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Role of Auxin and Polyamines in Adventitious Root Formation in Relation to Changes in Compounds Involved in Rooting

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ABSTRACT

An attempt was made to identify some cellular components that control adventitious root formation at the base of hypocotyl cuttings of mung bean (*Vigna radiata* L. cv. 105). Three phases of the adventitious root formation process were identified; induction (0–24 h), initiation (24–72 h), and expression (after 72 h). The lower peroxidase activity during the induction period (0–24 h) corresponded with the first peak of IAA at 24 h which indicated termination of the induction phase. A peak of peroxidase activity with low IAA levels at 72 h signaled the termination of the initiation phase. After 72 h, peroxidase activity declined and IAA levels increased slowly and this was characterized as the expression phase. IAA-oxidase activity could be inversely correlated with endogenous IAA levels. Endogenous putrescine and IAA increased simultaneously both at the induction and initiation phase, and might have a combined role in adventitious root formation. Spermidine and spermine levels did not change significantly throughout these phases. The pattern of diamine oxidase and polyamine oxidase (DAO/PAO) activities was inversely correlated with endogenous polyamine levels only during the induction phase. Levels of H_2O_2, which is involved in IAA and phenol oxidation and also in cell wall xylogenesis, were positively correlated with DAO/PAO activity because H_2O_2 is one of the end products of PA oxidation. An inverse relationship between phenol content and polyphenol oxidase activity was observed during rooting of mung bean cuttings. Compared with controls, IBA-(10^{-5} M) or PUT-(10^{-4} M) treated cuttings exhibited increased levels of IAA, putrescine, phenol and H_2O_2, decreased IAA-oxidase activity, and increased DAO/PAO, peroxidase, and polyphenol oxidase activities. An early appearance of the second peak of putrescine and phenol at the end of the initiation phase (72 h) was observed in cuttings treated with IBA or PUT. On the basis of IAA levels and peroxidase activity, there was no change in the duration of different rooting phases due to IBA or PUT treatments. However, on the basis of endogenous PUT levels, treatment with IBA or PUT reduced the total duration of the initiation and expression phases.

Key words: Auxin; Polyamines; Adventitious root formation

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Introduction

Developmental physiology in general is a poorly understood phenomenon. The process of adventitious root formation at the base of a stem cutting is influenced by a number of internal and external factors (Davis and others 1988). Because vegetative propagation is frequently practiced by nurserymen for maintaining fidelity and achieving quicker propagation of horticultural and floricultural plants it is necessary to understand the physiological and biochemical basis of adventitious root formation.

Many basic studies on adventitious root formation have been carried out under *in vitro* (Gaspar and others 1992, 1994, 1997; Klerk and Brugge 1992; Klerk 1996; Moncousin and Gaspar 1983) and also *in vivo* (Eriksen 1974; Friedman and others 1985; Jarvis 1986) conditions to distinguish and delineate the successive phases of adventitious root formation and regulation. Rooting appears to be a developmental process consisting of distinct stages, each with its own requirements and characteristics (Kevers and others 1997). Different researchers have recognized different phases of adventitious root formation based on physiology (Eriksen 1974; Friedman and others 1985; Gaspar and others 1992; 1994, 1997; Klerk and others 1999; Moncousin and Gaspar 1983; Smith and Thorpe 1975) and histology (Jasik and Klerk 1997; Sircar and Chatterjee 1973) either in cuttings or in plant parts under cultural conditions. But there are inconsistencies in delineating the different stages of the rooting process under *in vivo* and *in vitro* conditions because molecular biological data are lacking and biochemical studies are not always convincing.

Auxin has been shown to be intimately involved in the process of adventitious root formation (Haissig and others 1994; Wiesmann and others 1988, 1989) and the interdependent physiological stages of the rooting process (Gaspar and others 1997) are associated with changes in endogenous auxin concentrations (Heloir and others 1996). In addition, several studies have emphasized that polyamines play a role in rooting (Biondi and others 1990; Davis and others 1988; Hausman and others 1994), although the results are contradictory and fragmentary (Davis and others 1988) and the relative roles played by auxin and polyamine are far from clear. A possible interrelationship between auxin and polyamines in the control of rooting induction has been suggested by some workers (Gaspar and others 1997). It is also known that peroxidase activity regulates IAA catabolism and acts as a marker for the successive phases, typically with a minimum at the induction phase and a maximum at the initiation phase (Gaspar and others 1997). Most of the recent work attempting to delineate the different phases of rooting was performed under *in vitro* conditions.

The aim of the present work was to study the changes with time in the metabolism of IAA, polyamines, peroxidase activity, and some other compounds associated with adventitious root formation under *in vivo* conditions using mung bean hypocotyls as experimental materials.

Materials and Methods

Preparation of Plant Samples

Mung bean (*Vigna radiata* L. cv. 105) seedlings were grown in sand in a controlled growth room with 16 h photoperiod at 222 µmole m^{-2} s^{-1} intensity (400–700 nm) for 7 days. The hypocotyls of 7-d-old seedlings were excised 3 cm below the cotyledonary node, the cotyledons were removed, and the resulting cuttings consisting of the hypocotyl and the intact epicotyl, with a pair of primary leaves were used in rooting experiments reported here. Freshly prepared hypocotyl cuttings were put into 50 ml glass beakers containing 30 ml of test solution that covered the entire hypocotyl. The test solutions of IBA (10^{-5} M) or putrescine (10^{-4} M) were separately renewed from time to time. Control solutions were distilled water (pH 7.0). Cuttings were maintained in a controlled growth chamber (26 ± 1°C temperature, 16 h photoperiod and 80% RH) for 12 d, after which the adventitious roots were counted. The endogenous levels of free indole-3-acetic acid (IAA), putrescine (PUT), spermidine (SPD), and spermine (SPM) were analyzed along with the enzymes related to their oxidation from the hypocotyl region of cuttings from 0 to 5 d after excision at intervals of 24 h.

Fluorimetric Determination of Free IAA Content and IAA-oxidase Activity in Mung Bean Cuttings

A more specific fluorimetric assay method based on the reaction of IAA with acetic anhydride in the presence of perchloric acid as a catalyst, to form the fluorescent tricyclic derivative 2-methyl indole-α-pyrone (Plieninger and others 1964), was standardized. The high specificity of the assay is indicated by the fact that other indoles such as indole-3-butyric acid do not form detectable amounts of fluorescent derivatives in the α-pyrone assay (Stoessl and Venis 1970). The assay conditions were standardized using

the hypocotyl of *Vigna radiata* L cv. 105. For IAA extraction one g of hypocotyl tissue was collected randomly from 12 hypocotyls from 0 to 5 d after excision of seedlings and was extracted following the method of Knegt and Bruinsma (1973), slightly modified by Sinha and Basu (1981). The recovery efficiency was usually between 60 and 75% (Knegt and Bruinsma 1973). After the IAA was extracted, it was assayed fluorimetrically following the method of Mousdale and others (1978), with modifications as detailed below. The extracted IAA was dissolved in 2 ml redistilled methanol and then carefully evaporated to 0.2 ml. After cooling, the reaction was initiated by adding 0.1 ml acetic anhydride followed by 0.1 ml 60% (w/v) perchloric acid as a catalyst. After appropriate reaction time (optimum 10 min), the reaction was stopped by adding 4 ml of distilled water. The aqueous solutions were mixed and read immediately in an LS 30-Luminescence Spectrofluorimeter (Perkin-Elmer, UK) with the excitation wave length fixed at 440 nm and the emission wave length fixed at 520 nm. In the blank, methanol was used before acetic anhydride and perchloric acid. The amount of IAA was analyzed by a standard calibration curve prepared for the pyrone fluorimetric assay of IAA and expressed in terms of ng. g^{-1} f.wt.

Extraction and Assay of IAA-Oxidase (IAA-ox)

IAA-oxidase was extracted from one g of hypocotyl tissue following the method of Sinha and Basu (1981). The reaction mixture (10 ml) consisted of 6 ml citrate buffer (50 mM, pH 4.5), 1 ml dichlorophenol (1 mM), 1 ml $MnCl_2$ (1 mM), 1 ml IAA (120 µg), and 1 ml of crude enzyme solution. The mixture was incubated at 30°C for 1 h. After incubation, 0.1 ml of $NaHCO_3$ (N) was added to the reaction mixture and acidified to pH 3.0 by adding H_2SO_4 (9N). The residual IAA was then extracted to the organic phase by adding 2 ml diethyl ether. The ether part was separated and evaporated to dryness. Such residual IAA was then measured following the method of Mousdale and others (1978) as described. In the blank, instead of crude enzyme solution, 1 ml potassium phosphate buffer (50 mM, pH 5.3) was used. The enzyme activity was expressed in terms of µg IAA degraded (100 mg fresh weight)$^{-1}$ h^{-1}.

Extraction and Assay of Peroxidase (POX)

Peroxidase activity was measured according to the method of Kar and Mishra (1976) using pyrogallol as the substrate. The activity of the enzyme (Enzyme Unit, that is, E.U.) was calculated as ΔAXTV/tXv, where ΔA is the absorbance of sample after incubation minus absorption at zero time control, TV is the total volume of the filtrate, t is the time (min) of incubation with substrate, and v is the volume of filtrate actually taken for incubation (Fick and Qualset 1975). The enzyme activity was expressed in terms of E.U. mg^{-1} protein min^{-1}.

Extraction and Estimation of Polyamines

Polyamines were extracted, separated and detected after dansylation as described by Reggiani and others (1990) using silica plates (60 F_{254}, Merck, Germany) with cyclohexane-ethylacetate (3:2 v/v) as the solvent. Spots, demarcated under UV light, were scraped from the plates and extracted with ethylacetate. Fluorescence was measured in an LS 30-Luminescence Spectrofluorimeter (Perkin-Elmer, UK) at an excitation wave length of 360 nm and emission wave length of 506 nm and the results were compared with dansylated standards. Polyamine content was expressed as n mol.mg^{-1} protein.

Extraction and Assay of Diamine Oxidase (DAO) and Polyamine Oxidase (PAO)

To measure DAO activity, 1 g hypocotyl tissue was homogenized in 1.5 ml phosphate buffer (50 mM, pH 7.0) with 10^{-2} M catechol (inhibitor of peroxidase and catalase) in a prechilled mortar (Nag and others 2000). The extract was centrifuged at 10,000 rpm for 15 min at 4°C in a Beckman centrifuge (Model GS-15 R, USA). The clear supernatant fraction was used as the enzyme source during the assay. The enzymatic (DAO) reaction was started by adding 3 mM putrescine (for PAO, 3 mM spermine was used as substrate), potassium phosphate buffer (40 mM, pH 7.4), with 0.3 ml enzyme extract. The reaction was allowed to proceed for 30 min at 37°C and was terminated by adding 0.1 ml of 15% (w/v) titanium sulfate ($TiSO_4$) in 23% H_2SO_4 forming a brilliant orange yellow-colored complex that was used for measuring H_2O_2 in plant tissues (Mac Nevin and Uron 1953). The incubation mixture was then centrifuged at 10,000 rpm for 10 min and the absorbance of the orange-yellow-colored solution was taken at 410 nm in a UV-VIS Shaimadzu Spectrophotometer. In the control set, $TiSO_4$ was added prior to the enzyme solution. The enzyme activities were expressed in terms of ΔA/30 min.

Determination of Hydrogen Peroxide (H_2O_2) Content

The total H_2O_2 content was estimated using a titanium sulfate reagent following the method of Mac

Table 1. Effect of IBA (10^{-5} M) and PUT (10^{-4} M) on Adventitious Root Formation of Stem Cuttings of *Vigna radiata* L. cv. 105

Treatment & concentration	No. of root primordium	No. of primary roots	No. of secondary roots	Total root length (cm)
Control (H_2O)	12.7 ± 1.1	10.3 ± 1.4	10.1 ± 0.9	10.5 ± 1.3
IBA 10^{-5}	38.3 ± 2.1	14.4 ± 2.2	16.3 ± 1.6	13.6 ± 2.0
PUT 10^{-4}	12.8 ± 1.0	10.8 ± 1.4	13.9 ± 1.2	14.6 ± 1.9

Data are expressed as average values for 20 cuttings ± standard error

Nevin and Uron (1953) as modified by Mondal and Choudhuri (1981) and expressed in terms of µM H_2O_2 produced min^{-1}.

Extraction and Determination of Total Phenol Content

Total phenol was extracted and estimated by Folin-Phenol reagent (Bray and Thorpe 1954) and expressed as mg. g^{-1} f.wt.

Extraction and Assay of Polyphenol Oxidase (PPO) Activity

Polyphenol-oxidase was extracted and assayed following the method of Kar and Mishra (1976) using pyrogallol as the substrate and expressed in terms of E U. mg^{-1} protein.min^{-1}.

Determination of Protein Content

The amount of protein was determined by the method of Bradford (1976) with bovine serum albumin as the standard.

All the results are the mean of at least three samples from three independent experiments. The data were statistically analyzed for standard error.

Results

Effect of Growth Regulators on Rooting

Data from Table 1 shows that IBA (10^{-5} M) treatment had greater effects on the parameters of adventitious root formation studied in mung bean stem cuttings than PUT. Though the effect of PUT (10^{-4} M) was less than that of IBA (10^{-5} M), except in total root length (14.6 cm ± 1.9), it produced markedly better results on rooting parameters than controls.

Different Phases for Adventitious Root Formation

The present study shows that the endogenous content of free IAA in control cuttings of mung bean hypocotyls increased during the first 24 h and then decreased up to 48 h during adventitious root formation (Figure 1). After that, free IAA levels increased slowly up to 96 h and decreased thereafter. On the other hand, peroxidase showed lower activity during 0–48 h but a peak of peroxidase activity occurred at 72 h which corresponded to a decreased level of free IAA. After 72h, peroxidase activity declined in control cuttings.

Effects of IBA and PUT on Endogenous IAA Content and Activities of IAA-Oxidase (IAA-ox) and Peroxidase (POX)

IBA and PUT treatment produced similar trends in endogenous IAA levels during the different stages of adventitious root formation, as were observed in controls. However, IAA levels were higher in treated cuttings than in controls during adventitious root formation. Figure 2B shows that IAA-ox activity decreased rapidly from 0–24 h, increased from 24–48 h, declined again from 48–72 h, and then showed a gradual increase in the activity up to 120 h. Endogenous IAA levels (Figure 2A) were greater in IBA treatments than that in PUT treatments. IAA-ox activity remained higher in controls than in treated cuttings. IAA-ox activity was less in IBA-treated hypocotyls compared with PUT-treated tissues (Figure 2B). Figure 2C shows that there was no marked differences in POX activities during 0–24 h between control and treated cuttings. POX activity increased sharply from 48–72 h, reaching a peak at 72 h and declining thereafter. The POX activity in treated cuttings (IBA or PUT) was greater than controls but increased after 96 h rather than decreasing as observed in controls.

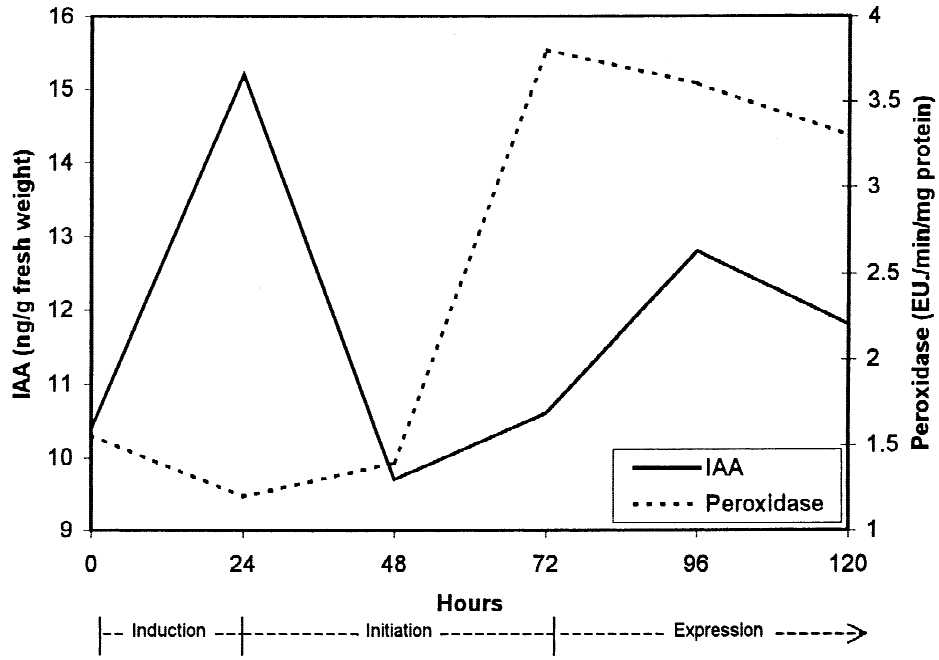

Figure 1. Phases and durations of adventitious root formation based on endogenous IAA levels and peroxidase activity in mung bean cuttings.

Putrescine (PUT), Spermidine (SPD), and Spermine (SPM) Content

Figure 3A shows that the level of PUT varied markedly throughout the phases of adventitious root formation but the trend of changes was very similar to that of IAA content (Figure 2A). In control cuttings, PUT showed a peak at 24 h. The level then began to decrease from 24–48 h, showed a second peak at 96 h, and gradually declined thereafter. In treated cuttings (IBA or PUT), the first peak appeared at 24 h like that of controls whereas the second peak appeared earlier (72 h) than that of controls (96 h). The level of PUT diminished after 72 h in both the treated cuttings. The PUT level was higher in both the treated cuttings than in controls, however, the levels of SPD (Figure 3B) and SPM (Figure 3C) showed no marked changes in control cuttings from different phases. In IBA- or PUT-treated cuttings, SPD and SPM levels both increased from 0–24 h but thereafter no marked differences from controls were noted. The levels of SPD and SPM remained lower than PUT levels in both treated and control cuttings.

DAO and PAO Activity and H_2O_2 Content

The present study shows that DAO activity, as measured by the new method standardized by the present authors (Nag and others 2000), gradually decreased up to 72 h, followed by a gradual increase after 72 h in control as well as treated cuttings. However, there was no marked difference in the activity of DAO between different treatments (Figure 4A). The activity of PAO, on the other hand, decreased sharply in both control and treated cuttings only during 0–24 h; beyond 24 h, it increased only in treated cuttings (IBA or PUT), whereas in controls it remained at the same level (Figure 4B). The H_2O_2 content in control mung bean cuttings was maximal at 0 h and then declined up to 96 h followed by a gradual increase (Figure 4C). In treated cuttings (IBA or PUT), although H_2O_2 levels were higher than controls, they decreased from 0–72 h and then increased slowly afterwards.

Phenol Content and Polyphenol Oxidase (PPO) Activity

The total phenol content from mung bean cuttings showed a rise from 0–24 h followed by a sharp decline from 24–48 h. Levels then started rising again during 48–72 h, attaining a maximum between 72–96 h. After 96 h, phenol content started declining again. Although a similar trend was noted in treated cuttings, phenol content remained higher in IBA- or PUT-treated cuttings compared with controls throughout the experiment (Figure 5A). An inverse relationship between phenol content and PPO activity was noticed at different stages of root formation in control as well as in treated cuttings (Figure 5B).

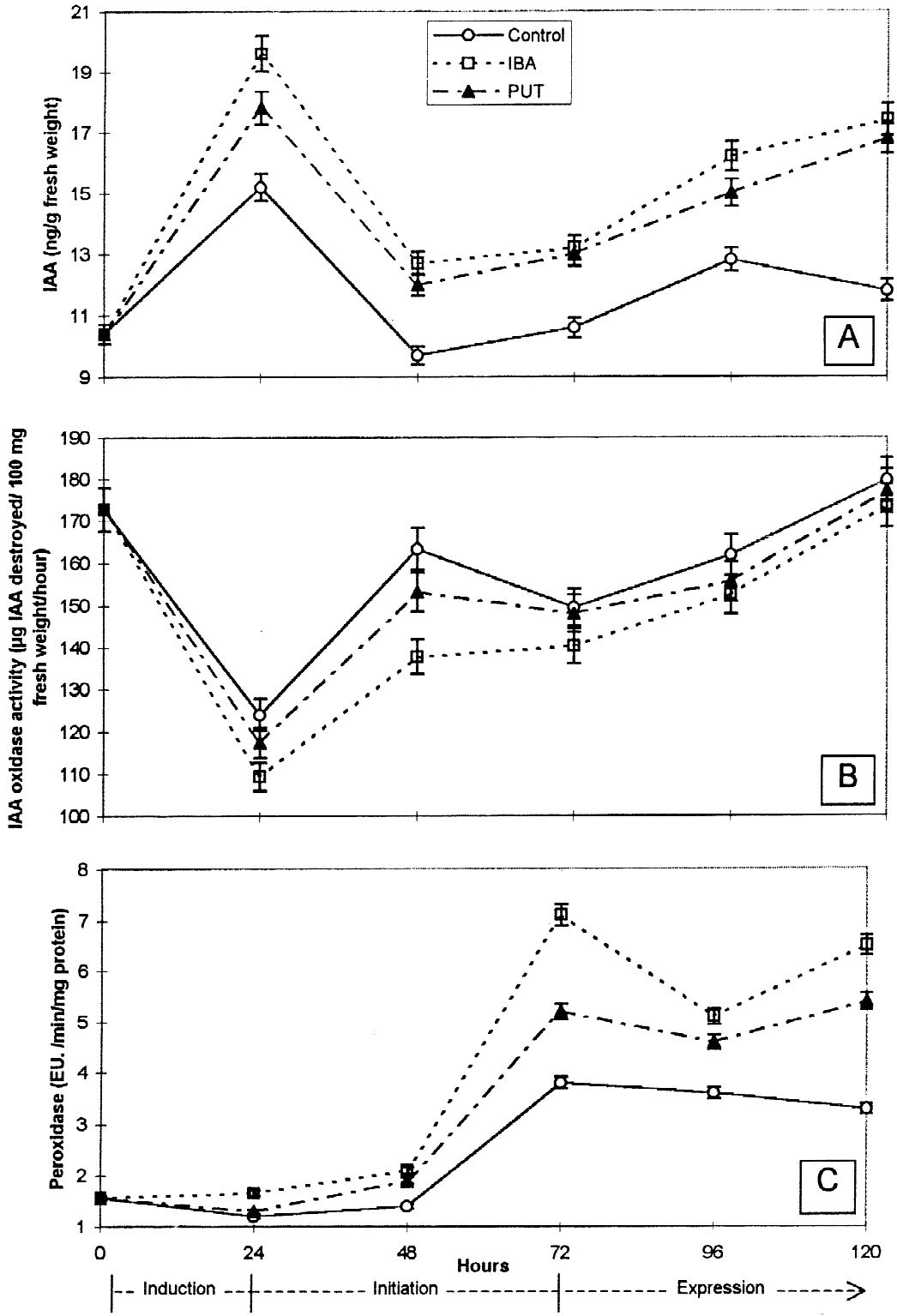

Figure 2. Changes in endogenous IAA content (**A**), IAA oxidase activity (**B**), and peroxidase activity (**C**) with time in mung bean hypocotyls treated with IBA (10^{-5} M) and PUT (10^{-4} M).

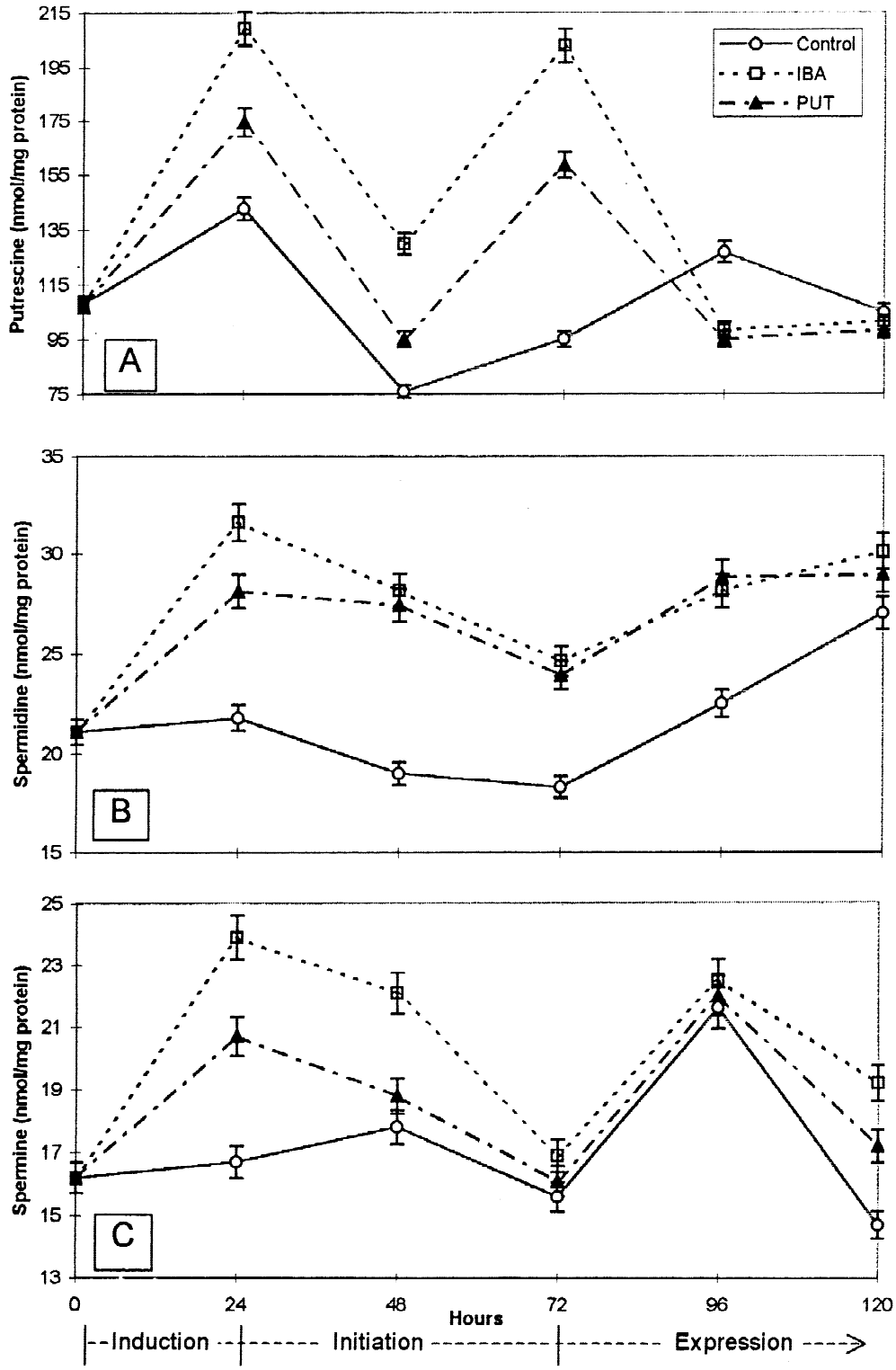

Figure 3. Changes in endogenous levels of putrescine (A), spermidine (B), and spermine (C) with time in mung hypocotyls treated with IBA (10^{-5} M) and PUT (10^{-4} M).

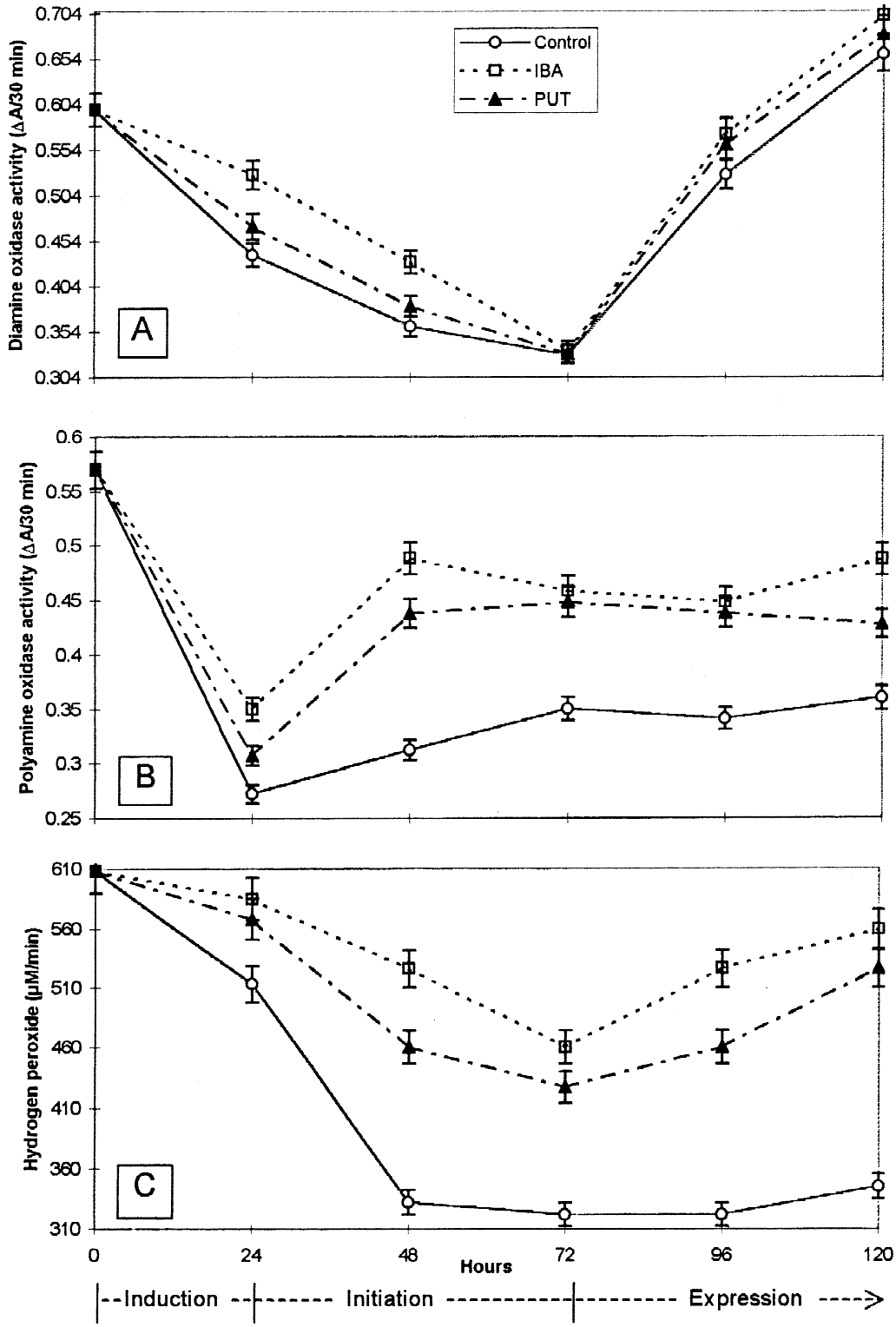

Figure 4. Changes in the activity of diamine oxidase (**A**), polyamine oxidase (**B**), and hydrogen peroxide content (**C**) with time in mung bean hypocotyls treated with IBA (10^{-5} M) and PUT (10^{-4} M). Enzyme activities were expressed in terms of ΔA/30 min where ΔA is the change of absorbance at 410 nm.

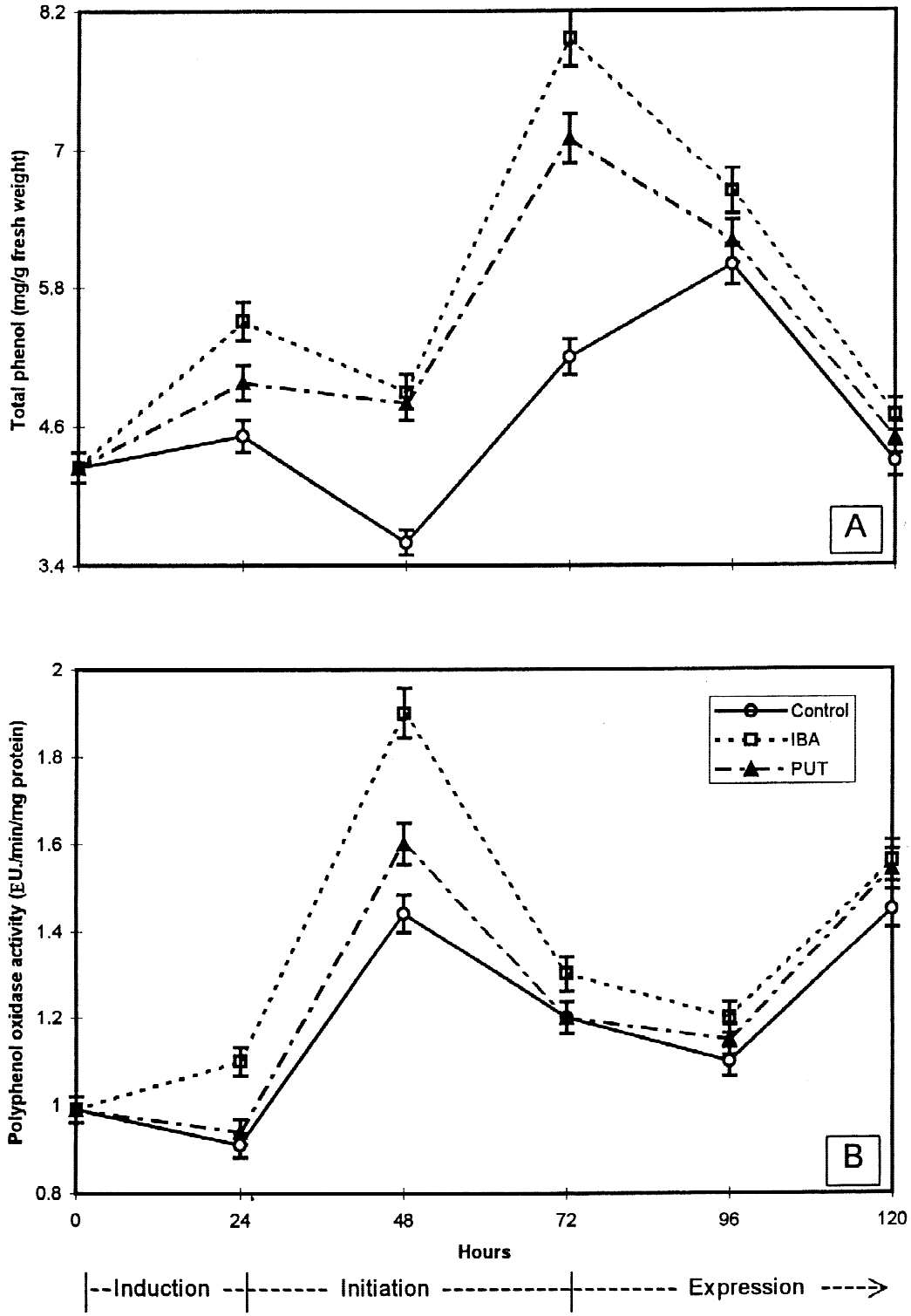

Figure 5. Changes in endogenous levels of phenol (**A**) and polyphenol oxidase activity (**B**) with time in mung bean hypocotyls treated with IBA (10^{-5} M) and PUT (10^{-4} M).

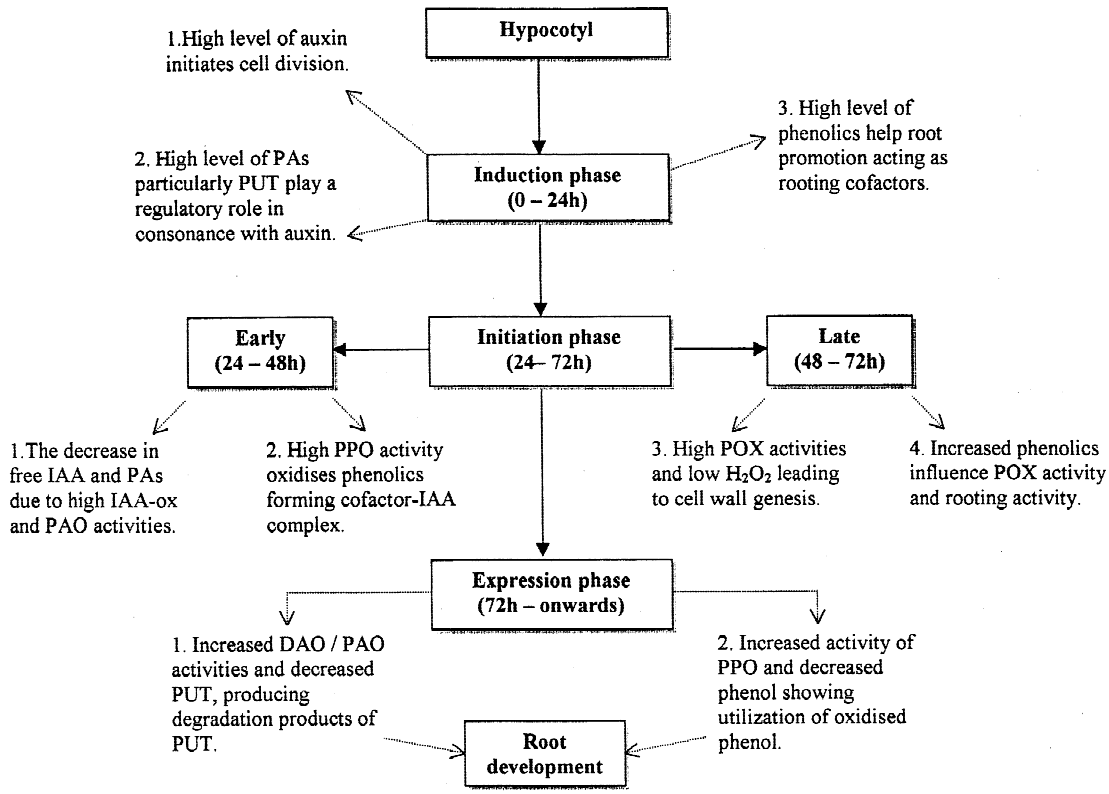

Figure 6. Probable interrelationships of IAA, PAs and some cellular compounds associated with adventitious root formation at different rooting stages.

Discussion

A schematic representation combining all the results is shown in Figure 6. This should help explain the interdependent physiological phases comprising the rooting process.

The data in Table 1 clearly reveal that IBA treatment produced significant effects on the parameters (number of root primordium, number of primary roots, number of secondary roots, and total root length) of rooting in hypocotyl cuttings of *Vigna radiata* L. cv. 105 compared to controls. Among the rooting parameters studied, IBA had a pronounced effect on the number of root primordium. PUT, on the other hand, produced marginal improvement over controls in all rooting parameters. Interestingly, the effect of PUT on total root length was more pronounced than IBA. However, in other parameters, the effect of IBA was significantly better than PUT. Similar observations were also reported by Nag and others (1999).

Gaspar and Hoffinger (1998) demonstrated in a number of different plant species (Gaspar and others 1994, 1997) that increasing endogenous free IAA levels always occur in the inductive phase of rooting (0–24 h) with the first peak of free IAA terminating the inductive phase and signaling the beginning of the initiation phase. A gradual increase (from 72 h) in free IAA content, reaching a peak at 96 h and declining thereafter, designates the expression phase. They also showed that POX activity generally peaked in a reverse trend compared to endogenous IAA levels. In the present study with mung bean hypocotyl cuttings, nearly identical trends in endogenous-free IAA levels and POX activities were observed as those demonstrated by Gaspar and his coworkers. Thus, the present study further corroborates the different rooting phases described by Gaspar and his coworkers based on endogenous IAA levels and POX activities.

IAA-ox activity may play a crucial role in regulating endogenous IAA levels (Gaspar 1995; Moncousin and Gaspar 1983). The present study with mung bean hypocotyls revealed a reverse correlation between endogenous IAA levels and IAA-ox activity in different phases of rooting. High levels of IAA and low IAA-ox activity during the induction period in IBA-treated cuttings appears to be responsible for better development of adventitious roots with IBA possibly serving as the source of free auxin (Wiesmann and others 1988, 1989). During the early phase of root initiation (24–48 h) in IBA-

treated cuttings, low levels of IAA were associated with high IAA-ox activity. Our finding agrees with the hypothesis that the adventitious root formation initially occurs in two phases: an auxin-sensitive phase and an auxin-insensitive phase (Hartmann and others 1993). In mung bean hypocotyls, this auxin-sensitive phase appears to extend from 0–24 h followed by the auxin-insensitive phase (beyond 24 h).The results further indicate that IAA is an essential factor for induction rather than initiation of roots. At the expression phase, however, IAA content remained low, which might be ascribed to high IAA-ox activity. According to some authors, the low free IAA levels might be explained by formation of IAA- conjugates by auxin protectors and not by high IAA-ox activity (Mato and Vieitez 1986; Nordstrom and Eliasson 1991). However, the present results lend support to high IAA-ox activity along with its cofactors (discussed later) as the cause of low free IAA levels in this phase.

A sharp increase in POX activity was observed at the late initiation phase (72 h) in IBA-treated cuttings. This might be taken as an index of better rooting performance by mung bean cuttings and might serve as a good marker for rooting ability in cuttings (Gaspar and others 1992; Moncousin and Gaspar 1983). In PUT-treated cuttings, a similar rise in POX activity was also observed although POX activity was lower than that of IBA-treated cuttings. Plant peroxidases are known to be involved in auxin metabolism as well as cell wall synthesis in the presence of H_2O_2 and phenol, and the present finding that POX activity rose later than IAA-ox activity indicates that POX activity is more involved in cell wall genesis at the later phase and obligatory step in root formation (Pan and Gui 1997; Pan and Tian 1999).

The role of PAs in adventitious root formation along with auxin is not clear (Davis and others 1988). Reports of IBA-induced increases in PUT levels are available in the literature (Friedman and others 1973, 1985). It seems that PAs play a regulatory role in conjuction with auxin in the early events of adventitious root formation that is, during the active cell division stage and initiation of root primordia in the induction phase. It has also been reported that PUT acts as a second messenger, correlating with the peak of mitotic activity (Tiburcio and others 1989). All these results further support the regulatory role of both IAA and PUT in adventitious root formation at the base of stem cuttings. In the present study, the decrease in PUT levels after the initiation phase indicates its turnover and/or degradation before the subsequent root growth in the expression phase. This is supported by the observation of Friedman and others (1985). It is interesting to note that in IBA- or PUT-treated hypocotyls, the second peak of PUT appears in the late initiation (48–72 h) phase instead of in the expression phase, as observed in the untreated control. This shows that treatment of hypocotyls with either IBA or PUT reduces the total duration of the initiation and expression phases. The rise of other PAs in control and treated cuttings, although following the trend of PUT, was not very significant.

The pattern of DAO/PAO activities during the induction phase (0-24 h) was positively correlated with endogenous PA levels but at later phases, such a correlation was not clearly discernible. The enzymes DAO/PAO are known to cause oxidation of diamines and polyamines resulting in H_2O_2 as one of the end products (Smith 1985). The changes in H_2O_2 levels could be correlated with DAO/PAO activity. H_2O_2 levels remained lowest during the late initiation phase but rose gradually thereafter when DAO/PAO also showed high activities. In IBA- or PUT-treated cuttings, DAO/PAO showed greater activities than that observed in untreated controls. Similarly, H_2O_2 levels also increased in treated hypocotyls compared to controls. It points out that the oxidation of PUT is more important in adventitious root formation.

According to Berthon and others (1993) phenol is involved in different steps of adventitious root formation. Phenolics cause modification of IAA-ox activities, and formation of covalently linked auxin-phenolic conjugates (rhizocaline) and appear to be involved in rooting (Haissig 1974; Pal 1990). Moreover, phenolics are known to act as rooting cofactors (Hess 1962).

The present study shows that there was an increase in total phenol in IBA- and PUT-treated cuttings, particularly in the induction and late initiation phase. The activity of PPO showed a reverse trend compared to that of phenol content. It is tempting to speculate that the rise in phenol content during the induction and expression phases and the early appearance of the peak of phenol content in IBA- or PUT-treated cuttings might be associated with better rooting performance in treated cuttings where phenolics play an important role as rooting cofactors (Hess 1962).

The data presented here seem, thus, to suggest that treatment with IBA and PUT affected POX and IAA-ox activities and maintained higher levels of endogenous IAA, PUT and phenol in the rooting zone, leading to an induction of better rooting in auxin and polyamine-treated cuttings. And both IAA and PAs (particularly PUT) play a role in conjunction with each other. It appears that the oxidized products of PUT (such as H_2O_2 and γ-amino

butyric acid) are more involved in the rooting performance of cuttings than PUT itself.

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