.

Explants require a minimum of 7 d on SIM prior to transfer to BM before any shoots are produced (the point of minimal determination). At this time the most advanced structures observed are meristematic domes with no apparent differentiation. Determination for shoot formation appears to occur prior to specialization of the meristematic region. As observed in several other systems (Christianson and Warnick, 1983; Flinn et al., 1988; Attfield and Evans, 1991b), the process of shoot determination is not synchronous; a further 11–14 d on SIM is required for a maximal shoot forming response (the point of maximal determination).

The time required for minimal shoot determination, as ascertained by transfer to basal medium, can be manipulated by altering the concentration of BA in SIM. Exposure to a higher concentration of BA reduces the time required for minimal determination by up to 3 d and greatly increases the number of shoots determined after short exposures to the inductive medium. Exposure to high concentrations of BA does not alter the rate at which tissue differentiation occurs. Explants cultured on BA8 appear similar to those cultured on SIM for the same length of time.

When an explant, in which few cells are able respond to an inductive medium, is exposed to a sequence of media, the growth regulator composition of the first medium can have effects carried over to the second medium (Halperin, 1986; Renaudin et al., 1990). The difference in the apparent timing of determination when explants are exposed to media containing different concentrations of BA highlights one of the problems encountered when studying organogenesis in multicellular explants. A high concentration of BA in the shoot-inducing medium may simply allow a greater internal concentration to build up within the tissues before transfer to basal medium. Exogenous hormones could be carried-over in the explant during transfer to the non-inductive medium, where they could continue to influence the regenerating cells for some time.

An alternative approach which may help to overcome problems associated with estimating the timing of determination in large explants, is to transfer to a medium containing an alternative inductive stimulus, rather than to a basal medium (Christianson and Warnick, 1983).

Exposure to BA8 and then transfer to RIM showed that despite shoots forming upon transfer to BM, explants are not strongly canalized with less than 7 d exposure to shoot-inducing conditions. If the small meristematic regions observed after 4–5 d culture on BA8 were strongly canalized for shoot formation, shoots would be expected, irrespective of the medium to which the explant was transferred.

Explants only require exposure to RIM for 3 or 4 d to induce root formation upon transfer to BM. Transfer from RIM to SIM increases the time required on RIM for root formation. Transfer to SIM has adverse effects on root formation, at days 3 and 4, suggesting that cells within the explant are not strongly canalized with less than 5 d on RIM. A similar occurrence was observed with tobacco leaf discs (Attfield and Evans, 1991b), where the time required for root determination was increased from 1 to 3 d. In both *B. x erythrophylla* petiole sections and tobacco leaf discs a brief exposure to RIM allows explants to form roots upon transfer to BM, but does not result in a change in the competence of the responding cells. These cells remain able to be influenced by exposure to an alternative inductive stimulus.
It is not known if the small meristematic regions that are observed in *B. x erythropylla* petiole sections, after 4 d on RIM, form shoots upon transfer to SIM or if they simply stop dividing and degenerate. But as explants were transferred to BM after 22 d and no roots subsequently formed, it can be assumed that these regions are at least inhibited from forming roots. The same applies to the meristematic regions which fail to form shoots, upon transfer to RIM, with less than 7 d exposure to SIM.

Shoot formation in both *Convolvulus arvensis* and tobacco leaf discs is preceded by callus formation (Christianson and Warnick, 1983; Attfield and Evans, 1991b). During the formation of callus, explants acquire 'competence', i.e. the ability to respond to an organogenic induction (Christianson and Warnick, 1985). This process is formally analogous to the commonly held concept of dedifferentiation of the explant (Christianson and Warnick, 1985). Callus formation is not a prerequisite for either root or shoot formation in *B. x erythropylla*, with organs forming directly from cells of the explant.

Unlike shoot formation, root formation from tobacco leaf discs is direct, with explants determined for rhizogenesis after 1 d of exposure to RIM, with 3 d required for a strongly canalized response (Attfield and Evans, 1991b). Rhizogenesis in the tobacco system appears to require little or no time interval to gain competence. If *B. x erythropylla* petiole sections are competent to respond to an inductive medium at the time of excision, one might expect more rapid determination than observed.

During the first 2 d of the inductive period, explants can be exposed to either SIM or RIM with no reduction in the total time, from excision, required for minimal determination of either type of organ. In contrast, exposure to basal medium results in the gradual loss of the explants ability to respond to either SIM or RIM. Preculture on basal medium has been shown to result in the gradual loss of competence in several other systems (Christianson and Warnick, 1983; Flinn et al., 1988; Attfield and Evans, 1991b).

*B. x erythropylla* petiole sections require exposure to growth regulators during the early stages of induction, but explants may be independent of the ratio of auxin to cytokinin in the medium, with both SIM and RIM capable of fulfilling the other’s role. Only later do explants become responsive to the specific inductive properties of the medium to which they are exposed. Hence growth regulator-induced changes may occur which induce a general state of competence. This general state of competence may be the result of metabolic changes within the explant, required for both types of organogenesis, which are initiated by both SIM and RIM, and occur prior to commencement of cell division in the epidermal layer. Studies are being carried out to investigate if such changes do occur.

References


Reynolds TL. 1989. Adventive organogenesis from somatic