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Gradients of Polyamines and Their Biosynthetic Enzymes in Coleoptiles and Roots of Corn[1]

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FRANÇOISE M. DUMORTIER, HECTOR E. FLORES, NARPAT S. SHEKHAWAT[2], AND ARTHUR W. GALSTON
Department of Biology, Yale University, New Haven, Connecticut 06511

ABSTRACT

The distribution of diamines, polyamines, and their biosynthetic enzymes arginine decarboxylase and ornithine decarboxylase in roots and coleoptiles of corn (*Zea mays* var Golden Cross Bantam) seedlings have been determined. Putrescine content, expressed on either a fresh weight or protein basis, increases from the tip to the base in both roots and coleoptiles. In roots, this gradient is paralleled by an activity gradient of arginine and ornithine decarboxylases. Spermidine is distributed equally along the length of coleoptiles; in roots, this is true only on a protein basis. Free spermine is detectable only in the root tip, but a bound form is present throughout the root and coleoptile. The results are compared with gradients in protein and DNA content and discussed in relation to the possible cellular roles of polyamines.

Diamines and PA[3] have long been implicated in the regulation of growth and development of microbial and mammalian cells (1, 8, 16). Recent evidence indicates that PA might play a similar role in plant growth, development, and senescence (4, 18; brief review in 13). Data indicating their possible importance for plant growth include the following. (a) Slices of dormant tubers of *Helianthus tuberosus* can be made to grow by either exogenous auxin or PA, and show a rapid enhancement of PA synthesis upon addition of auxin (4). (b) Rapidly growing cells are rich in PA, and tumor cells can contain up to 100 times the PA content of normal cells (3). (c) The sprouting of potato tubers and the germination of seeds is accompanied by a sharp increase in PA titer (19, 23). (d) The R-induced promotion of bud growth in etiolated pea seedlings is accompanied by an increase in ADC activity and PA content, while the simultaneously induced inhibition of 3rd-internode elongation is accompanied by a decrease of ADC activity and PA accumulation (9, 14). A similar correlation has been found for the GA_3-enhanced growth of light-grown dwarf pea seedlings (10). Using a system in which R effects could be decoupled from growth, Goren *et al.* (15) concluded that the change in ADC activity was not a result of the change in growth rate, and could be its cause. (e) The pollination-induced enlargement of tomato ovaries into fruits is dependent upon continued PA biosynthesis, since blockage of ODC by α-difluoromethylornithine, a specific inhibitor of ODC, prevents growth, and this inhibition is reversible by putrescine (7). (f) The senescence of excised oat leaves in darkness is correlated with declining ADC and PA levels (20). In light, senescence is arrested, and ADC levels rise. Senescence is also arrested by the application of 1 to 10 mM PA (17). The molecular basis of this growth factor-like action of PA is unknown, but might involve an effect of PA on DNA, RNA, and protein synthesis (3, 16, 18) or a PA-induced stabilization of membranes (21).

To obtain further information on the possible roles of PA in plant growth and developmental processes, we have investigated the distribution of PA and the levels of ADC and ODC activities along the axes of two actively growing organs of corn seedlings, (a) coleoptiles, in which no cell division or changing patterns of differentiation exist and where all growth is due to cellular elongation, and (b) roots, in which cell division is localized at the

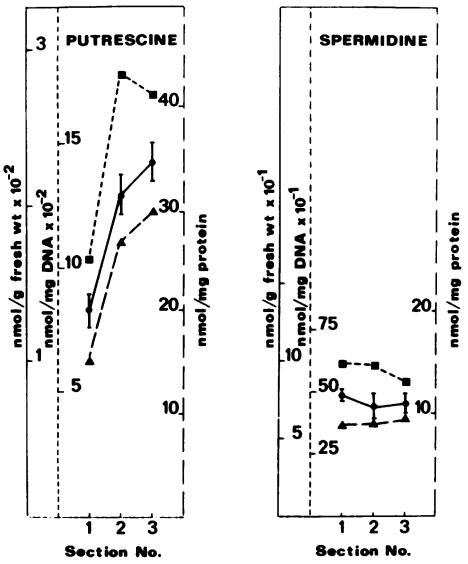

FIG. 1. Putrescine and spermidine content of different regions of the coleoptile. Coleoptiles were cut into three equal parts: No. 1, tip; No. 2, middle; No. 3, base. The results are expressed on a fresh weight basis (●——●), DNA basis (■- - -■), and protein basis (▲- -▲).

Table I. *Protein and DNA Content in Different Regions of the Coleoptile*
Section 1 is apical, 3 basal.

Section No.	Protein Content	DNA Content
	mg/g fresh wt	*μg/g fresh wt*
1	8.7 ± 0.6	126 ± 9
2	7.8 ± 2.3	117 ± 8
3	7.8 ± 0.6	135 ± 12

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[2] Government of India National Scholar, Department of Botany, Jodhpur University, Jodhpur, India.

[3] Abbreviations: PA, polyamine(s); R, red light; ADC, arginine decarboxylase; ODC, ornithine decarboxylase; PCA, perchloric acid; PLP, pyridoxal phosphate; DAPI, 4',6-diamidino-2-phenylindole; SAM, *S*-adenosylmethionine.

Table II. *Free and Bound PA in Different Regions of the Coleoptile*

Section 1 is apical, 3 basal.

Section No.	PA Content								
	Putrescine				Spermidine				Spermine (Bound)
	Free	Bound	Total	Bound	Free	Bound	Total	Bound	
	μmol/g fresh wt			%	*μmol/g fresh wt*			%	*μmol/g fresh wt*
1	104 ± 14	49 ± 10	155 ± 11	31	108 ± 27	31 ± 17	138 ± 39	22	41 ± 6
2	173 ± 27	22 ± 13	195 ± 22	11	103 ± 29	23 ± 16	120 ± 46	19	42 ± 11
3	208 ± 30	21 ± 10	229 ± 24	9	115 ± 32	31	129 ± 49	24	46 ± 17

Table III. *ADC and ODC Activity in Different Regions of the Coleoptile*

Measurements represent the average of two experiments (see Fig. 1).

Section No.	ADC Activity	Relative ADC Activity	ODC Activity	Relative ODC Activity
	nmol CO_2·mg protein^{-1}·h^{-1}		*nmol CO_2·mg protein^{-1}·h^{-1}*	
1	4.56	100	59	100
2	5.88	129	113	193
3	7.08	155	140	239

Table IV. *Protein and DNA Content in Different Regions of the Root*

Section 1 is apical, 6 basal.

Section No.	Protein Content	DNA Content
	mg/g fresh wt	*μg/g fresh wt*
1	44.7 ± 3.5	747 ± 17
2	8.1 ± 0.3	106 ± 8
3	4.6 ± 0.1	67 ± 4
4	4.6 ± 0.1	61 ± 4
5	5.5 ± 0.4	63 ± 5
6	5.3 ± 0.6	81 ± 8

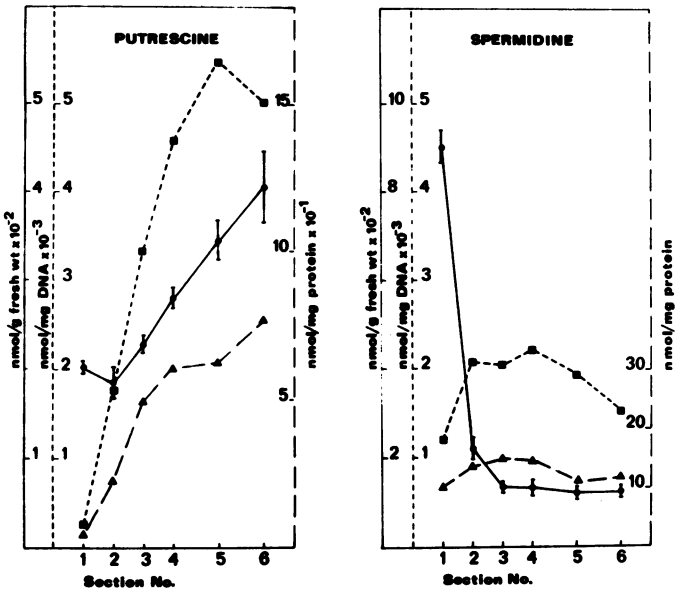

FIG. 2. Putrescine and spermidine content in different regions of the root. The first 2 cm (including the tip) were cut into four equal parts (No. 1–4) and the remaining basal piece into two equal parts (Nos. 5 and 6). The results are expressed on a fresh weight basis (●——●), DNA basis (■- - -■), and protein basis (▲- -▲).

apical meristem and orderly sequences of cell elongation and differentiation can be predicted.

MATERIALS AND METHODS

Plant Material. Seeds of *Zea mays* var Golden Cross Bantam were soaked for 10 h in tap water in the dark, and germinated between two layers of moist paper towel. After 24 h, they received 10 min of broad band R (600–900 nm) totalling 0.012 mJ/m^2 to inhibit first internode growth and were transferred to moist vermiculite. This R treatment was repeated daily; otherwise seedlings were grown in the dark at 27°C and about 70% RH. Roots were obtained from 3-d-old seedlings (length 3.5–4.0 cm) and coleoptiles from 5-d-old seedlings (length 3–4 cm).

Extraction and Quantification of PA. The tissues were extracted for 1 h in 5% ice-cold PCA (100 mg fresh weight/ml PCA), the extracts centrifuged for 20 min at 26,000*g*, and the supernatant fraction used directly for analysis of free PA. Bound PA were released by hydrolysis in 6 N HCl for 16 h at 110°C. Further analysis was done as for free PA. After dansylation, the PA were separated on TLC plates with chloroform:triethylamine (25:5, v/v), located, scraped, eluted, and quantified by fluorimetry as previously described (12). The identity of the PA was confirmed with a second TLC solvent system, cyclohexane:ethylacetate (5:4, v/v). PA content is expressed per unit fresh weight, protein, or DNA.

Extraction and Assay of ADC and ODC. For determination of ADC and ODC activity, the procedure described by Dai and Galston (9) was adopted with some modifications. Coleoptiles were homogenized in 0.2 M Tris-HCl buffer (pH 8; 50 mg fresh weight tissue/ml buffer). A pH of 8 appeared optimal for the assay of ADC as well as ODC in coleoptile extracts, 0.2 M Tris giving better results than 0.1 M. The homogenate was centrifuged for 20 min at 26,000*g* (at 4°C) and the supernatant fraction used in the enzyme assay.

The assay of ADC was carried out with a mixture of 100 μl crude enzyme extract and 10 μl of a solution of L-[U-^{14}C]arginine (New England Nuclear Co.; this solution had been diluted with unlabeled arginine to a concentration of 9 mM arginine, containing 20 μCi/ml). ODC activity was measured with a mixture of 100 μl crude enzyme extract and 10 μl of a solution of DL-[1-^{14}C]ornithine (New England Nuclear Co.; this solution had been diluted with unlabeled ornithine to a concentration of 66 mM ornithine, containing 20 μCi/ml). Addition of extra PLP proved to be inhibitory for both enzyme reactions; it was consequently omitted. Both reactions were stopped after 45 min incubation at 30°C by the addition of 0.2 ml 10% TCA. The $^{14}CO_2$ liberated was trapped on paper discs impregnated with 50 μl of 2 N KOH. Radioactivity on the discs was counted in 2 ml Biofluor in a scintillation counter.

The extraction of root tissue was carried out in 0.2 M Tris-HCl buffer (pH 8.5), the apparent pH optimum for ADC and ODC activity in root extracts (100 mg fresh weight/ml buffer). ADC and ODC assays of root extracts were carried out as described for

Table V. *Free and Bound PA in Different Regions of the Root*

Section 1 is apical, 3 basal.

Section No.	PA Content											
	Putrescine				Spermidine				Spermine			
	Free	Bound	Total	Bound	Free	Bound	Total	Bound	Free	Bound	Total	Bound
	nmol/g fresh wt			%	*nmol/g fresh wt*			%	*nmol/g fresh wt*			%
1	140 ± 7	61	201 ± 5	30	349 ± 23	107	457 ± 30	23	45 ± 3	41	86 ±7	48
2	202 ± 13	25	227 ± 15	11	114 ± 6	16	130 ± 9	12		19 ± 2		100
3	304 ± 21	35	339 ± 26	10	79 ± 5	20	99 ± 7	20		20 ± 2		100

Table VI. *ADC and ODC Activity in Different Regions of the Root*

See Figure 2.

Section No.	ADC Activity	Relative ADC Activity	ODC Activity	Relative ODC Activity
	nmol CO_2·mg protein^{-1}·h^{-1}		*nmol CO_2·mg protein^{-1}·h^{-1}*	
1	3.36 ± 0.15	100	16 ± 2	100
2	4.32 ± 0.02	129	58 ± 7	352
3	4.50 ± 0.04	134	67 ± 4	408
4	4.32 ± 0.18	129	67 ± 5	405
5	3.42 ± 0.09	102	64 ± 5	388
6	3.00 ± 0.12	89	47 ± 6	286

coleoptile extracts, except that for ODC 10 μl of 5 mM PLP was added to the assay mixture. Enzyme activities are expressed as nmol substrate converted·mg protein $^{-1}$·h^{-1}.

Determination of DNA and Protein Content. Protein content was determined by the Coomassie Blue method of Bradford (5), using γ-globulin as a standard.

DNA content was estimated by the DAPI-method, essentially as described by Brunk *et al.* (6) and Baer *et al.* (2). Tissue was extracted in pH 7 Tris buffer (10 mM Tris, 10 mM EDTA, 2 M NaCl) at a ratio of 100 mg fresh weight/ml buffer. After extraction for 1 h in an ice bath, samples were centrifuged for 20 min at 26,000*g* and the supernatant fraction used for determination of the DNA content. The fluorescence of the DAPI-DNA complex was measured at 450 nm in an Aminco-Bowman spectrophotofluorimeter with the activation wavelength set at 350 nm. DAPI was used at a final concentration of 100 ng/ml buffer (0.1 M NaCl, 10 mM EDTA, 10 mM Tris [pH 7]). For DNA quantification, three aliquots of 10 μl each of the DNA extracts were added successively to 3 ml of the DAPI solution and the fluorescence values noted each time. Afterwards, three aliquots of DNA standard solution (calf thymus DNA, 20 μg/ml) were added successively to the same cuvette. When the fluorescence values were plotted against the cumulative volume of aliquots added in the cuvette, two straight lines were obtained from which the DNA content in the extract could be calculated.

Analysis of PA content, determination of DNA and protein content and determination of enzyme activities were carried out with extracts from different plants. The data represent the mean ± SE of three experiments, except for ADC and ODC activity in the coleoptile (Table III), where the average of two assays is given. In this case, additional assays were carried out, but under slightly different conditions. The results were, however, similar to those presented.

RESULTS

Coleoptile. For extraction and quantification of PA, coleoptiles were, after removal of the leaf, cut into three equal parts, part No. 1 corresponding to the tip. Only putrescine and spermidine were found free in coleoptiles. Their distribution along the coleoptile axis is shown in Figure 1 and the corresponding distribution of proteins and DNA is given in Table I. Free putrescine content shows a gradient increasing from tip to base of the coleoptile. Inasmuch as the protein content and the DNA content per unit fresh weight were about the same in all parts, the putrescine gradient was obvious on fresh weight, protein, and DNA bases. By contrast, free spermidine was equally distributed along the axis of the coleoptile. The distribution of total PA along the coleoptile axis is shown in Table II. The amount of bound spermidine was about the same in all sections (~22% of the total spermidine content); the total putrescine content shows the same gradient as the free form, although the tip of the coleoptile contained somewhat more bound putrescine than the other regions. Spermine was present only in a bound form in all three sections.

The ADC and ODC activities found in extracts of the three different regions are shown in Table III. ADC activity was low compared to ODC activity, and both enzyme activities were higher at the base than at the apex, thus showing the same gradient as putrescine. Free putrescine content in the base was about twice as high as in the tip but the gradient in total putrescine was less pronounced and showed the same trend as ADC activity.

Root. For analysis of free PA, proteins and DNA, the first 2 cm of the root (including the tip) were cut into four equal parts numbered 1 to 4, while the remaining basal piece was cut into two equal parts, numbered 5 and 6. All parts contained putrescine and spermidine while free spermine was detectable only in the tip at a concentration of 73 ± 2.7 nmol/g fresh weight. The free putrescine and spermidine contents of the different sections is shown in Figure 2, and the corresponding distribution of proteins and DNA in Table IV. Putrescine content increased from the tip to the base on a fresh weight, protein, and DNA basis, but the gradient was most pronounced when expressed on DNA basis (the basal parts showing 20 times more putrescine than the tip). Spermidine content, as expressed per g fresh weight, was much higher in the tip than in the other regions. However, on a protein basis, the distribution of spermidine did not differ much along the root axis. DNA content was higher in the tip and base, which resulted in a higher spermidine/DNA ratio in the middle region (sections 2–4).

For estimation of bound *versus* total PA content, roots were cut into three equal sections. As was found for coleoptiles, relatively more putrescine was bound in the tip section and the percentage of bound spermidine was about the same in all sections (Table V). Spermine was present in a bound form in all sections but highest in section 1, which also contained free spermine.

As was found for coleoptiles, ADC activity was much lower than ODC activity (Table VI). Like the distribution of spermidine, ADC activity was about the same in the different parts of the root. ODC activity was 3 to 4 times lower in the tip than in other parts.

DISCUSSION

Determinations of ADC and ODC activity provide information relevant only to the time of assay, while determinations of levels

of PA reflects the balance of synthesis and degradation over the previous growing period of the plant. We should not therefore expect a strict correlation between the data on enzymic activities and PA content. Nevertheless, some parallelism exists between putrescine content and ADC or ODC activity in the coleoptile and the acropetal half of the root.

Reverse gradients of diamines and higher PA were first observed in etiolated pea seedlings (Flores *et al.*, in preparation). Hence, our observations in a monocot argue for generality of the phenomenon. Putrescine accumulation in older cells (such as basipetal root cells) could be due to limited conversion to spermidine and spermine because of low spermidine (and spermine) synthase activity. Such an explanation would also account for the observed distribution patterns of these higher PA. Low spermidine synthesis might also result from a diversion of SAM into increased ethylene biosynthesis, since SAM is a precursor for both spermidine (and spermine) and ethylene (11). SAM is also a methyl donor in many biochemical reactions, and might be depleted if such activity were to increase.

In roots, we could detect free spermine only in the tips, which are known to have meristematic activity, but not in more basal regions. In coleoptiles, where cell division does not occur, free spermine could not be detected at all. Inasmuch as spermine is the most efficient of all PA in stabilizing DNA *in vitro* (22), and is also effective in stimulating DNA synthesis and mitotic activity in oat leaf protoplasts (18), it might function in nuclear processes *in vivo*.

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