J Plant Growth Regul (1995) 14:49–59

Journal of
Plant
Growth
Regulation

Auxins and Polyamines in Relation to Differential *in Vitro* Root Induction on Microcuttings of Two Pear Cultivars

R. Baraldi,[1,*] G. Bertazza,[1] A. M. Bregoli,[2] F. Fasolo,[1] A. Rotondi,[1] S. Predieri,[1] D. Serafini-Fracassini,[2] J. P. Slovin,[3] and J. D. Cohen[4]

[1]Istituto di Ecofisiologia delle Piante Arboree da Frutto, CNR, Via Gobetti, 101, 40129 Bologna, Italy; [2]Dipartimento di Biologia Evoluzionistica Sperimentale, Università di Bologna, Via Irnerio, 42, 40126 Bologna, Italy; [3]Climate Stress Laboratory and [4]Horticultural Crops Quality Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, USDA, Beltsville, Maryland 20705, USA

Received July 7, 1994; accepted November 15, 1994

Abstract. The internal levels of indole-3-acetic acid (IAA) and polyamines (PAs) and the metabolism of indole-3-butyric acid (IBA) were studied in relation to the in vitro rooting process of two pear cultivars, the easy-to-root Conference and the difficult-to-root Doyenne d'Hiver. Doyenne d'Hiver required about a 10 times higher concentration of IBA to achieve a rooting percentage similar to that of Conference. One- or two-day exposures to IBA were sufficient to stimulate rooting but with different efficiency for each cultivar. Longer exposure to auxin strongly increased the root number in Conference, whereas root elongation was inhibited in both cultivars. The metabolism of IBA in both cultivars was not significantly different when IBA was used at a high concentration to stimulate maximal rooting in Doyenne d'Hiver. IBA was mainly conjugated into IBA glucose, which was accumulated, and a small amount was converted into free IAA in both cultivars. However, in Doyenne d'Hiver this metabolic pathway appears to be active only at a higher exogenous IBA concentration. At a high IBA concentration more callus was formed by Doyenne d'Hiver, indicating that the cells of Doyenne d'Hiver are not capable of responding to the hormone in the same manner as Conference cells. Anatomic observations indicated that the capacity to induce initial dividing cells was more efficient in Doyenne d'Hiver, but subsequently the number of root primordia formed and root development were much reduced relative to Conference. A possible correlation between these processes and an early increase followed by a decrease of free IAA was seen in Conference. By day 4, a significant increase in IAA conjugates and free putrescine was observed in Doyenne d'Hiver. This higher putrescine content may be related to the lower amount of root development. Together with previous studies these results indicate that differences in the uptake and metabolism of applied auxins may affect rooting ability and the subsequent development of adventitious roots in microcuttings of pear.

The formation of adventitious roots depends on numerous endogenous factors, among which growth substances such as auxins and polyamines are believed to play important roles (Altman 1989, Batten and Goodwin 1978). Auxins appear to be the primary phytohormones involved in this process since the application of synthetic auxins alone can stimulate root initiation. However, the promoting effect of auxins varies among species and cultivars, making it difficult to understand the mechanism of their regulatory action. Generally, indole-3-butyric acid (IBA) is considered to be a better rooting promoter than indole-3-acetic acid (IAA; Wiesman et al. 1988). It has been suggested that IBA has a higher resistance to oxidation because of the side chain length and therefore persists at the site of induction longer than IAA (Fawcett et al. 1960).

IBA has been shown to be converted to IAA by

Abbreviations: IBA, indole-3-butyric acid; IAA, indole-3-acetic acid; PA(s), polyamine(s); HPLC, high pressure liquid chromatography; GC-MS, gas chromatography-mass spectrometry; TCA, trichloroacetic acid; dansyl, 1-dimethylaminonaphthalene-5-sulfonyl; TLC, thin layer chromatography; TBA, terbutilic alcohol; IBAGluc, IBA glucose; IAAGluc, IAA glucose; IAAsp, IAA aspartate.

* Author for correspondence.

Table 1. Effect of IBA and IAA concentrations on rooting percentage, number of roots, and mean root length of Conference microcuttings. Values are the means of three experiments ± standard error. Microcuttings were kept in contact with hormone for the entire experimental period. Data were collected after 21 days.

Concentration (μM)	Rooting %		Root number/rooted shoot		Mean root length (mm)	
	IBA	IAA	IBA	IAA	IBA	IAA
0	31 ± 13	31 ± 13	2.1 ± 1.4	2.1 ± 1.4	18.9 ± 2.7	18.9 ± 2.7
0.5	39 ± 10	45 ± 13	2.2 ± 1.3	3.3 ± 1.1	15.2 ± 2.5	18.3 ± 2.1
1.5	84 ± 10	53 ± 15	3.4 ± 0.9	5.3 ± 1.1	17.1 ± 1.8	13.4 ± 1.6
5	89 ± 6	70 ± 13	4.5 ± 0.9	4.5 ± 0.8	8.1 ± 1.6	16.5 ± 1.7
15	95 ± 5	72 ± 6	6.3 ± 0.8	4.8 ± 0.9	4.6 ± 1.9	11.1 ± 1.8

Table 2. Effect of IBA and IAA concentrations on rooting percentage, number of roots, and mean root length of Doyenne d'Hiver microcuttings. Values are the means of three experiments ± standard error. Microcuttings were kept in contact with hormone for the entire experimental period. Data were collected after 21 days.

Concentration (μM)	Rooting %		Root number/rooted shoot		Mean root length (mm)	
	IBA	IAA	IBA	IAA	IBA	IAA
0	13 ± 4	13 ± 4	1.1 ± 0.1	1.1 ± 0.1	77.8 ± 8.5	77.8 ± 8.5
0.5	21 ± 10	25 ± 11	2.1 ± 0.9	1.1 ± 0.1	34.6 ± 7.6	59.3 ± 6.7
1.5	45 ± 13	41 ± 8	2.2 ± 0.7	1.7 ± 0.8	10.7 ± 7.6	34.6 ± 5.9
5	42 ± 11	63 ± 8	2.9 ± 0.7	2.5 ± 0.6	16.5 ± 5.2	31.1 ± 4.4
15	83 ± 7	67 ± 12	5.9 ± 0.5	3.2 ± 0.6	4.9 ± 3.2	23.7 ± 4.1

several plant species (Epstein and Lavee 1984, Van der Krieken et al. 1992, Wiesman et al. 1988). Conjugate formation after the application of IBA is possibly also important in rooting because conjugation can serve as a mechanism to protect auxins from oxidation and allow for the slow release of free auxin (Cohen and Bandurski 1982, Wiesman et al. 1989). Although some reports have attempted to correlate endogenous auxin levels and metabolism with adventitious root formation, conflicting information exists concerning changes in naturally occurring IAA. In some cases, an early increase in IAA levels followed by a decline was observed during the rooting process (Blakesley et al. 1991, Moncousin 1988, Norcini et al. 1985). In other cases, no increase in IAA concentration was found, but instead only a gradual decline was measured (Berthon et al. 1989, Blakesley et al. 1991).

Because the levels of free polyamines (PAs) in auxin-treated tissues have been shown to change before cell division or root formation (Sankhla and Upadhyaya 1988, Serafini-Fracassini et al. 1980) it is also important to understand the relationship of PA metabolism to changes in hormonal metabolism and levels. In addition, the need for free PAs to sustain cell division in plants has been well established (Bagni et al. 1982, Serafini-Fracassini 1991). Less, however, is known about their involvement in organogenesis. Even though application of PAs or PA inhibitors sometimes produces conflicting results, PAs do appear to be involved in rhizogenesis as an increase in free putrescine and spermidine levels as well as an increase in the activity of their biosynthetic enzymes occur in many different kinds of plant tissue cultures which have been induced to root (Chriqui et al. 1986, Desai and Metha 1985, Torrigiani et al. 1989, 1991). PA content is low when tissues are either completely differentiated or when rhizogenesis or callogenesis ceases to occur (Torrigiani et al. 1989). In addition to free PAs, bound PAs also appear to be involved in rhizogenesis; in fact, free and bound PAs increase when root meristems appear, much before the visible roots emerge (Biondi et al. 1990).

A clear correlation between rhizogenesis and growth substances is still more problematic in woody plants, in part because of marked variability among species and cultivars. In a preliminary study on in vitro adventitious root formation, Baraldi et al. (1993) suggested that auxin uptake and metabolism were related to rooting ability in pear microcuttings. In fact, the easy-to-root cultivar showed a faster and higher uptake of labeled IBA and the ability to convert IBA into free IAA very early during the root induction period. We now report results from further experiments carried out using the same two pear cultivars, the easy-to-root Conference and the difficult-to-root Doyenne d'Hiver. In this study

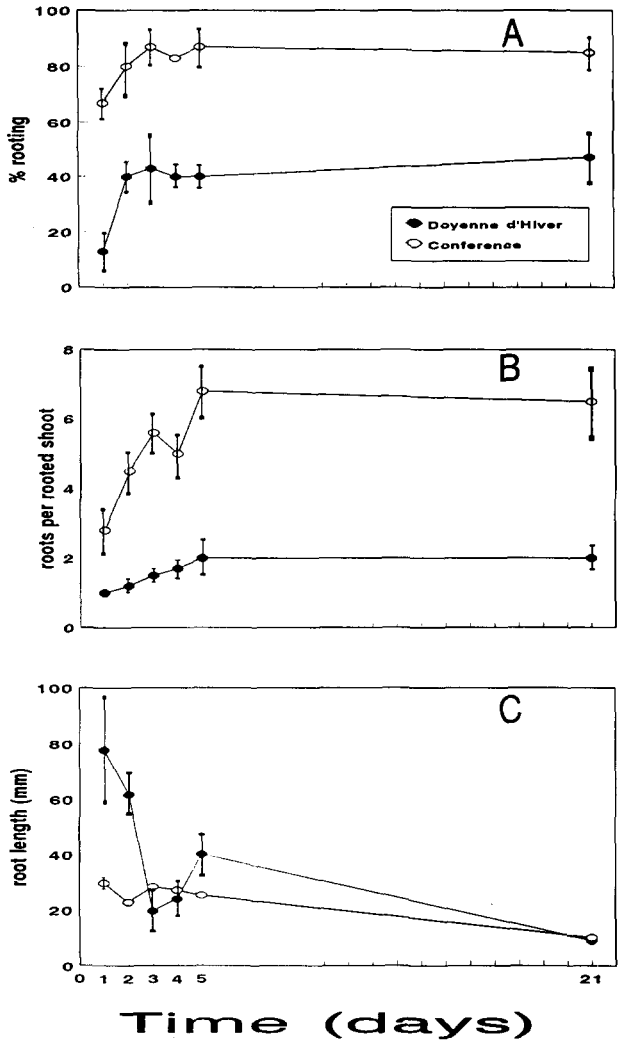

Fig. 1. Relationship between length of exposure to 1.5 μM IBA and rooting percentage (**A**), number of roots per rooted shoot (**B**), and root length (**C**) in Conference and Doyenne d'Hiver. Values are the means of three replicates/treatment. *Bars* represent ± SE (not drawn if smaller than symbols).

we have: (1) analyzed the role of exogenous auxins in in vitro rhizogenesis; (2) determined the internal levels of auxins and polyamines; and (3) related these changes to histologic data obtained during the rooting process. The few papers that have described physiologic parameters such as auxins, PAs, or nutrient uptake and translocation related to the rooting process in woody species such as *Malus*, *Vitis*, and *Olea* (Epstein and Lavee 1984, Moncousin 1988) wild cherry (Label et al. 1989), *Tuja occidentalis* L. (Bender et al. 1987), and *Prunus avium* (Biondi et al. 1990) often do not report detailed histologic information. No histologic information is available on rooting in *Pyrus communis*, although limited anatomic details on *Pyrus calleriana* were reported by Berardi et al. (1992). A few authors have studied the in vitro rooting process solely from a histologic point of view in woody species such as *Malus* (Hicks 1987, Sutter and Luza 1993, Zhou et al. 1992).

Materials and Methods

Rooting Response to Exogenous Auxins

Single shoots, 15 mm long, of two micropropagated pear cultivars, Conference and Doyenne d'Hiver, were excised from proliferating cultures and placed in 100 ml of rooting medium in glass jars (500 ml, Bormioli, Parma, Italy). The rooting medium consisted of half-strength Murashige and Skoog (1962) salts and Linsmaier and Skoog (1965) vitamins supplemented with 3% (w/v) sucrose and 0.65% (w/v) agar (B & V, Parma, Italy). For root induction, IBA and IAA were used at 0, 0.5, 1.5, 5.0 or 15 μM. The medium was adjusted to pH 5.7 and autoclaved at 120 °C for 20 min. Shoots in the rooting medium were kept in the dark for 7 days at 24 °C for root induction and then transferred to a 16-h/day photoperiod at a photosynthetic photon flux density of 60 μmol m^{-2} s^{-1}, provided by cool white fluorescent lamps, for the remainder of the experimental period (21 days).

Influence of Length of Exposure to IBA on Rooting

In the time course experiments, 20 single shoots were left in contact with 1.5 μM IBA in agar-solidified medium for 1, 2, 3, 4, 5, and 21 days. Subsequently, shoots were transferred to a similar medium lacking IBA and kept under the experimental conditions described above for 21 days total. Control shoots were kept in the IBA-lacking medium for the entire experimental period.

IAA and PA Content during the in Vitro Rooting Process

Stock cultures were stored at 4 °C. These cultures were removed from the cold, transferred to fresh medium, multiplied and grown in a growth chamber at 24 °C. From these proliferating cultures shoots were harvested for rooting. Endogenous auxins and PAs were determined for shoots cultured in the dark in 100 ml of agar-solidified rooting medium supplemented with 1.5 μM IBA for 7 days. Shoots, ranging from 0.5 to 1 g fresh weight, were excised at 0, 12 h, 1, 2, 4, or 7 days of culture, immediately frozen with liquid nitrogen, and ground to a fine powder.

For auxin analyses, samples were extracted in 65% isopropyl alcohol (v/v) with 0.2 M imidazole buffer at pH 7 to which [^{3}H]IAA as a radiotracer and [$^{13}C_6$]IAA (0.1–1 μg g^{-1} sample) as an internal standard for quantitative mass-spectral analysis were added. After overnight isotope equilibration, the analyses of free and conjugated IAA (esters + amides) were performed according to Chen et al. (1988). The instrument used for HPLC purification of IAA was a Beckman System Gold coupled to an UV

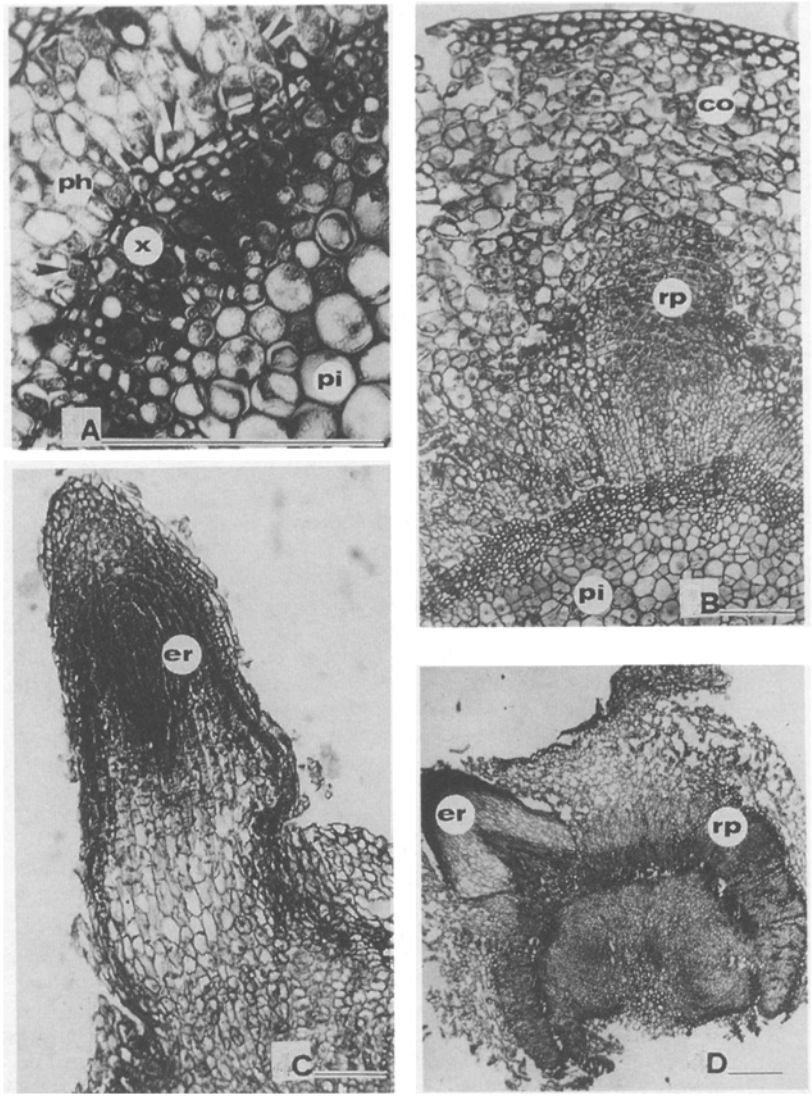

Fig. 2. Transverse sections of Conference and Doyenne d'Hiver microcuttings grown on a 1.5 μM IBA-enriched medium. *Bars* indicate 100 μm, except where indicated. *pi*, pith; *co*, cortex. Shown are the distribution and dimension of schlerenchyma fiber bundles in microcuttings of Conference (**A** and **C**) and Doyenne d'Hiver (**B** and **D**).

Table 3. In vitro rooting of Conference and Doyenne d'Hiver microcuttings during the first 8 days of subculture with 1.5 μM IBA. Values refer to the percentage of explants examined which had at least one initial cell or root primordium or adventitious-root.

	Days of culture						
	0	1	2	4	5	7	8
Conference							
Initial cells	0	0	50	33	29	22	7
Root primordia	0	0	0	15	29	44	35
Roots	0	0	0	0	0	0	46
Doyenne d'Hiver							
Initial cells	0	0	0	75	71	29	10
Root primordia	0	0	0	0	0	14	30
Roots	0	0	0	0	0	0	10

detector (Varian UV 50). Auxins were separated by chromatography on a 5-μm C_{18} Partisphere column (Whatman, 110 × 5 mm, inner diameter), at 1 ml min^{-1} with 20% acetonitrile/water and 1% acetic acid.

Quantitative auxin analyses were carried out on a GC-MS (Hewlett Packard 5890-5970) equipped with a 12-m Chrompack CPSil 19 capillary column (inner diameter 0.25 mm; film thickness 0.25 μm). The carrier gas was helium at 1 ml min^{-1}, GC injector was at 280 °C, and the oven temperature was increased from 50 to 110 °C at a rate of 30 °C min^{-1} then at a rate of 6 °C min^{-1} until 280 °C. The source temperature was 270 °C, and ionizing voltage was 70 eV. Ions monitored were *m/z* 130 and 136 for the base peak (quinolinium ion) and 189 and 195 for the molecular ion of the methyl ester of IAA and methyl ester of [$^{13}C_6$]IAA, respectively. Ratios of 130:136 and 189:195 were used to calculate endogenous levels of IAA and to verify the analysis.

For PA determinations, samples were extracted in 3 volumes of 5% cold TCA plus 5 mM 1,7-diaminoheptane as internal standard. Extracts were centrifuged for 15 min at 3,000 ×g and the supernatants washed three times with diethyl ether (1:1, v/v) to eliminate lipids. The pellets were washed twice in 3 volumes of TCA and resuspended in the original volume of TCA. Aliquots (0.3 ml) of this suspension and of the original supernatant were hydrolyzed at 110 °C for 18 h in 6 N HCl. The hydrolysates were dried under vacuum at 60 °C and resuspended in the original volume of TCA. Aliquots (0.1 ml) of the original supernatants (from which free PAs were determined), the hydrolyzed supernatants (from which the TCA-soluble bound PAs were determined by subtracting the free levels), and the hydrolyzed pellets (containing the TCA-insoluble bound PAs) were dansylated in the dark for 18 h at room temperature with 2 volumes of dansylchloride solution (3 mg ml^{-1} in acetone). The pH was adjusted to 8.6 with 50 mg of $NaHCO_3$. The excess of dansylchloride was converted to dansylproline by adding 0.1–0.3 ml of proline (15 mg ml^{-1} distilled water). After a 30-min incubation, dansylated PAs were extracted with 5 volumes of anhydrous benzene and cochromatographed with dansylated standards on TLC precoated plates of Silica Gel 60 with a concentration zone (Merck). Chloroform/triethylamine (5:1, v/v) or cyclohexane/ethylacetate (3:2, v/v) was used as running solvent. Fluorescence of PA spots, scraped and eluted in 2 ml of acetone, were measured using a Jasco FP-550 spectrofluorometer (excitation 360 nm, emission 506 nm).

The experiments were repeated three times, and the analyses were performed on three samples for each trial.

Histoanatomic observations were performed on shoots to follow root formation and development. For these observations, five shoots were sampled at 0, 1, 2, 4, 5, 7, 8, and 21 days, fixed in FAA (37% formaldehyde, glacial acetic acid, 100% ethanol, distilled water 10:5:50:35 by volume), dehydrated with TBA,

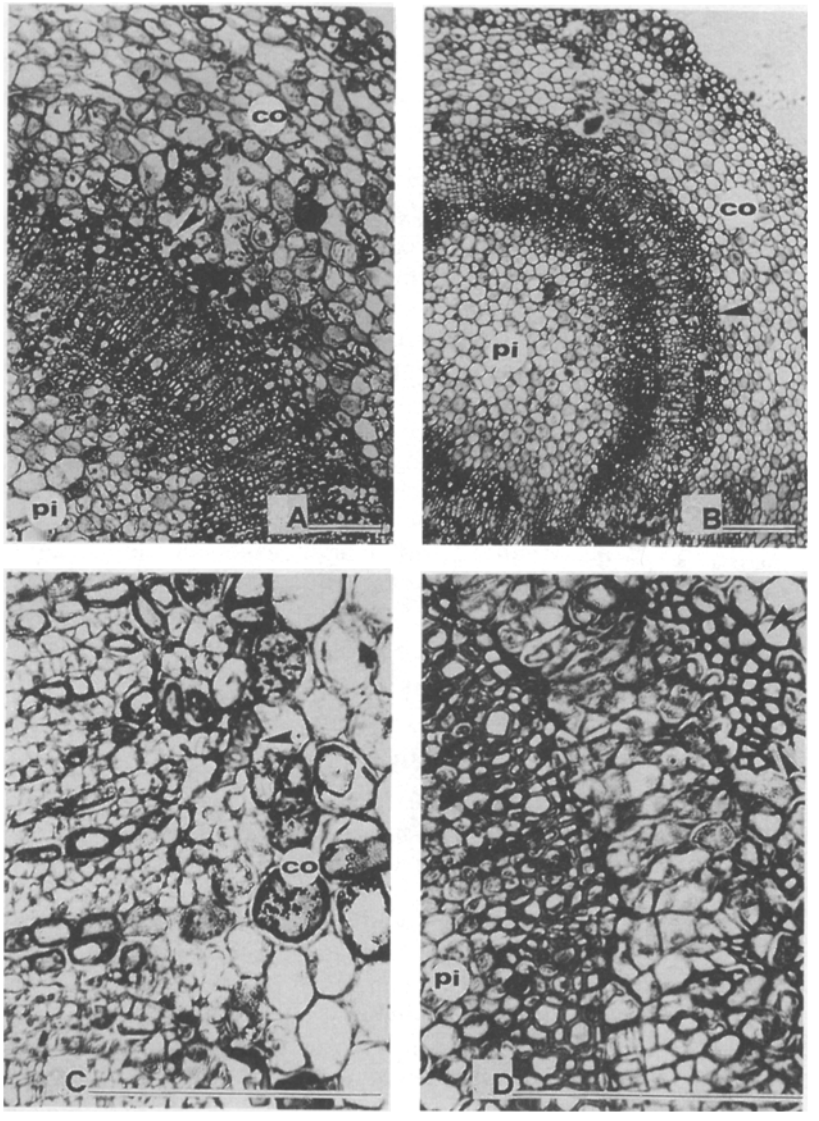

Fig. 3. Transverse sections as in Figure 2. Shown is the development of adventitious roots in Conference in response to auxin treatment. **A**, transverse section at day 2; *arrows* designate the position of initial cells. **B**, root primordium at day 4. **C**, protruding root at 8 days. **D**, microcutting at day 8 showing the asynchrony of the rooting process: one root primordium and one emergent root are present at the same time. *Bar* indicates 100 μm in **A**, **B**, and **C** and 300 μm in **D**. *ph*, phloem; *x*, xylem; *pi*, pith; *co*, cortex; *rp*, root primordium; *er*, emergent root.

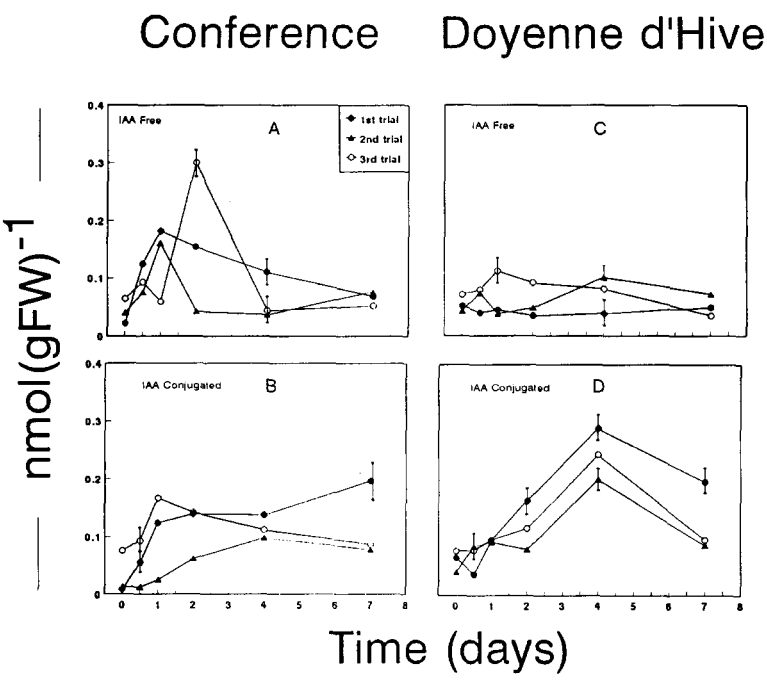

Fig. 4. Changes in the level of endogenous free (**A** and **C**) and conjugated (**B** and **D**) IAA in 1.5 μM IBA-treated microcuttings of Conference (*left*) and Doyenne d'Hiver (*right*) during root induction. *Bars* represent ± SE (not drawn if smaller than symbols).

then embedded in paraffin. Cross-sections of 8–10 μm were stained with safranin-fast green (Jensen 1962).

Effect of the High IBA Concentration on Auxin Metabolism

To evaluate IBA metabolism in microcuttings of both cultivars, single shoots were placed in the dark for 24 h in 1.5-ml disposable centrifuge tubes containing 20 μl of liquid rooting medium to which 15 μM [^{14}C]IBA (prepared from [^{14}C]indole, Research Products International, Mt. Prospect, IL, 50 mCi/mmol, by the method of Cohen and Shulze 1981) was added. The centrifuge tube was left uncapped for about 2 h under aseptic conditions to increase the uptake of label by transpiration. Shoots were washed, immediately frozen with liquid nitrogen, and ground in 80% acetone/water (v/v) using a mortar and pestle. Analyses of the labeled IBA and metabolite extracts were performed by TLC and radioimaging and identified by R_F of standards. The extracts were applied to Silica Gel 60 TLC plates, which were developed in methyl ethyl ketone:ethyl acetate:ethanol:water (3:5:1:1, v/v; Labarca et al. 1965). After drying, the plates were placed in an AMBIS model 1000 for radioactivity imaging and counting.

For all of the rooting experiments, the rooting percentage (expressed as the percentage of shoots producing at least one root), the average number of roots per rooted shoot, and root length were recorded after 21 days. Each treatment consisted of 30 explants, and experiments were conducted three times. Histoanatomic observations were also preformed on the shoots to evaluate the effect of high IBA concentration on root formation.

Results

Rooting Response to Exogenous Auxins

Thirty percent of the Conference explants rooted even without auxin in the rooting medium (Table 1). A much lower value was observed for Doyenne d'Hiver (Table 2). When treated with 1.5 μM IBA, 84% of the Conference shoots rooted, and increasing the hormone concentration did not increase root production significantly (Table 1). When IAA was added to the rooting medium at 1.5–15 μM rooting percentages varied between 53 and 72%. The highest rooting percentage for Doyenne d'Hiver was obtained with 15 μM IBA (83%) or 5–15 μM IAA (63–67%; Table 2). At all of the other concentrations tested for both auxins, root production was lower. The higher concentrations of either auxin generally enhanced the number of roots per rooted shoot and also reduced root length, especially in Doyenne d'Hiver.

Influence of Length of Exposure to IBA on Rooting

For Conference shoots, root initiation occurred with 1 day of auxin application (Fig. 1A). In contrast, microcuttings of Doyenne d'Hiver were significantly stimulated only after at least 2 days of exposure (40%).

In shoots of both cultivars the number of roots per rooted microcuttings generally increased up to the 5-day treatment (Fig. 1B). However, the microcuttings of Conference produced about six roots after exposure to IBA-enriched medium for 5 or more days, whereas under the same experimental conditions, the number of roots per rooted shoot of Doyenne d'Hiver did not exceed two. The presence

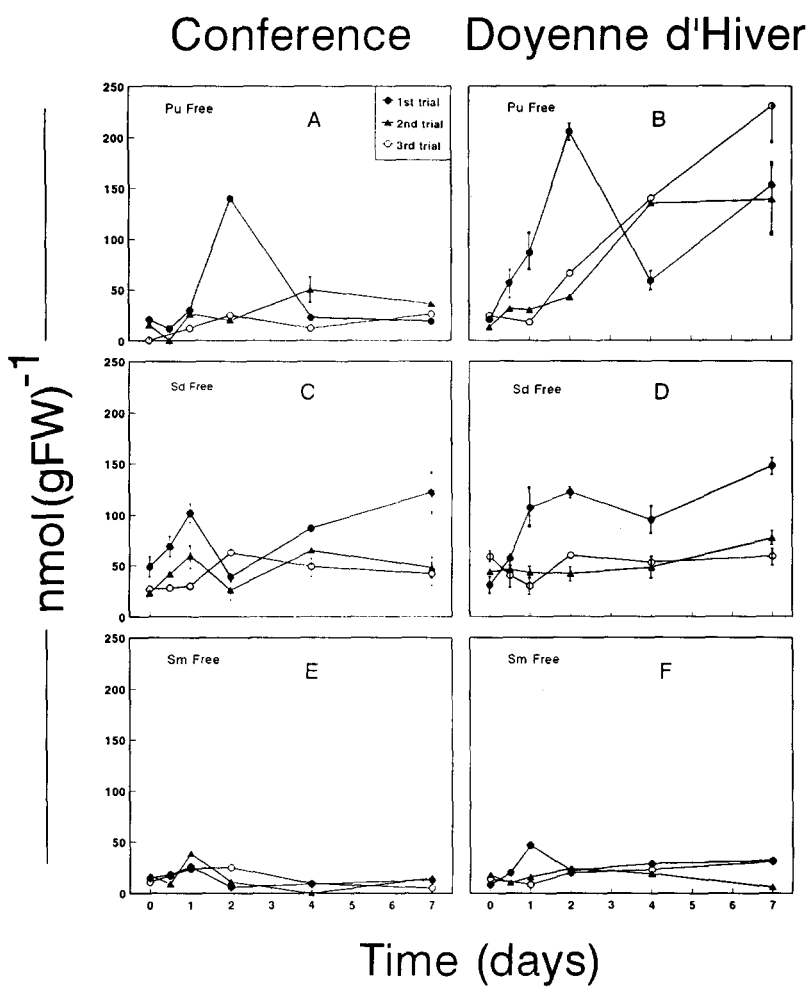

Fig. 5. Changes in the level of endogenous free PA in 1.5 μM IBA-treated microcuttings of Conference (*left*) and Doyenne d'Hiver (*right*) during root induction. Free putrescine (**A** and **B**), spermidine (**C** and **D**), and spermine (**E** and **F**). *Bars* represent ± SE (not drawn if smaller than symbols).

of auxin for the entire rooting period did not increase the root number while significantly inhibiting root elongation in both cultivars (Fig. 1C).

Progress of Adventitious Root Development

All of the histologic observations related to the rooting process were made within a narrow band of 0.5 mm above the base of the microcuttings, which is the region of root emergence. No evidence of rooting activity was found in more apical sections of the shoot. Shoots harvested from multiple shoot cultures had very limited secondary tissue development prior to root induction, and no root initial cells could be detected at that time. Very few single phloem fibers were observed on Conference shoots (Fig. 2, **A** and **C**), whereas 100% of Doyenne d'Hiver microcuttings showed sclerenchyma fiber bundles external to the phloem (Fig. 2, **B** and **D**). Anatomically, these fiber bundles, however, are not a mechanical hindrance for the centrifugal root development arising from the initial cells since no primordia were formed in the competent areas located innermost of the correspondent fiber clusters.

In Conference shoots, the first anatomic changes were observed 3 days after 1.5 μM IBA treatment, when some shoots showed cells in the cambial zone or in the phloem adjacent to it which had more densely stained cytoplasm and nuclei (Table 3 and Fig. 3A). On day 4, the first root primordia were detected, some of which had started to differentiate and extend outward into the cortex (Fig. 3B).

At day 8, 35% of shoots had at least one primordium, and 46% of them had at least one emergent root at the shoot surface. At that time the root cap and the first tracheary elements of the roots were visible (Fig. 3C). The appearance of initial cells and root primordia occurred later in Doyenne d'Hiver than in Conference. Although 75% of the Doyenne

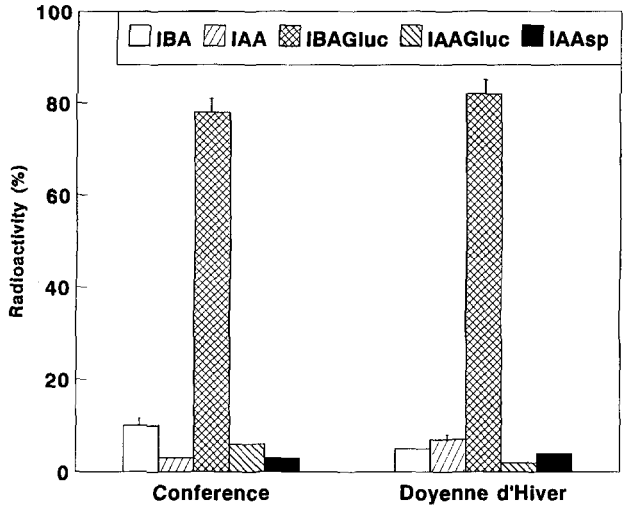

Fig. 6. Distribution of the total extractable radioactivity detected on TLC at day 1 in 15 μM IBA-treated shoots of Conference and Doyenne d'Hiver. *Bars* represent ± SE.

d'Hiver shoots had initial cells on day 4, by day 8 only 10% of them had emergent roots (Table 3). The processes of initiation and development of adventitious roots were not synchronous. In fact, in sections of both cultivars we observed different stages of root development in a single microcutting (Fig. 3D).

IAA and PA Content during the in Vitro Rooting Process

The analysis of both free and conjugated IAA (esters and amides) of 1.5 μM IBA-treated shoots showed that at the time of excision, auxin levels were relatively low in both cultivars (Fig. 4). In Conference shoots, free IAA levels always increased severalfold over the first 1 or 2 days and then decreased to the initial levels by day 7 (Fig. 4A). Microcuttings of Doyenne d'Hiver did not exhibit any consistent variation in endogenous free IAA content throughout the rooting period and always remained at low levels (Fig. 4C). Conjugated IAA increased slightly in Conference microcuttings during the root induction period (Fig. 4B), whereas in Doyenne d'Hiver a steady increase of IAA conjugates was observed over the first 4 days, followed by a decrease at the time when the first root primordia were observed (Table 3).

In Doyenne d'Hiver shoots free putrescine levels increased six- to tenfold in the first 7 days of culture (Fig. 5B), whereas in Conference there was, in general, little change (Fig. 5A). As seen in Table 3, root primordia initiation was delayed in Doyenne d'Hiver relative to Conference; therefore, although putrescine levels were essentially the same at the beginning of the experiment, the putrescine content in Doyenne d'Hiver was actually higher than in Conference at the onset of root primordia formation. In Conference microcuttings spermidine double in coincidence with the peak of IAA occurring at day 1 or 2 (Fig. 5C). No consistent trends between cultivars were noted in free spermine levels (Fig. 5, E and F). The levels of bound PAs were always very low in both cultivars (data not shown).

Effect of High IBA Concentration on Auxin Metabolism

Auxin metabolism was studied in microcuttings treated with 15 μM IBA, a concentration that allowed a similar rhizogenetic response in both cultivars.

At 24 h after application, all of the labeled IBA was taken up by both cultivars. Only 5 and 10% of the total radioactivity from [^{14}C]IBA was found as free IBA in Doyenne d'Hiver and Conference cultivars, respectively (Fig. 6). About 80% of the ^{14}C label chromatographed as IBAGluc; smaller percentages of radioactivity, corresponding to other metabolites, were observed in both cultivars. The R_F of these metabolites suggested that they were free IAA, IAAGluc, and IAAsp. The relative amounts of free and conjugated IAA were not statistically different between the cultivars. Histologic studies of cuttings of both cultivars in 15 μM IBA showed high meristematic activity in the region of the phloem and the involvement of medullary parenchyma in rhizogenesis (Fig. 7A). It is important to note that this high IBA concentration also caused undesirable callus formation at the shoot base, particularly in Doyenne d'Hiver, reducing the survival of plants during the acclimatization phase. In addition, some root primordia forked and developed into two emergent roots (Fig. 7, B and C) with a clear vascular connection with the stem by day 8 (Fig. 7D).

Discussion

The data presented here demonstrate that microcuttings provide a useful model for conducting physiologic experiments. In fact, one can work with a large population of uniform plant material, microcuttings readily take up compounds from the media, and experiments can be conducted under

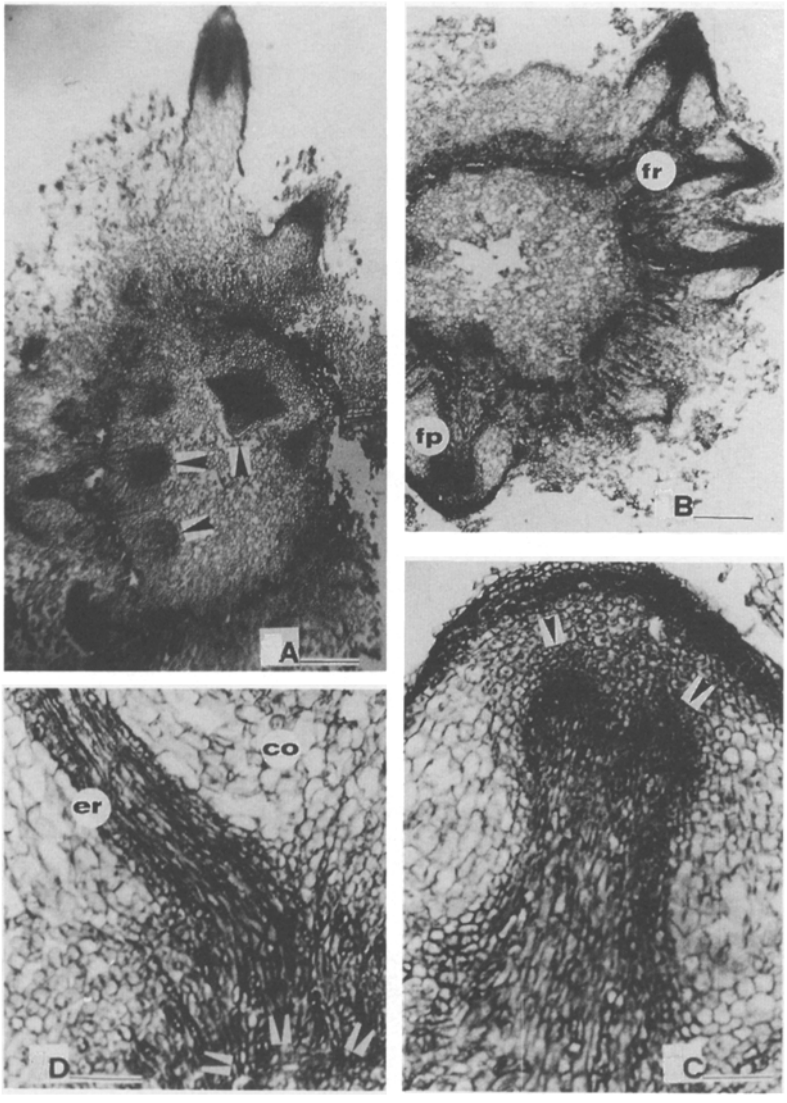

Fig. 7. Transverse section of Conference microcutting at day 8 after high IBA treatment (15 μM). Several root primordia and roots are evident; *arrows* show root primordium in the pith (**A**). Forked root primordia and forked roots (**B**). Higher magnification of the forked primordium (**C**). *Arrows* show vascular connection between stem and root (**D**). *Bar* indicates 300 μm in **A** and **B** and 100 μm in **C** and **D**. *fr*, fork root; *fp*, fork root primordia; *er*, emergent root; *co*, cortex.

sterile conditions that eliminate artifacts due to microbial metabolism.

A previous work (Baraldi et al. 1993) had shown that at 1.5 μM IBA only Conference metabolized the IBA to detectable levels of IAA. We now show that Doyenne d'Hiver is capable of this conversion but at a 10 times higher concentration of the applied IBA. This concentration induces similar rooting percentages in both cultivars, although the macroscopic growth of callus observed, particularly with Doyenne d'Hiver, may suggest a different response of the competent cells to the exogenous hormone. The critical treatment period for root induction in Conference at the IBA concentration of 1.5 μM is 1 day in contrast to the 2-day exposure required to see stimulation of rooting percentage in Doyenne d'Hiver. This difference may be due in part to the reduced rate of uptake and failure to convert IBA to IAA by Doyenne d'Hiver as compared with Conference at that concentration. Auxin stimulation of the high number of initial cells with the subsequent poor root development in Doyenne d'Hiver may be explained if the first anatomic changes that take place only result in vascular development instead of root primordia, as has been reported to occur by Lovell and White (1986) and Mitsuhashi-Kato et al. (1978). The anatomic differences (the presence of fibers outside the phloem) are not a mechanical hin-

drance for the centrifugal root development arising from the initial cells as observed in olive (Lovell and White 1986).

There is a peak in IAA levels in Conference before root induction. Based on this timing, the magnitude of the change, and the observation that an increase in IAA levels does not occur in Doyenne d'Hiver, we suggest that changes in the levels of endogenous IAA are related in a critical way to the rooting process in pear microcuttings. Increased levels of conjugated IAA appeared to be related to the lower rooting seen in Doyenne d'Hiver. The high putrescine levels, attained only in Doyenne d'Hiver, strongly support its possible inhibitory effect in root formation as also observed by Tiburcio et al. (1987). In our plant material spermidine did not peak as markedly as observed in *Nicotiana* thin cell layers before the appearance of root meristemoids (Torrigiani et al. 1989).

In conclusion, root production in both cultivars is not anatomically hindered, and both cultivars are capable of metabolizing IBA to similar compounds. This ability is, however, dependent on the exogenous IBA concentration, which must be higher for the difficult-to-root cultivar. Results from the analysis of PAs showed that increased levels of putrescine were associated with the lower rooting found in Doyenne d'Hiver.

Acknowledgments. We are grateful to M. M. Altamura and A. Fabbri for helpful discussion. We also thank R. H. Zimmerman and P. Davies for reading the manuscript and for the helpful comments. This research is part of a binational project between the National Research Council of Italy and the United States Department of Agriculture, Agricultural Research Service. This research was partially supported by National Research Council of Italy, Special Project RAISA, Subproject N.2, Paper N 1986 and USDA-NRI Grant 91-03079.

References

Altman A (1989) Polyamines and plant hormones. In: Bachrach U and Heimer MY (eds) The physiology of polyamines. Vol 2. CRC Press, Boca Raton, pp 121–145

Bagni N, Serafini-Fracassini D, Torriggiani P (1982) Polyamines and cellular growth processes in higher plants. In: Wareing PF (ed) Plant growth substances 1982. Academic Press, London, pp 473–482

Baraldi R, Bertazza G, Predieri S, Bregoli A, Cohen JD (1993) Uptake and metabolism of indole-3-butyric acid during the in vitro rooting phase in pear cultivars (*Pyrus communis*). Acta Hort 329:289–291

Batten DJ, Goodwin PB (1978) Phytohormones and the induction of adventitious roots. In: Letham DS, Goodwin PB, Higgins TJV (eds) Phytohormones and related compounds: A comprehensive treatise. Vol 2. Amsterdam: Elsevier North-Holand Biomedical Press, pp 137–145

Bender L, Harry IS, Yeung EC, Thorpe TA (1987) Root histology, nutrient uptake and translocation in tissue culture plantlets and seedlings of *Thuja occidentalis* L. Trees 1: 232–237

Berardi G, Neri D, Maiorino A, Adversi R (1992) In vitro rooting of *Pyrus calleryana*. Acta Hort 300:181–188

Berthon JY, Maldiney R, Sotta B, Gaspar T, Boyer N (1989) Endogenous levels of plant hormones during the course of adventitious rooting in cuttings of *Sequoiadendron giganteum* (Lindl.) in vitro. Biochem Physiol Pflanzen 184:405–412

Biondi S, Diaz T, Iglesias I, Gamberini G, Bagni N (1990) Polyamines and ethylene in relation to adventitious root formation in *Prunus avium* shoot cultures. Physiol Plant 78: 474–483

Blakesley D, Weston GD, Elliott MC (1991) Endogenous level of indole-3-acetic acid and abscisic acid during the rooting of *Cotinus coggygria* cuttings taken at different times of the year. Plant Growth Regul 10:1–12

Chen KH, Miller AN, Patterson GW, Cohen JD (1988) A rapid and simple procedure for purification of indole-3-acetic acid prior to GC-SIM-MS analysis. Plant Physiol 86:822–825

Chriqui D, D'Orazi D, Bagni N (1986) Ornithine and arginine decarboxylases and polyamine involvement during in vivo differentiation and in vitro dedifferentiation of *Datura innoxia* leaf explants. Physiol Plant 68:589–596

Cohen JD, Bandurski RS (1982) Chemistry and physiology of the bound auxins. Annu Plant Physiol 33:403–430

Cohen JD, Schulze A (1981) Double standard isotope dilution assay. I. Quantitative assay of indole-3-acetic acid. Anal Biochem 112:249–257

Desai HV, Metha AR (1985) Changes in polyamine levels during shoot formation, root formation, and callus induction in cultured *Passiflora* leaf disks. J Plant Physiol 119:45–53

Epstein E, Lavee S (1984) Conversion of indole-3-butyric acid to indole-3-acetic acid by cuttings of grapevine (*Vitis vinifera*) and olive (*Olea Europea*). Plant Cell Physiol 25:97–103

Fawcett CH, Wain RL, Wightman F (1960) The metabolism of 3-indolylalcane carboxilic acids, and their amides, nitriles and methyl esters in plant tissues. Proc R Soc Lond B Biol Sci 152:231–254

Hicks GS (1987) Adventitious rooting of apple microcuttings in vitro: An anatomical study. Can J Bot 65:1913–1920

Jensen WA (1962) Botanical Histochemistry. WH Freeman, San Francisco, pp 55–99

Labarca C, Nicholls PB, Bandurski RS (1965) A partial characterization of indoleacetylinositols from *Zea mais*. Biochem Biophys Res Commun 20:641–645

Label PH, Sotta B, Miginiac E (1989) Endogenous levels of abscisic acid and indole-3-acetic acid during in vitro rooting of Wild Cherry explants produced by micropropagation. Plant Growth Regul 8:325–333

Linsmaier E, Skoog F (1965) Organic growth factor requirements of tobacco tissue cultures. Physiol Plant 18:100–127

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

Mitsuhashi-Kato M, Shibaoka H, Shimokoriyama M (1978) The nature of the dual effect of auxin on root formation in *Azukia* cuttings. Plant Cell Physiol 19:1535–1542

Moncousin C (1988) Adventitious rhizogenesis control: New development. Acta Hort 230:97–104

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 15:473–497

Norcini JG, Heuser CW, Hamilton RH (1985) Changes in free and conjugated indole-3-acetic acid during initiation and early development of adventitious root in mung bean. J Am Soc Hort Sci 110:528–533

Sankhla N, Upadhyaya A (1988) Polyamines and adventitious root formation. In: Davis TD, Haissing BE, Sankhla N (eds) Adventitious root formation in cuttings. Dioscorides Press, Portland, pp 202–213

Serafini-Fracassini D (1991) Cell cycle-dependent changes in polyamine metabolism. In: Slocum RD and Flores HE (eds) Biochemistry and physiology of polyamines in plants. CRC Press, Boca Raton, pp 143–156

Serafini-Fracassini D, Bagni N, Cionini PG, Bennici A (1980) Polyamines and nucleic acids during the first cell cycle of *Helianthus tuberosus* tissue after the dormancy break. Planta 148:332–337

Sutter EG, Luza J (1993) Developmental anatomy of roots induced by agrobacterium rhizogenes in *Malus pumila* M.26 shoots grown in vitro. Int J Plant Sci 154:59–67

Tiburcio AF, Kaur-Sawhney R, Galston AW (1987) Effect of polyamine biosynthetic inhibitors on alkaloids and organogenesis in tobacco callus cultures. Plant Cell Tissue Organ Cult 9:111–120

Torrigiani P, Altamura MM, Capitani F, Serafini-Fracassini D, Bagni N (1989) De novo root formation in thin layers of tobacco: Changes in free and bound polyamines. Physiol Plant 77:294–301

Torrigiani P, Altamura MM, Capitani F, Falasca G, Bagni N (1991) Rhizogenesis from tobacco thin layers: Effect of various inhibitors of polyamine biosynthesis. Giorn Bot Ital 125:865–874

Van der Krieken WM, Breteler H, Visser MHM (1992) The effect of the conversion of indolebutyric acid into indoleacetic acid on root formation on microcuttings of *Malus*. Plant Cell Physiol 33:709–713

Wiesman Z, Riov J, Epstein E (1988) Comparison of movement and metabolism of indole-3-acetic acid and indole-3-butyric acid in mung bean cuttings. Physiol Plant 74:556–560

Wiesman Z, Riov J, Epstein E (1989) Characterization and rooting ability of indole-3-butyric acid conjugates formed during rooting of mung bean cuttings. Plant Physiol 91:1080–1084

Zhou J, Wu H, Collet GF (1992) Histological study of initiation and development in vitro of adventitious roots in microcuttings of apple rootstocks of M26 and EMLA9. Physiol Plant 84:433–440