Plant Physiol. (1982) 69, 411–415
0032-0889/82/69/0411/05/$00.50/0

Relation of Polyamine Biosynthesis to the Initiation of Sprouting in Potato Tubers[1]

Received for publication June 29, 1981 and in revised form October 5, 1981

RAVINDAR KAUR-SAWHNEY, LIU-MEI SHIH, AND ARTHUR W. GALSTON
Department of Biology, Yale University, New Haven, Connecticut 06511

ABSTRACT

The polyamines putrescine, spermidine, and spermine and their biosynthetic enzymes arginine decarboxylase, ornithine decarboxylase and *S*-adenosyl-L-methionine decarboxylase are present in all parts of dormant potato (*Solanum tuberosum* L.) tubers. They are equally distributed among the buds of apical and lateral regions and in nonbud tissues. However, the breaking of dormancy and initiation of sprouting in the apical bud region are accompanied by a rapid increase in ornithine decarboxylase and *S*-adenosyl-L-methionine decarboxylase activities, as well as by higher levels of putrescine, spermidine, and spermine in the apical buds. In contrast, the polyamine biosynthetic enzyme activities and titer remain practically unchanged in the dormant lateral buds and in the nonbud tissues. The rapid rise in ornithine decarboxylase, but not arginine decarboxylase activity, with initiation of sprouting suggests that ornithine decarboxylase is the rate-limiting enzyme in polyamine biosynthesis. The low level of polyamine synthesis during dormancy and its dramatic increase in buds in the apical region at break of dormancy suggest that polyamine synthesis is linked to sprouting, perhaps causally.

The PA,[2] Put, Spd, and Spm are present throughout the microbial, animal, and plant worlds (3, 7). In microbial and plant cells, Put is derived either from arginine via ADC and the intermediate Agm, or from ornithine by ODC. In mammalian cells, Put synthesis occurs only by the latter pathway. The Put thus formed is converted successively to Spd and Spm through propylamino group transfer from SAM mediated by SAMDC in all the above types of cells (3, 11). These amines have been implicated in several important processes involved in cell growth and development, especially those involving nucleic acids (3, 7, 11, 27).

Both the activity of PA biosynthetic enzymes and PA titer have been reported to increase dramatically during rapid growth in many plant systems, such as germinating seeds of *Zea*, *Pisum*, *Triticum*, and *Tragopogon* (30), developing seedlings of *Phaseolus* (4) and *Lathyrus* (21, 22), ovaries of tomato, and rapidly dividing tobacco cells in suspension culture (12), during crown gall-tumor development (6) and embryogenesis of carrot suspension cells (18, 19). Although little is known about the activities of PA biosynthetic enzymes in dormant tissues such as tubers and senescencing plant parts, contents of PA in these systems are extremely low (1, 26). We have observed that activities of PA biosynthetic enzymes and PA titer decrease during aging and senescence of oat leaves (17). Furthermore, exogenous application of the PA or their precursor amino acids retards senescence of leaves in several monocotyledonous and dicotyledonous plants (16) and of protoplasts from oat leaves (2, 10, 14, 15). PA appear to inhibit senescence by preventing Chl, protein, and RNA breakdown in leaves (16) and by increasing macromolecular synthesis and mitotic activity in protoplasts (15). The antisenescence properties of PA and their correlation with cell proliferation and differentiation lend support to the contention that they act as growth factors (3, 5, 11). Despite these observations, the relative roles of individual PA biosynthetic enzymes in dormant and actively dividing plant tissues is not well understood. We have, therefore, examined the activities of ADC, ODC, and SAMDC and the endogenous levels of Put, Spd, and Spm in dormant and actively growing tissues of potato tubers.

MATERIALS AND METHODS

Plant Materials. Idaho Russet baking potatoes (*Solanum tuberosum* L.) (U.S. No. 1) were purchased from the local supermarket. The tubers were firm, with no sign of sprouting, and were therefore regarded as dormant. Medium-size tubers were selected and allowed to sprout by storing them in the dark at room temperature for about 2 months. Samples of tissue were taken from dormant, initial, and advanced (profuse) sprouted tubers. Three areas from the same tuber showing different sprouting activity were selected for sampling (Fig. 1): (A) bud tissue in the apical region (hereafter referred to as apical buds) which developed sprouts; (B) bud tissue from the lateral region (hereafter referred to as lateral buds) which remained dormant throughout the storage period; and (C) nonbud tissue which does not develop sprouts. Tissue sections (7 mm in diameter, 3 mm in length) containing the skin and outer cortex were removed. During the periods when sprouting occurred, the sprouts were excised with the apical bud tissue and assayed together. Six to eight tubers were used for each experiment and slices of each selected area were randomized. Suitable amounts were selected for determining ADC, ODC, and SAMDC activities and PA titer.

Extraction and Measurements

Polyamine Biosynthetic Enzymes. For each extraction, approximately 5 g (9 discs) of tissue was homogenized in chilled mortars with 1.5 ml of 100 mM phosphate buffer at pH 7.6. The homogenates were centrifuged at 26,000*g* for 15 min at 4°C and the resulting clear supernatant fractions were assayed for enzyme activities.

Enzyme assays were done by modification of conventional methods (8, 18, 24) in 12 × 75 mm polystyrene culture tubes sealed with polyethylene caps. A filter paper disc (6 mm diameter) impregnated with 50 μl 2 N KOH was supported on a 22-gauge syringe needle through the cap and used to trap the $^{14}CO_2$ liberated. ADC activity was determined by measuring the release of $^{14}CO_2$ from the substrate, L-[U-^{14}C]arginine. The reaction mixture

[1] Supported by grants from the National Science Foundation and Binational Agricultural Research and Development Fund to A. W. G.

[2] Abbreviations: PA, polyamine; Put, putrescine; Spd, spermidine; Spm, spermine; ADC, arginine decarboxylase; Agm, agmatine; ODC, ornithine decarboxylase; SAM, *S*-adenosyl-L-methionine; SAMDC, *S*-adenosyl-L-methionine decarboxylase.

contained 100 μl crude enzyme and 10 μl 20 μCi/ml of L-[U-^{14}C]arginine (300 mCi/mmol, ICN Pharmaceuticals) diluted with unlabeled L-arginine to give a final concentration of 9 mM. ODC activity was similarly measured by using DL-[1-^{14}C]ornithine as the substrate. Each reaction mixture consisted of 100 μl crude enzyme, 10 μl 20 μCi/ml DL-[1-^{14}C]ornithine (54.9 mCi/mmol, New England Nuclear Co.) diluted with unlabeled L-ornithine to a final concentration of 66 mM, and 10 μl 5 mM pyridoxal phosphate. SAMDC activity was determined by measuring the release of $^{14}CO_2$ from SAM [^{14}C]carboxyl. The reaction mixture contained 100 μl crude enzyme, 10 μl 20 μCi/ml SAM [^{14}C]-carboxyl (60 mCi/mmol, New England Nuclear Co.) containing unlabeled SAM, to give a final concentration of 2.7 mM and 10 μl 1 mM pyridoxal phosphate. Later experiments showed that omission of pyridoxal phosphate did not change the activities of ODC and SAMDC. The enzyme activities were linear with increasing concentrations of crude enzyme and with time of incubation up to 60 min. The temperature for optimal activity was between 35 to 40°C. Thus, for all three enzymes, the reaction mixtures were incubated for 45 min at 37°C, and CO_2 liberated by injecting 0.2 ml 10% TCA into each reaction tube through the syringe needle. The tubes were stoppered and incubated for an additional 45 min in the water bath. The liberated $^{14}CO_2$ was determined by placing the paper disc into 2 ml Econofluor in mini-vials and counting in a scintillation counter. Enzyme activity was expressed as nmol of $^{14}CO_2$ liberated/g fresh weight.

Polyamine Extraction. PA from different parts of the potato tuber were extracted by homogenizing 5 g tissue with 2 ml 10% $HClO_4$ in a prechilled mortar. The homogenates were placed on ice for about 1 h and then centrifuged at 26,000*g* for 15 min. The resulting supernatant fractions were used for measuring PA content.

Polyamine Content. The endogenous levels of PA were determined by HPLC. The PCA-soluble extracts were benzoylated by the method of Redmond and Tseng (23). The details adapted for plant tissues have been described by Flores and Galston (9).

The benzoylated samples were separated in a Beckman (Altex) Model 322 liquid chromatograph equipped with an ODS reverse phase column and a 254-nm absorbance detector. Conversion of the chromatographic peaks to amounts of each of the PA was done by an Altex C-RIA integrator.

The HPLC determinations of PA levels were further confirmed by TLC. Briefly, the PCA extracts were dansylated according to the method of Seiler (25) and separated on silica gel thin layer plates (Whatman, LK6D). These plates, equipped with a preadsorbent which packs the components of the mixture at the origin, permit sharp separations. For separation of the PA, 20 μl benzene extract was spotted on the TLC plate and developed in solvents containing chloroform:triethylamine (25:2, v/v) or cyclohexane: ethyl acetate (5:4, v/v). PA standards were similarly dansylated and chromatographed. The dansylated PA separated well, permitting their removal by scraping off of the fluorescent spots. PA were then eluted with 2 ml ethyl acetate and quantified fluorometrically with the aid of an Aminco-Bowman spectrophotofluorimeter, in which the emission at 500 nm was recorded after excitation at 350 nm.

With both HPLC and TLC, standard curves, run together with tissue extracts in each experiment, were reproducible within 5%. The limit of detectability was 25 to 50 pmol. PA concentrations were expressed as μg/g fresh weight.

Potato tubers were used from two different harvest periods. In each instance, enzyme assays and PA levels were determined in triplicate. The data presented below are means of two to four experiments.

RESULTS AND DISCUSSION

Enzyme Activities in Dormant and Sprouting Potato Tubers. When firm dormant potato tubers are kept in the dark at room temperature, sprouting starts to occur after about 30 days and is profuse after about 60 days (Fig. 1). These stages are described for convenience as dormant, initial sprouting, and profuse sprouting (Fig. 1). Under our storage conditions, the apical buds sprouted while most of the lateral buds remained dormant. Measurements of enzyme activities at dormancy and progressive stages of sprouting (Fig. 2) show that ODC, SAMDC, and ADC activities are approximately the same in the apical and lateral buds and nonbud tissues of the dormant tuber. However, with initiation of sprouting after 30 days of storage, ODC and SAMDC activities in the apical buds increase and continue to increase with progressive sprouting. ODC activity at advanced sprouting after 60 days was more than four times and SAMDC more than two times the respective activities at the dormant stage. ADC activity, on the other hand, showed a slight increase with initiation of sprouting and then declined to the value at the dormant stage. Dialysis of the enzyme extracts did not alter the enzyme activities, suggesting that the higher ODC and SAMDC activities at sprouting are not due to a low molecular weight activating molecule. Likewise, the low ADC activity is not due to a low molecular weight inhibitor. The above observations were confirmed by mixing (1:1, v/v) extracts from buds of dormant tubers with sprouted apical buds which showed approximately half the initial values. The activities of ODC and SAMDC in the lateral buds and nonbud tissues remained essentially the same, whereas ADC activity decreased with progressive sprouting (Fig. 2).

The maximum increase in enzyme activities clearly occurred at the initiation of sprouting and was limited to the apical buds; ODC activity increased more than 300% from dormant to initial sprouting and about 200% from initial sprouting to profuse sprouting (Fig. 3). SAMDC showed a similar trend. These findings suggest that the high activities of the PA biosynthetic enzymes, ODC and SAMDC, are related to active cell division initiated by the breaking of dormancy. Similar correlation of ODC and SAMDC activities with cell division have been shown in other plant systems, such as developing ovaries of tomato, actively dividing tobacco cells in suspension cultures (12) and germinating corn seeds (28). Correlation of ODC and SAMDC activities with cell proliferation has also been reported in animal tissues (3, 7), while low enzyme activity with declining cell activity has been observed in resting and aging animals, in nongrowing tissues (3, 7) and in senescing leaves (17). The observed 5-fold increase in ODC activity at the profuse sprouting stage suggests that ODC is the primary enzyme involved in polyamine biosynthesis during sprouting of the potato tuber. In plants, polyamine biosynthesis via ADC has thus far been considered the more probable pathway, partly because more work has been done with ADC rather than ODC (3, 27). A recent investigation (12), however, shows much

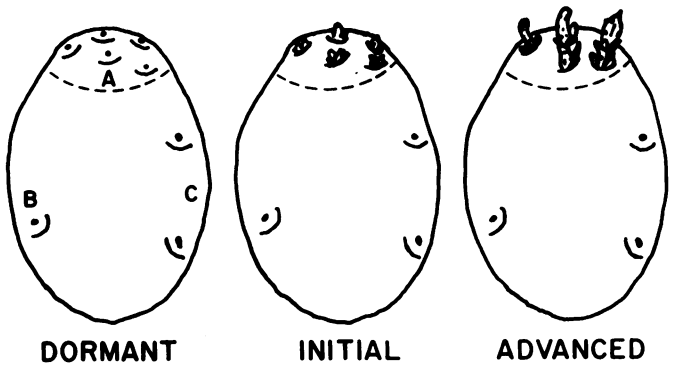

FIG. 1. Diagram showing progressive stages of sprouting in potato tubers. Sampling procedure: (A), bud tissue in the apical region (apical buds); (B), bud tissue in the lateral region (lateral buds); (C), nonbud tissue. Sprouts were assayed together with the apical bud tissue.

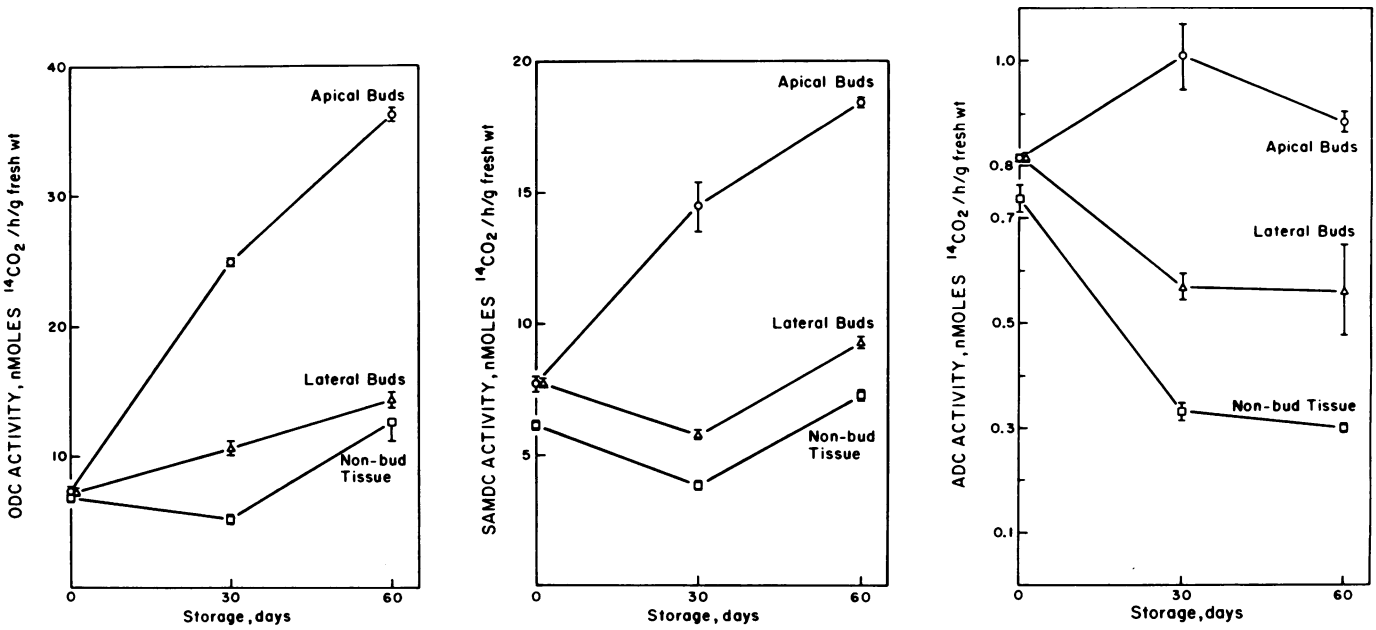

FIG. 2. Activities of ODC, SAMDC, and ADC in apical and lateral buds and nonbud tissue of dormant and sprouting potato tubers at different stages of sprouting: 0 day, dormant; 30 days, initial sprouting; 60 days, profuse sprouting.

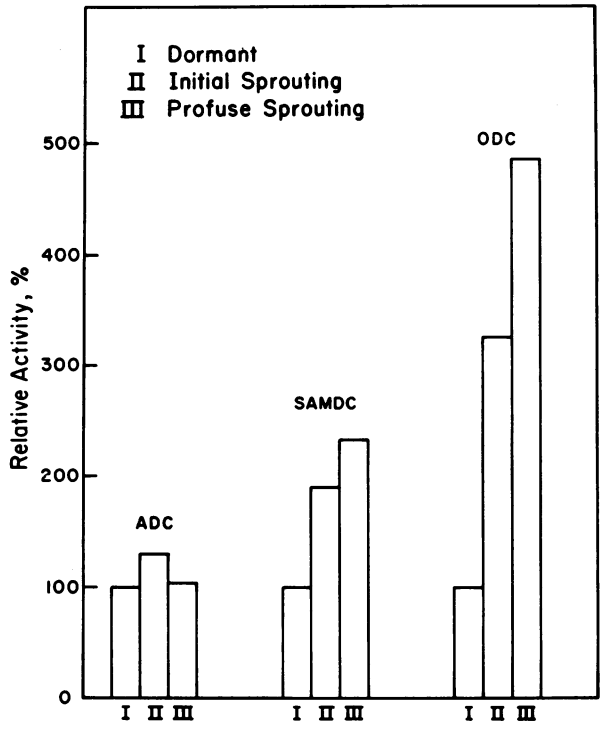

FIG. 3. Comparison of ADC, SAMDC, and ODC activities in apical buds of dormant and sprouting potato tubers.

higher activities of ODC than ADC in developing ovaries of tomato and in the exponential growth phase of tobacco cells in suspension cultures. In animal systems also, ODC is considered the key enzyme in PA biosynthesis (3, 7). Our observations with the potato tuber, together with those of Heimer *et al.* (12) suggest that either ODC or ADC can act as the rate-limiting enzyme for PA biosynthesis in different actively growing plant tissues.

Polyamine Titer. Measurements of PA levels show that Put, Spd, and Spm are present in tissues from dormant and sprouting potato tubers (Table I). Cadaverine was not detected either by HPLC or TLC. In dormant tubers, PA titer in apical buds, lateral buds, and nonbud tissues is essentially the same (Table I). However, at break of dormancy accompanying initiation of sprouting, PA in the apical buds increased sharply, and like the enzyme activities, continued to increase with progressive sprouting. Spm increased more than three times, Spd about two times, and Put about 1.5 times the initial values at dormancy. At profuse sprouting, the increase in Spm titer was the highest. Levels of Spm in the apical buds increased by more than 10 times, while Spd and Put increased by about three and two times, respectively, when compared with the initial levels (Fig. 4). Furthermore, the HPLC profiles of PA show that peaks of the individual PA are well resolved and that PA titer increases with progressive sprouting.

Table I. *Polyamine Content in Progressive Stages of Sprouting Potato Tubers*

PA extracts in PCA were benzoylated, separated, and quantified by HPLC.

PA	Type of Tissue	PA Content in Tuber Tissue		
		Dormant	Initial sprouting	Profuse sprouting
		μg/g fresh wt[a]		
Agm	Apical buds	40.4 ± 2.1	65.3 ± 3.1	181.9 ± 8.7
	Lateral buds	40.4 ± 2.1	50.6 ± 1.7	55.6 ± 2.0
	Nonbud tissue	20.8 ± 2.0	32.8 ± 1.7	37.8 ± 6.6
Put	Apical buds	4.5 ± 0.7	6.4 ± 0.1	9.3 ± 0.3
	Lateral buds	4.5 ± 0.7	4.4 ± 0.3	4.5 ± 0.1
	Nonbud tissue	6.0 ± 0	4.5 ± 0.2	4.7 ± 0.4
Spd	Apical buds	7.7 ± 1.7	14.5 ± 1.6	20.7 ± 2.3
	Lateral buds	7.7 ± 1.7	9.6 ± 0	9.9 ± 0.1
	Nonbud tissue	9.0 ± 0.4	8.5 ± 0.6	7.3 ± 0.1
Spm	Apical buds	1.0 ± 0.2	3.4 ± 0.5	10.2 ± 1.0
	Lateral buds	1.0 ± 0.2	2.1 ± 0	3.2 ± 0.2
	Nonbud tissue	0.6 ± 0.1	1.0 ± 0.3	2.3 ± 0.1

[a] ±SE; $n = 6$ to 12.

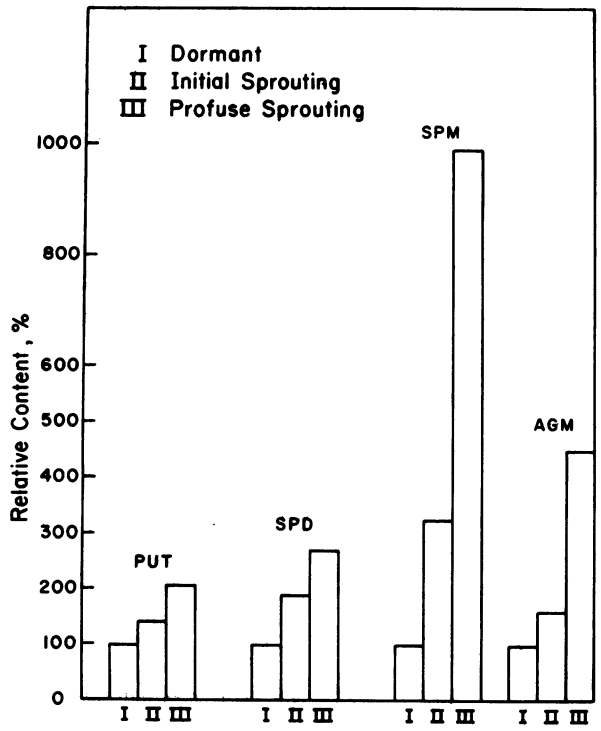

FIG. 4. PA content in apical buds of dormant and sprouting potato tubers.

Spd and Spm are the first to rise significantly, followed by Agm and Put. A similar trend was observed when samples were analyzed by TLC (data not presented).

The profuse sprouting in the apical buds appears to suppress growth of lateral buds. Consequently, in the lateral buds the endogenous levels of most of the PA, like the biosynthetic enzymes, remain essentially the same during sprouting as in the dormant stage. Similarly, PA titer in the inactive nonbud tissue remains unchanged. In both types of tissues however, Spm titer increased with sprouting, but the levels are much lower than those in the apical buds (Table I).

The high activities of ODC and SAMDC and the corresponding increases in PA levels during initial sprouting are consistent with the view that increased synthesis of PA is one of the primary events controlling the inception of growth in previously dormant tissue. This is further supported by the correlation of low activities of ODC and SAMDC and low titer of PA with no sprouting in lateral buds inhibited by apical dominance. Correlation of high endogenous levels of PA with break of dormancy by hormones or by slicing has also been reported in tubers of Jerusalem artichoke (5, 26).

To investigate further the role of ADC in PA synthesis during sprouting in potato tuber, we determined levels of Agm (best resolved by HPLC) which is an intermediate in putrescine synthesis from arginine mediated via ADC. Although there was no appreciable rise in ADC activity during sprouting, Agm levels increased sharply in the apical buds. In contrast, both ADC activity and Agm levels remained essentially unchanged in lateral buds and nonbud tissues. In apical buds, the Agm titer increased by about five times in the advanced stage of sprouting as compared with the dormant stage (Table I). The high levels of Agm during sprouting might depress ADC activity by end-product inhibition, or the conversion of Agm to Put might constitute the rate-limiting step in this tissue. Although the lack of correlation between Agm and ADC activity during sprouting is puzzling, the results indicate that the observed increased level of putrescine does not directly involve ADC. These results further support the contention that ODC is the rate-limiting enzyme in Put synthesis in potato tubers.

The high localized PA titer in advanced sprouting and the low titer in dormant tubers suggest that PA, like the well-known hormones ABA, gibberellins, and cytokinins, can act as growth factors. PA probably differ from hormones, however, in being relatively immobile intercellularly (Young and Galston, in preparation). ABA levels generally increase with dormancy, whereas giberellins and cytokinins increase with break of dormancy. Such changes in endogenous levels of these hormones have been well established in seeds, and vegetative and flower buds, which like the potato tuber, undergo a period of dormancy. In the potato tuber also, dormancy is associated with low endogenous levels and sprouting with high levels of growth promoting hormones (13, 29). The increased PA synthesis during potato tuber sprouting observed here suggests that PA are also involved in the break of dormancy, perhaps as second messengers. The action of exogenous PA in preventing senescence of excised leaves (1, 16), protoplasts (2), and chloroplast thylakoids (20) support their proposed physiological role.

Acknowledgment—We are grateful to Hector Flores for expert advice and help on the HPLC separation of PA.

LITERATURE CITED

1. ALTMAN A, U BACHRACH 1981 Involvement of polyamines in plant growth and senescence. *In* CM Caldarera, V Zappia, U Bachrach, eds, Advances in Polyamine Research, Vol 3. Raven Press, New York, pp 365–375
2. ALTMAN A, R KAUR-SAWHNEY, AW GALSTON 1977 Stabilization of oat leaf protoplasts through polyamine-mediated inhibition of senescence. Plant Physiol 60: 570–574
3. BACHRACH U 1973 Function of Naturally Occurring Polyamines. Academic Press, New York
4. BAGNI N 1970 Metabolic changes of polyamines during the germination of *Phaseolus vulgaris*. New Phytol 69: 159–164
5. BAGNI N, D SERAFINI-FRACASSINI 1973 The role of polyamines as growth factors in higher plants and their mechanism of action. *In* Plant Growth Substances. Hirokawa Publishing Co, Tokyo, pp 1205–1271
6. BAGNI N, D SERAFINI-FRACASSINI 1979 Polyamines and Plant Tumors. Ital J Biochem 28: 392–394
7. COHEN SS 1971 Introduction to the Polyamines. Prentice-Hall, Inc, Englewood Cliffs, NJ
8. FELDMAN MJ, CC LEVY, DH RUSSELL 1972 Purification and characterization of *S*-adenosyl-L-methionine decarboxylase from rat liver. Biochemistry 11: 671–677
9. FLORES HE, AW GALSTON 1982 Analysis of polyamines in higher plants by high performance liquid chromatography. Plant Physiol. In press
10. GALSTON AW, A ALTMAN, R KAUR-SAWHNEY 1978 Polyamines, ribonuclease and the improvement of oat leaf protoplasts. Plant Sci Lett 11: 69–79
11. GALSTON AW, R KAUR-SAWHNEY 1970 Polyamines and plant cells. What's New in Plant Physiol 11: 5–8
12. HEIMER YM, Y MIZRAHI, U BACHRACH 1979 Ornithine decarboxylase activity in rapidly proliferating plant cells. FEBS Letters 104: 146–148
13. HEMBERG T 1954 Studies on the occurrence of free and bound auxins and of growth-inhibiting substances in the potato tuber. Physiol Plant 7: 312–322
14. KAUR-SAWHNEY R, WR ADAMS JR, J TSANG, AW GALSTON 1977 Leaf pretreatment with senescence retardants as a basis for oat protoplast improvement. Plant Cell Physiol 18: 1309–1317
15. KAUR-SAWHNEY R, HE FLORES, AW GALSTON 1980 Polyamine-induced DNA synthesis and mitosis in oat leaf protoplasts. Plant Physiol 65: 368–371
16. KAUR-SAWHNEY R, AW GALSTON 1979 Interaction of polyamines and light on biochemical processes involved in leaf senescence. Plant Cell Environ 2: 189–196
17. KAUR-SAWHNEY R, L SHIH, HE FLORES, AW GALSTON 1982 Relation of polyamine synthesis and titer to aging and senescence in oat leaves. Plant Physiol 69: 405–410
18. MONTAGUE MJ, TA ARMSTRONG, EG JAWORSKI 1979 Polyamine metabolism in embryogenic cells of *Daucus carota*. II. Changes in arginine decarboxylase activity. Plant Physiol 63: 341–345
19. 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
20. POPOVIC RB, DL KYLE, AS COHEN, S ZALIK 1979 Stabilization of thylakoid membranes by spermine during stress-induced senescence of barley leaf discs. Plant Physiol 64: 721–726
21. RAMAKRISHNA S, PR ADIGA 1975 Amine levels in *Lathyrus sativus* seedlings during development. Phytochemistry 14: 63–68
22. RAMAKRISHNA S, PR ADIGA 1975 Arginine decarboxylase from *Lathyrus sativus* seedlings. J Biochem 59: 377–386

23. REDMOND JW, A TSENG 1979 High-pressure liquid chromatographic determination of putrescine, cadaverine, spermidine and spermine. J Chromatogr 170: 479–481
24. RUSSELL DH, SH SNYDER 1968 Amine synthesis in rapidly growing tissues: Ornithine decarboxylase activity in regenerating rat liver, chick embryo and various tumors. Proc Natl Acad Sci USA 60: 1420–1427
25. SEILER N, M WIECHMANN 1967 Die Mikrobestimmung von Spermin and Spermidin als 1-dimethylamino-naphtalin-5-sulfon-säure-Derivate. Z Physiol Chem 348: 1285–1290
26. SERAFINI-FRACASSINI D, N BAGNI, PG CIONINI, A BENNICI 1980 Polyamines and nucleic acids during the first cell cycle of *Helianthus tuberosus* tissue after the dormancy break. Planta 148: 332–337
27. SMITH TA 1977 Recent advances in the biochemistry of plant amines. *In* L Reinhold, JB Harborne, T Swain, eds, Progress in Phytochemistry, Vol 4. Pergamon Press, Oxford, pp 27–81
28. SUZUKI Y, E HIRASAWA 1980 *S*-adenosylmethionine decarboxylase of corn seedlings. Plant Physiol 66: 1091–1094
29. VAN STADEN J, GG DIMALLA 1978 Endogenous cytokinins and the breaking of dormancy and apical dominance in potato tubers. J Exp Bot 29: 1077–1084
30. VILLANUEVA VR, RC ADLAKHA, AM CANTERA-SOLER 1978 Changes in polyamine concentration during seed germination. Phytochemistry 17: 1245–1249