Polyamines and Root Formation in Mung Bean Hypocotyl Cuttings[1]

I. EFFECTS OF EXOGENOUS COMPOUNDS AND CHANGES IN ENDOGENOUS POLYAMINE CONTENT

Received for publication March 5, 1982 and in revised form May 25, 1982

RA'ANAN FRIEDMAN, ARIE ALTMAN[2], AND URIEL BACHRACH
Department of Horticulture, The Hebrew University of Jerusalem, Rehovot 76 100, Israel (R. F., A. A.); and Department of Molecular Biology, The Hebrew University and Hadassah Medical School, Jerusalem, Israel (U. B.)

ABSTRACT

The effect of several polyamines (putrescine, spermidine, and spermine), their precursors (L-arginine and L-ornithine), and some analogs and metabolic inhibitors (L-canavanine, L-canaline, and methylglyoxal-bis [guanylhydrazone]) on root formation have been studied in mung bean (*Vigna radiata* [L.] Wilczek) hypocotyl cuttings.

Exogenously applied polyamines did not promote adventitious root formation. Rooting was inhibited by L-canavanine and L-canaline, and this inhibition was reversed by the corresponding amino acids L-arginine and L-ornithine. Methylglyoxal-bis (guanylhydrazone), an inhibitor of *S*-adenosylmethionine decarboxylase and polyamine biosynthesis, was also found to inhibit root formation. All compounds at concentrations of $>10^{-4}$ molarity completely inhibited natural root formation, whereas at $<10^{-5}$ molarity only the indole-butyric acid-induced root formation was inhibited.

Indole-butyric acid-induced root formation was accompanied by a considerable increase in polyamine levels, more than 2-fold of the control. Whereas senescing (unrooted) cuttings evinced a rapid decline in polyamine content during 48 hours, indole-butyric acid treatment resulted in elevated levels of putrescine and increased putrescine to spermidine ratio. The changes in polyamines were dependent on indole-butyric acid concentration and were organ specific.

Cuttings excised from plants either senesce and deteriorate, or resume active cell division and regenerate adventitious roots. It is well established that root formation involves intensive mitotic activity and metabolic changes, and is dependent on an array of endogenous physiological factors (15, 16). Whereas auxins seem to be a universal 'inducer' of adventitious roots (1), other factors are also involved and these factors may become limiting under specific conditions. Thus, inhibitors (15), rooting 'cofactors' (15, 16), auxin antagonists (8, 19), and nutrients (16, 23) have been shown to modulate root formation. In addition, it is still unclear which hormone (auxin) messengers are involved, and what are the primary events in auxin-induced root formation.

Recent studies, including our own, indicate that PA[3] may play a significant role in plant growth and senescence (4), similar to their activity in mammalian tissues and procaryotes (7, 10, 31). It has been shown that active growth of germinating seeds, of habituated and crown gall tissues, of pollen tubes, of potato buds and tomato fruits (6, 14, 18, 29, 33), as well as embryo and organ differentiation (4, 21, 22), are correlated with significant changes in PA content and metabolism. In addition, PA have been shown to respond to environmental and hormonal stimuli (12, 18), and to play an important role in protoplast, tissue, and organ senescence (2, 3, 17).

It, therefore, seems that cuttings may serve as an important experimental system to elucidate the involvement of PA in their senescence or in auxin-induced root formation. In addition to the effect of exogenous PA, endogenous changes in PA, and possible inhibitors of PA biosynthesis were also investigated. Both L-canavanine and L-canaline, the structural analogs of L-arginine and L-ornithine, respectively (25, 26), may affect putrescine formation from these two amino acids. Similarly, MGBG can block spermidine and spermine biosynthesis by inhibiting *S*-adenosylmethionine decarboxylase (24). L-Canavanine has been shown previously to inhibit IAA-dependent elongation of *Avena* coleoptiles (9), and growth of *Phaseolus* roots (34) and soybean cell suspensions (11). The present paper is the first in a series of studies on polyamines and root formation.

MATERIALS AND METHODS

Plant Material and Rooting Experiments. Mung bean (*Vigna radiata* [L.] Wilczek) seedlings were grown in vermiculite in a controlled phytotron (27°C day and 22°C night temperature, 78% RH, 16 h photoperiod). Seven- to 9-d-old seedlings were excised 2.5 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.

Freshly prepared hypocotyl cuttings were introduced into glass vials containing 15 ml of test solution which covered the entire hypocotyl. This solution was renewed after 24 h, and then at 48-h intervals. Control solution consisted of 1 mM phosphate buffer at pH 5.8, to which various compounds were added, as mentioned. IBA was used at 5×10^{-5} M, unless otherwise mentioned. PA, L-arginine, L-ornithine, L-canavanine, L-canaline, and MGBG were used at the mentioned concentrations, all in buffer. Unless otherwise stated, all treatments were typically performed during the initial 24-h period, after which the cuttings were transferred to buffer solution. Cuttings were maintained in a controlled growth chamber (24 ± 1°C, 16 h photoperiod of cool white fluorescent

[1]This research was supported by a grant from the United States-Israel (Binational) Agricultural Research and Development Fund (BARD).

[2]Author to whom correspondence should be addressed.

[3]Abbreviations: PA, polyamines (including di- and polyamines); IBA, indole-3-butyric acid; MGBG, methylglyoxal-bis (guanyl hydrazone).

light at 3600 lux) for 5 to 6 d, after which the adventitious roots (longer than 0.5 mm) were counted.

Ten separate cuttings were used for each treatment, and all experiments were repeated two to four times.

Polyamine Determination. Free polyamines were extracted by grinding 100 to 150 mg fresh weight of plant material in 2 ml cold 4% (v/v) $HClO_4$ using mortar and pestle. The extract was centrifuged for 25 min at 12,000*g*, and the supernatant was used for PA determination by the dansyl reaction (27). To a 0.2-ml aliquot of the $HClO_4$ extract, sufficient Na_2CO_3 was added to bring it to a pH of approximately 7.0, followed by an overnight incubation in the dark (room temperature) with 0.4 ml dansyl-chloride (30 mg/ml, in acetone). Excess dansyl chloride was converted to dansyl proline by a 30-min incubation with 0.1 ml L-proline (100 mg/ml), after which dansylated PA were extracted in 0.5 ml toluene. Twenty to 150 μl aliquots of toluene extracts were spotted on activated (1 h at 110°C) TLC plates precoated with 375 μm silica gel G-60 (Merck), and co-chromatographed with dansylated PA standards. Plates were developed in ethyl acetate:cyclohexane (2:3, v/v) or, occasionally, in benzene:triethylamine (5:1, v/v), and the spots were traced with long wave UV lamp. Dansylated derivatives were extracted in ethyl acetate and fluorescence measured in a Perkin-Elmer Fluorescence Spectrophotometer 204 (excitation at 365 nm, emission at >500 nm).

Protein Determination. The $HClO_4$ pellet was rinsed twice with 80% (v/v) acetone, and solubilized in 1 N NaOH (45 min at 37°C). Protein was determined according to Lowry *et al.* (20), with BSA standards.

RESULTS

Root Formation. Initially it was found desirable to look at the effect of exogenous PA on natural and on IBA-induced root formation. In neither case did putrescine or spermine (and spermidine, data not presented) promote root formation (Table I). Putrescine at 10^{-4} M and more so spermine, inhibited root formation, whereas higher concentrations (>5×10^{-4} M) were found to be toxic.

Since PA are synthesized in plants from L-arginine and L-ornithine, the effect of these precursors and their analogs on root formation was studied. Whereas L-arginine did not affect natural root formation and partially inhibited IBA-induced root formation (Table II), its analog, L-canavanine, totally prevented root formation in control cuttings and significantly inhibited IBA-induced root formation. Arginine completely reversed the inhibition and this effect was concentration dependent. The above mentioned results were more evident with 10^{-5} M IBA, as compared with its higher concentration. Similar results were found with L-ornithine and its analog, L-canaline. Root formation and inhibition is also illustrated (Fig. 1). In an attempt to clarify the nature of the L-canavanine inhibition, the timing effect of IBA and L-canavanine application was investigated (Table III). Root formation was completely arrested by 10^{-4} M L-canavanine, regardless of its time of application with respect to IBA application; thus, a later IBA treatment did not overcome the initial canavanine effect, neither did an IBA pretreatment prevent the canavanine inhibition. Surprisingly, the low canavanine concentration treatment resulted in a significant promotion of IBA-induced root formation when applied after IBA but inhibited it when administered prior to IBA. MGBG, another possible inhibitor of PA biosynthesis, significantly inhibited root formation (Table IV). The MGBG effect was found to be specific: inhibition of root initiation at the base of the cutting, resulting in a second wave of root formation above the damaged area of the hypocotyl (Fig. 1).

Polyamine Content. IBA treatment of cuttings resulted in remarkable increase in endogenous polyamine content, more than 2-fold of the control (Table V). This increase was especially evident in the hypocotyl and epicotyl, and a considerable increase in the putrescine/spermidine ratio was found in all organs. A study of PA changes over a 48-h period (Table VI) clearly indicates a continuous decrease in putrescine and spermidine levels in untreated cuttings which produce only few roots and presumably senesce, but this decrease in putrescine is prevented by IBA treatment. These changes are reflected in the putrescine to spermidine ratio.

Polyamine levels were further followed as a function of IBA concentrations. Whereas continuous increase in root formation was found at IBA concentrations higher than 5×10^{-6} M, putrescine level of hypocotyls peaked at 5×10^{-6} M IBA where only slight promotion of rooting was observed (Fig. 2). Putrescine and spermidine levels dropped dramatically at 5×10^{-5} M IBA, followed by a second increase. It should be noted, however, that the highest IBA concentration was supraoptimal, resulting in abnormal root formation and tissue hypertrophy. Similar changes in putrescine content were found in the epicotyl section of the cutting (data not reported). On the other hand, continuous increase in putrescine content was observed in leaves of cuttings treated with increasing IBA concentrations (Fig. 3).

DISCUSSION

Although accumulating evidence suggests that polyamines are intimately involved in plant growth and senescence (1, 4, 13), it is

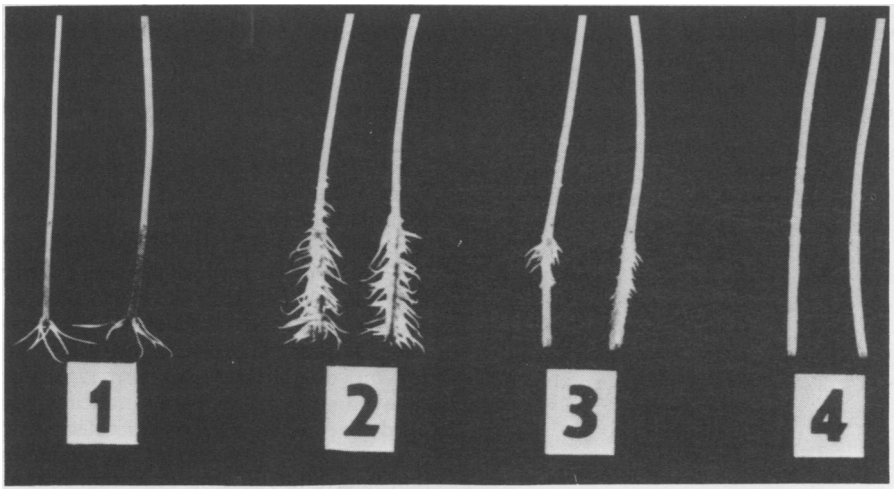

FIG. 1. Root formation in mung bean hypocotyl cuttings. 1, Control; 2, IBA, 5×10^{-5} M; 3, IBA, 5×10^{-5} M + MGBG, 10^{-4} M; 4, IBA, 5×10^{-5} M + L-canavanine, 10^{-4} M.

Table I. *Effect of Exogenous Polyamines on Root Formation*

Cuttings were treated with polyamines during the initial 24-h period. Values within a column are significantly different when followed by a different letter (P = 0.05, multiple range test).

Treatment	No. of Roots/Cutting	
	Control	IBA (5×10^{-5} M)
Control	6.7 d	38.7 a
Putrescine		
10^{-5} M	6.7 d	26.5 b
10^{-4} M	6.1 d	25.7 b
Spermine		
10^{-5} M	6.3 d	24.7 b
10^{-4} M	7.9 d	18.8 c

Table II. *Effect of Polyamine Precursors and Their Analogs on Root Formation in Mung Bean Cuttings*

Cuttings were treated with the various compounds during the initial 24-h period, and then transferred to buffer (±SD of the mean).

Treatment	No. of Roots/Cutting		
	Control	IBA	
		10^{-5} M	5×10^{-5} M
Control	6.7 ± 2.7	15.7 ± 7.0	22.1 ± 6.4
Arginine			
10^{-5} M	7.9 ± 2.7	9.3 ± 9.6	18.5 ± 6.0
10^{-4} M	8.6 ± 3.1	11.2 ± 5.3	13.4 ± 5.7
Canavanine			
10^{-4} M	0	4.6 ± 5.3	14.1 ± 4.1
10^{-4} M + arginine 10^{-5} M	0	7.1 ± 5.2	13.3 ± 7.6
10^{-4} M + arginine 10^{-4} M	0	12.8 ± 9.8	27.5 ± 10.9
Ornithine			
10^{-5} M	7.3 ± 3.7		
10^{-4} M	7.9 ± 3.5		
Canaline			
10^{-4} M	0	4.6 ± 5.0	8.4 ± 6.5
10^{-4} M + ornithine 10^{-5} M	0	9.5 ± 6.3	18.1 ± 8.2
10^{-4} M + ornithine 10^{-4} M	0	11.2 ± 6.1	16.5 ± 6.6

Table III. *Timing of the Canavanine Effect on Root Formation in Mung Bean Cuttings*

Experiment 1: Cuttings were pretreated with L-canavanine combined with IBA for 24 h (A), or pretreated with IBA for 24 h, followed by treatment with L-canavanine for additional 72 h (B). Experiment 2: Cuttings were pretreated with L-canavanine alone for 24 h, followed by treatment with IBA for additional 72 h. Multiple range test, $p = 0.05$, the two experiments analyzed separately.

Treatment	No. of Roots/Cuttings	
	Control	IBA (5×10^{-5} M)
Experiment 1		
Control	5.6 c	15.6 b
(A) 0–24 h		
Canavanine, 10^{-5}	7.5 c	21.8 b
Canavanine, 10^{-4}	0 d	0.4 d
(B) 24–96 h		
Canavanine, 10^{-5}	7.9 c	32.7 a
Canavanine, 10^{-4}	0.4 d	0 d
Experiment 2		
Control	6.3 b	25.5 a
Canavanine, 10^{-5}	7.3 b	3.8 b
Canavanine, 10^{-4}	0 c	0 c

Table IV. *Effect of MGBG on Root Formation in Mung Bean Cuttings*

Cuttings were treated with MGBG during the initial 24-h period (multiple range test, P = 0.05).

	No. of Roots/Cutting	
	Control	IBA (5×10^{-5} M)
Control	8.4 ± 2.0 c	27.1 ± 8.4 a
MGBG, 10^{-5} M	9.1 ± 2.9 c	18.7 ± 5.5 b
MGBG, 10^{-4} M	3.3 ± 4.3 d	15.3 ± 6.3 b

Table V. *Effect of IBA on Polyamine Contents in Mung Bean Cuttings*

Cuttings were separated to the corresponding organs after a 96-h rooting period. Number of roots per cutting: control, 7; IBA, 24. IBA applied at 10^{-5} M.

	Putrescine		Spermidine		Putrescine to Spermidine	
	Control	IBA	Control	IBA	Control	IBA
	nmol/mg protein				*ratio*	
Hypocotyl	95.1	203.5	22.5	37.9	4.23	5.37
Epicotyl	105.1	214.0	36.7	57.0	2.86	3.75
Leaves	34.2	50.0	9.1	9.5	3.76	5.26

not yet clear whether they have a precise regulatory role, or do PA changes only accompany growth processes. Untreated mung bean cuttings produce only few roots and occasionally senescence, whereas IBA-treated hypocotyls form many roots and show vigorous growth (Table II, Figs. 1 and 2). The initiation phase occurs during the first 24 h, followed later by differentiation and elongation of the roots (1, 16) and, thus, this system seems appropriate for elucidating the role of PA. Fully developed cuttings with roots (5 d) evince a remarkable increase in putrescine and spermidine in response to IBA treatment (Table V), and a corresponding increase in putrescine to spermidine ratio, indicative of active cell division and growth (7). Moreover, early changes in PA content of hypocotyls are detected before any growth of adventitious roots occurs (Table VI); thus, the considerable initial drop in PA level (and especially of putrescine) of senescing cuttings is prevented by an IBA treatment, which also results in higher putrescine to spermidine ratio already during the first 48 h. Such early changes in proteins and nucleic acids have been previously reported for root formation (5), and it is possible that these changes are related to PA content and metabolism, similar to other biological systems (7, 10, 31). It is noteworthy that the low content of free PA (10^{-8}–10^{-7} mol per g fresh weight) is the range of some other endogenous growth regulators. For exogenous treatments, however, PA are usually applied at 100- to 1000-fold concentrations (2, 3, 17, 18).

The lack of response to applied PA (Table I) suggests that either PA are not involved in root formation, or that applied PA do not reach the site of action, or that endogenous levels of PA are optimal for root production. However, the inhibition of IBA-induced root formation by L-canavanine and L-canaline, and its reversal by the corresponding amino acids (Tables II and III), indicate that PA may be necessary for root formation. It is well established that putrescine is synthesized in plants from arginine and ornithine (28) and that the antimetabolite canavanine may retard growth by competing with arginine and effectively inhibit enzymes of arginine metabolism (25). Moreover, the first report of canavanine effect in plants indicated that it inhibited IAA-dependent elongation of *Avena* coleoptiles, and a specific role for arginine was suggested (9). Recent reports (11, 34) clearly show that canavanine inhibits growth of several other plant tissues. It is noteworthy that arginine has been previously found to be active as an auxin-cofactor in root initiation on *Hibiscus* cuttings (32),

Table VI. *Effect of IBA on PA Content in the Hypocotyl of Mung Bean Cuttings*

Cuttings were pretreated with 10^{-5} M IBA for 24 h and then incubated for additional 24 h in buffer. The entire hypocotyl was used for PA determination.

Time	Putrescine		Spermidine		Putrescine		Spermidine		Putrescine to Spermidine	
	Control	IBA	Control	IBA	Control	IBA	Control	IBA	Control	IBA
h	*nmol PA/g fresh wt*				*nmol PA/mg protein*				*ratio*	
0	41.9		19.1		46.4		21.1		2.19	
24	28.4	37.1	15.8	18.1	33.1	44.2	18.3	21.4	1.80	2.05
48	25.7	49.3	12.9	13.4	25.4	42.8	12.4	11.5	1.99	3.68

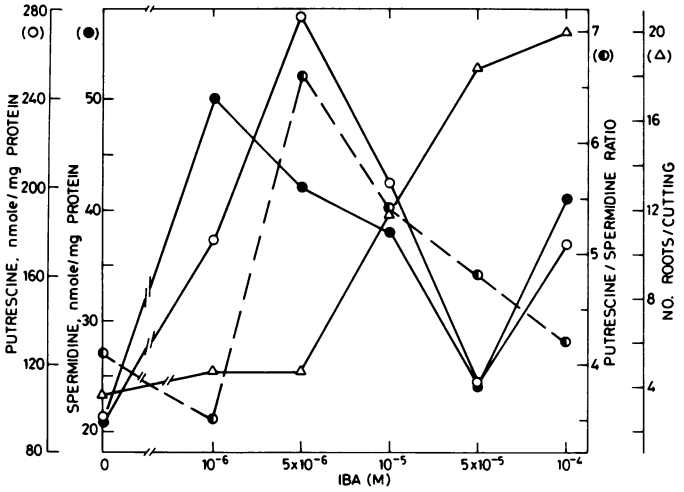

FIG. 2. Rooting and polyamine content of hypocotyls of mung bean cuttings treated with different IBA concentrations. Plant material was extracted for PA determination at end of a 96-h rooting period.

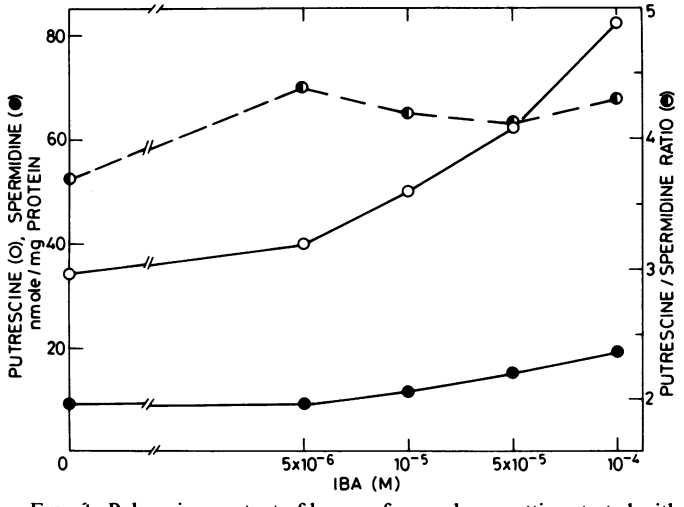

FIG. 3. Polyamine content of leaves of mung bean cuttings tested with different IBA concentrations. Plant material was extracted at end of a 96-h rooting period.

thus supporting the present data and conclusions. In addition, it was recently found (A. Altman and R. Friedman, unpublished data) that canavanine inhibits putrescine accumulation in IBA-treated cuttings. The ineffectivity of arginine in reversing the canavanine inhibition in control cuttings, unlike its effect on IBA-induced root formation (Table II), as well as the dependence of the effectivity of canavanine on its timing with IBA treatment (Table III), point to some, yet unclear, interrelationships of auxin-induced root formation, PA content, and inhibitors of PA biosynthesis. Similar conclusions can be drawn from the data of Figures 2 and 3 as to the effect of IBA concentrations on root formation and on PA content. Thus, the major changes in PA occur in the hypocotyl where roots are formed and less so in leaves, and the highest putrescine to spermidine ratio occurs at IBA concentration, which is only slightly inductive for root formation, whereas massive root growth may lead to relatively greater utilization of putrescine. MGBG, an inhibitor of spermidine and spermine biosynthesis, has been previously shown to inhibit *S*-adenosylmethionine decarboxylase activity of etiolated *Lathyrus sativus* seedlings (30) and in the present study it effectively retarded root formation (Table IV).

The foregoing suggests that polyamines are involved in auxin-induced root formation, although their specific role has yet to be elucidated in order to establish a causal relationship. Data of polyamine metabolism that support this conclusion will be presented in a subsequent paper.

Acknowledgments—The skillful technical assistance of Mrs. N. Levin and H. Wiener is gratefully acknowledged.

LITERATURE CITED

1. ALTMAN A 1972 The role of auxin in root initiation in cuttings. The International Plant Propagation Society 22: 280–294
2. ALTMAN A 1982 Retardation of radish leaf senescence by polyamines. Physiol Plant 54: 189–193
3. ALTMAN A, R KAUR-SAWHNEY, AW GALSTON 1977 Stabilization of oat leaf protoplasts through polyamine-mediated inhibition of senescence. Plant Physiol 60: 570–574
4. ALTMAN A, U BACHRACH 1981 Involvement of polyamines in plant growth and senescence. *In* CM Caldarea, V Zappia, U Bachrach, eds, Advances in Polyamine Research, Vol 3. Raven Press, New York, pp 365–375
5. ANZAI T 1975 Two phases in adventitious root formation in *Phaseolus mungo* hypocotyl cuttings, a phase sensitive to an inhibitor of RNA or protein synthesis and a phase sensitive to an inhibitor of DNA synthesis. J Exp Bot 93: 580–588
6. AUDISIO S, N BAGNI, D SERAFINI FRACASSINI 1976 Polyamines during the growth *in vitro* of *Nicotiana glauca* habituated tissue. Z Pflanzenphysiol 77: 146–151
7. BACHRACH U 1973 Function of Naturally Occurring Polyamines. Academic Press, New York
8. BATTEN DJ, PB GOODWIN 1981 Auxin transport inhibitors and rooting of hypocotyl cuttings from etiolated mung bean *Vigna radiata* (L.) Wilczek seedlings. Am Bot 47: 497–503
9. BONNER J 1949 Limiting factors and growth inhibitors in the growth of the *Avena* coleoptiles. Am J Bot 36: 323–332
10. COHEN SS 1971 Introduction to Polyamines. Prentice-Hall, Englewood Cliffs, NJ
11. CONSTABLE E, JW KIRKPATRICK, KN KAO, KK KARTHA 1975 The effect of canavanine on the growth of cells from suspension cultures and intergeneric heterokaryocytes of canavanine sensitive and tolerant plants. Biochem Physiol Pflanz 168: 319–325
12. DAI Y, R KAUR-SAWHNEY, AW GALSTON 1982 Promotion by gibberellic acid of polyamine biosynthesis in internodes of light-grown dwarf peas. Plant Physiol 69: 103–105
13. GALSTON AW, R KAUR-SAWHNEY 1980 Polyamine and plant cells. What's New in Plant Physiology 11: 5–8
14. HEIMER YM, Y MIZRAHI, U BACHRACH 1979 Ornithine decarboxylase activity in rapidly proliferating plant cells. FEBS Lett 104: 146–148
15. HESS CE 1969 Internal and external factors regulating root initiation *In* WY Whitting, ed, Root Growth. Proceedings of the 15th Easter School, Nottingham. Butterworth, London, pp 42–52
16. HEUSER CW, CE HESS 1972 Endogenous regulators of root initiation in mung bean hypocotyls. J Am Soc Hort Sci 97: 392–396
17. KAUR-SAWHNEY R, AW GALSTON 1979 Interaction of polyamines and light on

biochemical processes involved in leaf senescence. Plant Cell Environ 2: 189–196
18. KAUR-SAWHNEY R, L SHIH, AW GALSTON 1982 The relation of polyamine biosynthesis to the initiation of sprouting in potato tubers. Plant Physiol 69: 411–415
19. KEFFORD NP 1973 Effect of a hormone antagonist on the rooting of shoot cuttings. Plant Physiol 51: 214–216
20. LOWRY OH, NJ ROSEBROUGH, AL FARR, RJ RANDALL 1951 Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275
21. MONTAGUE MJ, JW KOPPENBRINK, EG JAWORSKI 1978 Polyamine metabolism in embryogenic cells of *Daucus carota*. I. Changes in intracellular content and rates of synthesis. Plant Physiol 62: 430–433
22. MONTAGUE MJ, TA ARMSTRONG, EG JAWORSKI 1979 Polyamine metabolism in embryonic cells of *Daucus carota*. II. Changes in arginine decarboxylase activity. Plant Physiol 63: 341–345
23. NANDA KK, MK JAIN, S MALHOTRA 1971 Effect of glucose and auxins on rooting etiolated stem segments of *Populus nigra*. Physiol Plant 24: 387–391
24. RAINA A, K TUOMI, RL PAJULA 1981 Synthesis of macromolecules and viruses in cells treated with inhibitors of polyamine synthesis. *In* CM Caldarea, V Zappia, U Bachrach, eds, Advances in Polyamine Research, Vol 3. Raven Press, New York, pp 163–173
25. ROSENTHAL GA 1977 The biological effects and mode of action of L-canavanine, a structural analogue of L-arginine. Q Rev Biol 152: 155–178
26. ROSENTHAL GA 1978 The biological and biochemical properties of L-canaline, a naturally occurring structural analogue of L-ornithine. Life Sci 23: 93–98
27. SEILER N, M WIECHMANN 1965 Zum Nachweis von Aminen in 10^{-10} Mol Masstab. Trennung von 1-Dimethylaminonaphthalin-5-sulfonsäure-amiden auf Dunnschicht Chromatogrammen. Experientia 21: 203–204
28. SMITH TA 1979 Arginine decarboxylase of oat seedlings. Phytochemistry 18: 1447–1452
29. SPERANZA A, N BAGNI 1977 Putrescine biosynthesis in *Agrobacterium tumefaciens* and in normal and crown gall tissues of *Scorzonera hispanica*. Z Pflanzenphysiol 81: 226–233
30. SURESH MR, PR ADIGA 1977 Putrescine-sensitive (artifactual) and insensitive (biosynthetic) *S*-adenosylmethionine decarboxylase activities of *Lathyrus sativus* seedlings. Eur J Biochem 79: 511–518
31. TABOR CW, H TABOR 1978 1,4-Diaminobutane (putrescine) spermidine and spermine. Annu Rev Biochem 45: 285–306
32. VAN OVERBEEK J, S GORDON, L GREGORY 1946 Analysis of the function of the leaf in the process of root formation in cuttings. Am J Bot 33: 100–107
33. VILLANUEVA VR, RC ADLAKHA, AN CANTERA-SOLER 1978 Changes in polyamine concentrations during seed germination. Phytochemistry 17: 1245–1249
34. WEAKS TE JR, GE HUNT 1974 The effect of canavanine on the growth of isolated roots of four plant species. Bot Gaz 135: 45–49