Patterns of Adventitious Root Formation in English Ivy

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Abstract. Adventitious root formation by debladed petiole cuttings of English ivy (*Hedera helix* L.) proceeds via a direct rooting pattern for the easy-to-root juvenile phase, while the difficult-to-root mature phase roots through an indirect rooting pattern. Juvenile petiole cuttings treated with α-naphthaleneacetic acid (NAA, 100 μM) plus the polyamine biosynthesis inhibitor, difluoromethylarginine (DFMA, 1 mM), formed an increased number of roots per cutting initiated by the indirect rooting pattern. The increased root formation and change in rooting pattern were reversed by the addition of putrescine (1 mM). Delaying auxin application to petiole cuttings for 15 days also induced juvenile petioles to root by the indirect pattern. This could be reversed by rewounding the base of the cutting prior to auxin application after day 15. The data support the use of the terms "competent root-forming cells" and "induced competent root-forming cells" to describe the target cells for the initial events of root formation for the direct and indirect rooting patterns, respectively.

Two patterns of adventitious root formation in cuttings have been recognized for both herbaceous and woody species (Hartmann et al. 1990). One pattern involves the direct formation of adventitious root primordia from cells associated with or in close proximity with the vascular system. The second pattern is the indirect formation of adventitious roots involving an interim period of undifferentiated cell divisions. These cell divisions are usually initiated in parenchyma or epidermal cells. Selected cells within these subsequent cell divisions eventually organize to initiate an adventitious root primordium.

In general, herbaceous species and easy-to-root woody species form adventitious roots through the direct pattern of root formation, while species that are difficult-to-root form roots through the indirect pattern (Hartmann et al. 1990). However, it is more accurate to describe root formation in many difficult-to-root species as occurring through both the direct and indirect pattern (Davies et al. 1982; Girouard 1967a, Gronroos and Arnold 1987). English ivy (*Hedera helix* L.) exhibits relatively stable ontogenetic phases where the juvenile phase is easy-to-root and the mature phase is difficult-to-root (Hackett 1988). In English ivy, it has also been determined that easy-to-root juvenile cuttings form roots predominantly through the direct rooting pattern, while difficult-to-root mature phase cuttings form roots via the indirect pattern (Girouard 1967a,b, Geneve et al. 1988). The objective of the current study was to use an in vitro system for rooting debladed petioles of English ivy (Geneve et al. 1988) to determine if the easy-to-root condition was strongly linked to the direct pattern of root formation and to better understand the cells involved in the initial stages of adventitious root formation.

Materials and Methods

The in vitro debladed petiole system for evaluating root formation in the juvenile and mature phases of English ivy has been described in detail, previously (Geneve et al. 1988). Leaves from the two phases were collected from plants grown in a greenhouse. The lamina was separated from the petiole and the petioles disinfected for 10 min in 0.5% NaOCl containing 0.01% Alconox detergent. Petioles were prepared aseptically to a uniform 2.3-cm length and placed upright in a 25-ml Erlenmyer flask containing three pieces of Whatman no. 1 filter paper saturated with 1 ml of Romberger medium. Petioles were placed directly on a medium containing 100 μM α-naphthaleneacetic acid (NAA) alone or in combination with 1 mM putrescine or difluoromethylarginine (DFMA). Petioles were also incubated on a basal medium without growth regulators for 3, 5, 7, 9, 12, or 15 days prior to being moved to a medium containing 100 μM NAA, or incu-

bated on an NAA medium for 0, 1, 3, 5, 7, 9, or 21 days prior to being moved to basal medium.

The localization of radiolabel from ^{14}C-NAA (α-naphthaleneacetic acid-carboxyl-^{14}C; specific activity 2.5 mCi/mmol, 1 Ci = 37 GBq; Pathfinder Lab, St. Louis, MO, USA) was evaluated in petioles in relationship to petiole orientation and wounding. Petioles were placed with the base (basipetal) or top (acropetal) portion of the petiole in contact with the medium containing 100 nmol ^{14}C-NAA. Additionally, petioles were wounded in the middle of the petiole by making a single transverse cut through one-half of the petiole at a 45° angle. The petioles were removed from the medium after 9 days and rinsed in deionized water to remove excess medium from the surface of the petiole. Each petiole was cut into three equal parts (~7 mm) and dried for 48 h at 50°C before being combusted in a Packard Tri-Carb 306 sample oxidizer. Collected $^{14}CO_2$ was quantified using a Beckman LS 9000 liquid scintillation system.

Each experiment was a completely randomized design with subsampling containing 15 petioles per treatment. Each experiment was repeated at least three times. Erlenmyer flasks were placed in a single growth chamber at 27°C with light provided for 16 h from fluorescent lamps at approximately 200 $\mu mol \cdot s^{-1} \cdot m^{-2}$.

Hand sections of the basal portion of the petioles were evaluated under a microscope to determine the type of rooting pattern found for each treatment. For the photomicrographs, petioles were fixed in FAA (formalin:acetic acid:ethanol), dehydrated using a tertiary-butanol series, and imbedded in Paraplast M. The 12-μm microtome sections were stained using safranin and fast green (Johansen 1940).

Results and Discussion

Debladed petioles from the juvenile phase of English ivy normally form adventitious roots through the direct pattern of root formation (Fig. 1) (Girouard 1967a,b, Geneve et al. 1988). However, juvenile petioles treated with NAA + DFMA were also able to initiate root primordia via the indirect pattern for root formation (Table 1, Fig. 2). In NAA + DFMA-treated cuttings, the number of roots formed was significantly greater than the control petioles treated with NAA alone. The pattern of root formation and the increase in root number per petiole could be reversed by the application of putrescine to the NAA + DFMA-treated petioles (Table 1). Delaying NAA application to petiole cuttings for 15 days also induced juvenile petioles to root by the indirect pattern. This could be reversed by incubating petioles on the basal medium for 15 days, then rewounding (removal of ~2 mm) the base of petioles prior to NAA application (Table 1). Putrescine alone did not affect rooting or the root formation pattern in either mature or juvenile phase petioles. Mature phase petioles failed to root in response to any treatment combination (data not shown).

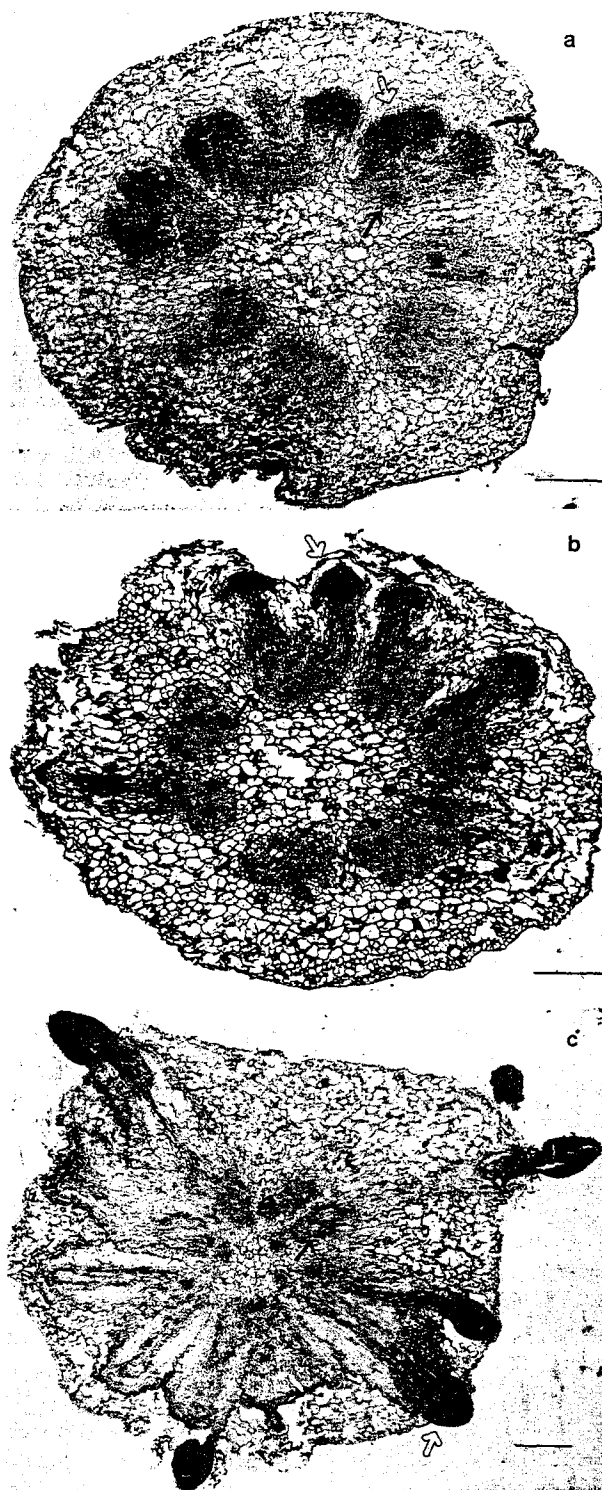There was no link between the easy-to-root condition and the pattern of root formation in juvenile

Fig. 1. The direct pattern of adventitious root formation in juvenile petioles of English ivy. Photomicrographs are cross sections of the lower (basal) section of the petiole treated with NAA (100 μM) after (a) 12 days, (b) 15 days, and (c) 18 days. Root primordia (open arrows) formed from phloem parenchyma cells associated with the vascular bundle (solid arrows). Reference bar equals 500 μm.

Table 1. Mean number of roots per petiole and the pattern of root formation in juvenile petioles of English ivy.

Treatment	Mean number of roots	Pattern of root formation
1. NAA (100 µM for 21 days)	9.2[a]	Direct
2. NAA (100 µM) + putrescine (1 mM) for 21 days	9.4[b]	Direct
3. NAA (100 µM) + DFMA (1 mM) for 21 days	12.7[c]	Indirect
4. NAA (100 µM) + DFMA (1 mM) + putrescine (1 mM) for 21 days	9.6[b]	Direct
5. Basal medium for 15 days prior to transfer to NAA (100 µM)	4.5[c]	Indirect
6. Basal medium for 15 days, recut base of petiole then move to NAA (100 µM)	7.9[d]	Direct

[a] Treatment means compared to control (NAA for 20 days) using Dunnett's test.
[b] Nonsignificant; [c,d] significant at 0.01, 0.05 level, respectively.

English ivy cuttings. Rather, the potential for root formation was inherent in the juvenile phase regardless of the rooting pattern. Also, the usual pattern of root formation in juvenile petiole cuttings was altered to the indirect pattern by using DFMA, a specific inhibitor of arginine decarboxylase. Previous studies with the debladed petiole rooting system have demonstrated a significant reduction in endogenous putrescine and spermidine levels after DFMA treatment (Geneve and Kester 1991). The reversal of this effect by additional putrescine suggested that putrescine was required for juvenile petioles to form roots via the direct pattern of root formation (Table 1). However, putrescine did not induce mature petioles to form roots implying that differential polyamine biosynthesis was not specifically responsible for the difference in rooting potential between the juvenile and mature phases of English ivy.

The anatomical events leading to adventitious root formation have been described as a series of three or four stages. Hartmann et al. (1990) characterized rooting as proceeding through a period of dedifferentiation, followed by formation of root initials leading to root primordia differentiation, and finally, growth and emergence of the root. The initial dedifferentiation period requires critical evaluation when determining the significant differences between easy and difficult-to-root cuttings.

Patterns for adventitious root formation observed for this study using English ivy support the use of the terms "competent root-forming cells" (CRFC) to describe the target cells involved in direct root primordia initiation and "induced competent root-
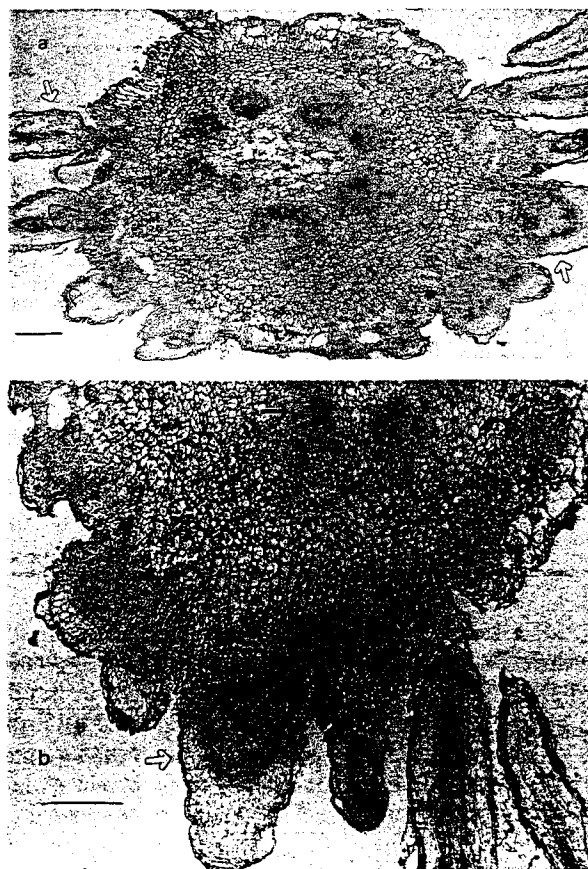

Fig. 2. The indirect pattern of adventitious root formation in juvenile petioles of English ivy treated with NAA (100 µM) plus DFMA (1 mM). (a) Cross section illustrating a petiole with 13 root primordia (open arrows) initiated from peripheral cell divisions in cortical parenchyma cells. (b) Close up of root primordia (open arrow) showing indirect pattern formation which lack a direct connection with the vascular bundle (solid arrow). Reference bar equals 500 µM.

forming cells" (ICRFC) to describe the cells involved in the indirect rooting pattern as detailed in Fig. 3. This terminology is similar to the description of direct and indirect patterns observed in somatic embryogenesis (Sharp et al. 1980, Williams and Maheswaran 1986). "Pre-embryogenic determined cells" (PEDC) is used to describe the direct pattern and "induced embryogenically determined cells" (IEDC) for the indirect pattern. To describe cells having the potential to form adventitious roots the term "competent" is used rather than "determined." The concept of competency better agrees with the scheme for organ pattern formation proposed by Christianson and Warnick (1988) and agrees with the use of "determined" to describe the later stage of root primordium development (Lovell and White 1986). Competency is the ability to re-
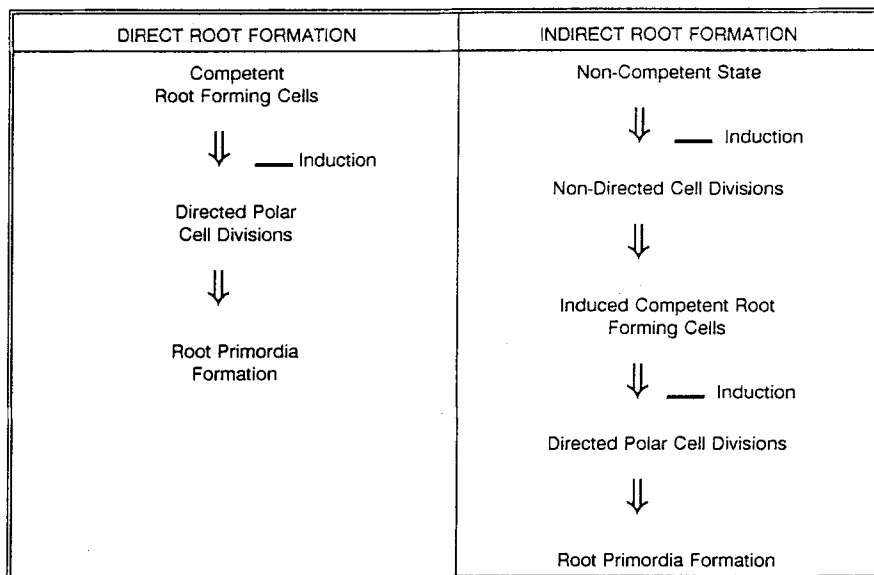
DIRECT ROOT FORMATION	INDIRECT ROOT FORMATION
Competent Root Forming Cells ⇓ —— Induction Directed Polar Cell Divisions ⇓ Root Primordia Formation	Non-Competent State ⇓ —— Induction Non-Directed Cell Divisions ⇓ Induced Competent Root Forming Cells ⇓ —— Induction Directed Polar Cell Divisions ⇓ Root Primordia Formation

Fig. 3. Proposed scheme for adventitious root primordium formation through the direct or indirect pattern of organ formation.

Table 2. The effect of timing of auxin application on root formation in juvenile English ivy petiole cuttings.

Number of days on basal medium prior to NAA (100 μM) treatment	Mean number of roots	Pattern of root formation
0	8.2[a]	Direct
3	8.4[b]	Direct
6	6.4[c]	Direct, indirect
9	5.1[c]	Direct, indirect
12	5.2[c]	Direct, indirect
15	4.5[d]	Indirect
18	3.1[d]	Indirect
21	2.1[d]	Indirect

Number of days on NAA (100 μM) medium prior to being moved to basal medium	Mean number of roots	Pattern of root formation
0	0[d]	—
1	1.2[d]	Direct
3	3.7[d]	Direct
5	5.0[d]	Direct
7	7.2[c]	Direct
9	8.8[b]	Direct
21	9.2	Direct

[a] Treatment means compared to control (NAA for 21 days) using Dunnett's test.
[b] Nonsignificant; [c,d]significant at .05, .01 level, respectively.

posure to NAA resulted in a higher number of roots formed.

Species possessing CRFC exhibit the direct pattern of root formation. CRFC are target cells competent to form adventitious roots in the presence of an inducer (Fig. 3). The inducer (in most cases, auxin), when applied exogenously or as it accumulates endogenously at the base of excised cuttings, initiates polar cell divisions which eventually lead directly to the formation of a root primordium. Tripepi et al. (1983) provided evidence for the early events occurring in CRFC. Using mung bean cuttings treated with radiolabeled uridine or thymidine, they were able to show that there was a localized increase in both DNA and RNA synthesis is specific cells throughout the hypocotyl associated with the sites of potential root primordia formation. They termed these sites, "rooting zone parenchyma." This synthesis was observed even though roots were only formed at the base of the cutting, where auxin would be accumulating. Increased synthesis was also observed with or without exogenous auxin, leading the authors to suggest that the localized increase in nucleic acid synthesis was a response to wounding. Using the proposed terminology, these target cells would be identified as CRFC. These CRFC have the potential to form root primordia, but only in the presence of auxin. This is supported by the observation that untreated mung bean cuttings formed a limited number of adventitious roots at the base of the cutting, while cuttings treated with NAA formed roots throughout the length of the hypocotyl (Tripepi et al. 1983), expressing the full potential of CRFC.

In the juvenile phase of English ivy, there was a

spond to an inductive signal, and cells from juvenile petioles exhibited a competent state by responding to as little as a 1 day application of auxin for the formation of roots (Table 2). However, longer ex-

Table 3. The effect of petiole orientation and wounding on the localization of radiolabel from ^{14}C-NAA in juvenile petioles of English ivy after 12 days of culture.

	Orientation[a]					
	Acropetal			Basipetal		
	nmoles of radiolabel from ^{14}C-NAA per segment					
	Top	Middle	Basal	Top	Middle	Basal
− Wound	15.07[b] ± 2.3	1.72 ± 0.4	5.19 ± 0.9	0.84 ± 0.1	1.14 ± 0.1	24.38 ± 2.6
+ Wound	15.93 ± 1.7	4.32 ± 0.5	4.98 ± 0.2	0.33 ± 0.3	1.15 ± 0.1	21.52 ± 2.3
	Mean number of roots					
	Top	Middle	Basal	Top	Middle	Basal
− Wound	4.4 ± 0.7	0	2.4 ± 0.8	0	0	8.8 ± 0.4
+ Wound	2.9 ± 0.1	1.75 ± 0.2	1.25 ± 0.5	0	0	9.2 ± 0.6

[a] Basipetal orientation had the basal (basipetal) and acropetal orientation had the top (acropetal) portion of the petiole in contact with the medium.
[b] Mean values ± SE.

close relationship between the location of adventitious roots and localized accumulation of radiolabel from ^{14}C-NAA (Table 3). Adventitious roots could be induced at the base (basipetal) or base and top (acropetal) portion of the petiole depending on the orientation of the petiole to the medium. Roots could also be induced to form in the middle of the petiole coincident with a wound in that location. In all cases, adventitious roots formed where radiolabel from ^{14}C-NAA was accumulating in a polar manner (Table 3). Although the possible conjugation of NAA and the exact cellular localization of the auxin was not determined in this study, the data suggest that there were many CRFC throughout the length of the petiole with the potential to respond to auxin to form adventitious roots. However, only the CRFC which receive a threshold amount of auxin respond to form adventitious roots.

The present study with English ivy suggests that both wounding and polyamine biosynthesis play an integral part in the maintenance of the competent state. The prevention of polyamine biosynthesis by DFMA or the delay in application for the inducer (auxin) for 15 days causes target cells in the juvenile phase of English ivy to lose their competent state and for root primordia formation to proceed via the indirect rooting pattern (Table 1). The resultant loss of the competent state by delaying auxin application appeared to be gradual with a transition phase where roots formed by both the direct and indirect pattern (Table 2).

A loss of the competent state could be used to explain the observation of Gronroos and Von Arnold (1987) working with hypocotyl cuttings of *Pinus contorta* rooted in vitro. Hypocotyl cuttings treated with indolebutyric acid (IBA) formed roots

via the direct pattern presumably from CRFC. However, cuttings that were not treated with auxin required a longer time to root and formed roots indirectly from callus "wound" tissue. The period of time where endogenous auxin levels may have been below a critical threshold required to induce CRFC to initiate root primordia may have led to a loss of the competent state.

In the absence or loss of a competent state, adventitious root formation would take place via an indirect rooting pattern (Fig. 2). The target cells for adventitious root primordia formation during the indirect rooting pattern would be found within the actively dividing cells of the cortical parenchyma. These target cells would be termed "induced-competent root-forming cells" (ICRFC). In the case of indirect root formation, there would be two periods when induction must take place (Fig. 3). First, an inducer (auxin + wounding) would be required to initiate nondirected cell division in the cortical parenchyma. During these cell divisions, a cell or group of cells (ICRFC) would become competent to respond to an inducer (auxin) to initiate polar cell divisions leading to root primordia formation.

For petiole explants of English ivy, the potential to form ICRFC was related to the overall rooting potential of the cutting. Juvenile phase petioles induced to form ICRFC have a greater potential to form adventitious root primordia compared to the difficult-to-root mature phase when cultured under similar conditions (Table 1). In fact, the greater number of roots formed in juvenile petioles treated with NAA + DFMA may be the result of an increased number of target cells located in dividing parenchyma cells (Table 1 and Fig. 2). Conversely, the poor rooting performance of difficult-to-root

species may be directly related to the inability to form ICRFC. The study of the events leading to ICRFC in the easy-to-root juvenile phase of English ivy may provide useful insight into the difficult-to-root condition observed in many woody species.

References

Christianson ML, Warnick DA (1988) Organogenesis in vitro as a developmental process. HortScience 23:515–519

Davies FT Jr, Lazarte JE, Joiner JN (1982) Initiation and development of roots in juvenile and mature leaf bud cuttings of *Ficus pumila* L. Am J Bot 69:804–811

Hackett WP (1988) Donor plant maturation and adventitious root initiation. In: Davis JD, Haissig BE, Sankhla N (eds) Adventitious root formation in plants. Dioscorides Press, Portland, OR, pp 11–28

Hartmann HT, Kester DE, Davies FT Jr (1990) Plant propagation: Principles and practices, 4th ed. Prentice Hall, Englewood Cliffs, NJ

Geneve RL, Hackett WP, Swanson BT (1988) Adventitious root initiation in de-bladed petioles from the juvenile and ma-

ture phases of English ivy. J Am Soc Hortic Sci 113:630–635

Geneve RL, Kester ST (1991) Polyamines and adventitious root formation in the juvenile and mature phase of English ivy. J Exp Bot 42:71–75.

Girouard RM (1967a) Initiation and development of adventitious roots in stem cuttings of *Hedera helix*. Anatomical studies of the mature growth phase. Can J Bot 45:1883–1886

Girouard RM (1967b) Initiation and development of adventitious roots in stem cuttings of *Hedera helix*. Anatomical studies of the juvenile growth phase. Can J Bot 45:1887–1889

Gronroos R, Von Arnold S (1987) Initiation of roots on hypocotyl cuttings of *Pinus contorta* in vitro. Physiol Plant 69:227–236

Johansen DL (1940) Plant microtechnique. McGraw Hill, New York

Lovell PH, White J (1986) Anatomical changes during adventitious root formation. In: Jackson MB (ed) New root formation in plants and cuttings. Martinus Nijhoff Publishers, Boston, pp 111–140

Sharp WR, Sondahl MR, Caldas LS, Maraffa SB (1980) The physiology of in vitro asexual embryogenesis. Hortic Rev 2:268–310

Tripepi RR, Heuser CW, Shannon JC (1983) Incorporation of tritiated thymidine and uridine into adventitious root initial cells of *Vigna radiata*. J Am Soc Hortic Sci 108:469–474

Williams EG, Maheswaran G (1986) Somatic embryogenesis: Factors influencing coordinated behavior of cells as an embryogenic group. Ann Bot 57:443–462