Plant Physiol. (1985) 79, 515–519
0032-0889/85/79/0515/05/$01.00/0

Activities of Arginine and Ornithine Decarboxylases in Various Plant Species[1]

Received for publication March 22, 1985 and in revised form June 10, 1985

HELENA BIRECKA*, ALAN J. BITONTI, AND PETER P. MCCANN
Department of Biological Sciences, Union College, Schenectady, New York 12308 (H.B.), *and Merrell Dow Research Institute, Cincinnati, Ohio 45215* (A.J.B., P.P.M.)

ABSTRACT

In extracts from the youngest leaves of *Avena sativa*, *Hordeum vulgare*, *Zea Mays*, *Pisum sativum*, *Phaseolus vulgaris*, *Lactuca sativa*, and four pyrrolizidine alkaloid-bearing species of *Heliotropium*, the activities of ornithine decarboxylase, close to V_{max}, ranged between traces and 1.5 nanomoles per hour per gram fresh weight when based on putrescine formed during incubation with labeled ornithine. The arginine decarboxylase activities in the same extracts ranged between 8 and 8000 nanomoles per hour per gram fresh weight being lowest in the borages and highest in oat and barley. α-Difluoromethylornithine and α-difluoromethylarginine inhibited ornithine and arginine decarboxylases, respectively, in all species. Agmatine, putrescine, spermidine, and spermine were found in all, diaminopropane in eight, and cadaverine in three species.

No correlation was observed between arginine or ornithine decarboxylase level and the levels of total polyamines. The *in vitro* decarboxylase activities found in the borages cannot explain the high accumulation of putrescine-derived pyrrolizidines in their youngest leaves if the pyrrolizidines are produced *in situ* from arginine and/or ornithine as precursors; other possibilities are discussed.

In assays of ornithine decarboxylase, an interference of decarboxylation not due to this enzyme was observed in extracts from all species. In arginine decarboxylase assays, the interfering decarboxylation as well as the interference of arginase were apparent in two species. Addition of aminoguanidine was needed to suppress oxidative degradation of putrescine and agmatine during incubation of extracts from pea, bean, lettuce, *Heliotropium angiospermum*, and *Heliotropium indicum*.

When assayed for ADC[2] and/or ODC using [1-^{14}C]- or [U-^{14}C]-labeled Arg and/or Orn, respectively, leaf extracts from oat, tomato, and especially form *Heliotropium angiospermum* exhibited significant decarboxylation of the substrates that was not due to activities of the assayed enzymes (6). Additional artifacts such as oxidative degradation of Agm and Put as well as conversion of Arg into Orn were observed in extracts from *H. angiospermum*, especially in Tris·HCl buffer. However, addition of AG, the amine oxidase inhibitor, at 0.1 to 0.2 mM and of Orn, the competitive inhibitor of arginase, at 20 mM permitted estimation of ADC and ODC activities on the basis of Agm and Put recovered from the reaction mixtures by thin layer electrophoresis. The presence of the inhibitors did not affect the formation of the amines in reaction mixtures containing oat or tomato leaf extracts.

The aim of this study was to evaluate the importance of the above artifacts in enzyme assays of other plant species and to estimate ODC and ADC activities in those species. Special attention has been paid to *Heliotropium*. In previous experiments (6), *H. angiospermum* revealed rather low ODC and ADC activities in leaves with very high levels of alkaloids containing a pyrrolizidine moiety which—as strongly indicated by many studies (5)—derives from two molecules of Put. The four *Heliotropium* species examined here differ significantly in the pyrrolizidine alkaloid levels (4, 7). Since the interfering decarboxylation was less pronounced in phosphate than in Tris·HCl buffer, phosphate buffer was used throughout this study.

MATERIALS AND METHODS

Plant Material. Seeds of *Avena sativa*, cv Garry; *Hordeum vulgare*, cv Boone; *Zea mays*, cv Truckers White; *Pisum sativum*, cv Alaska and the dwarf cv Little Marvel; *Phaseolus vulgaris*, cv Commador; and *Lactuca sativa*, cv Black Seeded Simpson were supplied by the Carolina Biological Supply Company and germinated in flats in a mixture of peat and vermiculite on a windowsill with southern exposure. First leaves (green cotyledons including apices, in the case of lettuce) were sampled on the 4th day after germination. *Heliotropium* plants were grown in the greenhouse from seeds or cuttings as previously described (6). *H. angiospermum* (2165.6)[3] youngest leaves, including apices, were sampled from the same flowering plants as in the previous experiments. Flowering *H. procumbens* (2118) and *H. indicum* (2112) originated from Mexico, while *H. spathulatum* (2175) originated from California. The sampled leaves were immediately frozen and kept at −80°C until analyzed.

Analyses. Homogenization, $(NH_4)_2SO_4$ fractionation, dialysis, ODC and ADC assays, and thin layer electrophoresis procedures as well as the sources of chemicals were identical as previously described (6). Spectrapor membrane tubings with mol wt cutoff of 3500 and Na phosphate buffer (pH 8.0) were used; in some cases buffers with different pH were also applied. The added substrates corresponded to 1.0 and 2.5 mM final concentration. Extraction of amino acids and polyamines as well as the HPLC procedures were also the same as previously used. However, in addition to compounds previously separated, Lys, cadaverine, and N^1-acetyl derivatives of Put, Spd, and Spm were also monitored in the leaf extracts. The compounds, with retention time values in parentheses, separated on the Ultrasphere ODS column

[1] This work was performed during a sabbatical (H. B.) at Merrell Dow Research Institute, Cincinnati, OH.

[2] Abbreviations: ADC, arginine decarboxylase; ODC, ornithine decarboxylase; Arg, arginine; Orn, ornithine; Lys, lysine; Agm, agmatine; Put, putrescine; Spd, spermidine; Spm, spermine, Dap, diaminopropane; AG, aminoguanidine; DFMA, α-D'L-difluoromethylarginine; DFMO, α-DL-difluoromethylornithine.

[3] M. Frohlich collection numbers (vouchers are deposited in the Union College Herbarium).

Table I. *Free Arg, Orn, Lys, and Polyamine Contents of Leaves*

Plant[a]		Dry Matter	Soluble Protein	Amino Acids			Polyamines[b]						Pyrrolizidine Alkaloids
Family	Species			Arg	Orn	Lys	Agm	Put	Spd	Spm	Dap	Total	
		% fresh wt		*nmol/g fresh wt*									*μmol/g fresh wt*
Gramineae	*Avena sativa*	12.1	0.66	154	37	477	133	153	31	22	406	745	
	Hordeum vulgare	9.2	0.43	215	32	298	110	302	30	42	23	537	
	Zea mays	13.8	0.62	266	115	2257	41	383	237	50	137	848	
Leguminosae	*Pisum sativum*, cv Alaska	15.2	1.83	6154	355	2256	253	428	837	679	ND[c]	2197	
	Pisum sativum, Little Marvel	15.6	1.88	3979	273	2430	193	502	731	450	ND	1876	
	Phaseolus vulgaris	18.8	1.01	3232	106	3090	175	347	650	368	<2	1540	
Compositae	*Lactuca sativa*	5.6	0.24	4293	22	229	23	173	200	21	ND	417	
Boraginaceae	*Heliotropium angiospermum*	21.1	0.91	283	63	144	27	266	85	51	<2	429	34
	Heliotropium spathulatum	12.2	0.25	23	58	52	6	204	14	22	<2	246	20
	Heliotropium procumbens	18.3	0.59	280	32	96	5	213	16	7	<2	241	3
	Heliotropium indicum (A)	19.8	0.68	184	208	293	23	262	42	34	<2	361	21
	Heliotropium indicum (B)	18.5	0.59	45	92	127	17	143	31	30	<2	221	5

[a] Gramineae first leaves; Leguminosae—first leaves including apices; *L. sativa*—green cotyledons including apices. All sampled on the 4th d after germination. *Heliotropium* plants were at the beginning of flowering; the samples represent the youngest leaves including apices. In the case of *H. indicum*, mature leaves were also sampled—*H. indicum* (B). [b] Traces of cadaverine were detected in corn, both cultivars of pea, and in *H. procumbens* leaves. N^1 acetyl derivatives of Put, Spd, or Spm were not detected in any of the tested species. [c] ND, not detectable.

Table II. *Activities of ODC in Leaf Extracts*

ODC was assayed using L-[U-^{14}C]Orn in the presence of 0.1 mM AG.

Extract	Basis for Calculations	Orn	DFMO	Plant Species											
				A. sativa	*H. vulgare*	*Z. mays*	*P. sativum*, Alaska, Little Marvel	*Ph. vulgaris*	*L. sativa*	*Heliotropium*					
										Angiospermum	*Spathulatum*	*Procumbens*	*Indicum*		
													A	B	
		mM	*1 mM*	*nmol/h^{-1}·g^{-1} fresh wt*											
Crude	[^{14}C]CO_2	1.0	−	12.7	2.9	12.3	3.2	3.9	3.7	5.3	23.5	2.0	2.9	50.5	17.5
			+	11.9	2.5	12.7	2.3	2.5	3.0	4.7	21.1	2.2	2.7	53.0	16.1
		2.5	−	24.1	3.7	18.0	5.1	7.2	5.7	9.9	39.3	3.5	5.0	99.1	32.2
	[^{14}C]Put	1.0	−	1.0	0.5	0.7	1.0	1.2	<0.1	1.1	1.1	ND	1.3	<0.1	<0.1
			+	<0.1	<0.1	<0.1	<0.1	<0.1		<0.1	<0.1		<0.1		
		2.5	−	0.9	0.5	0.8	1.3	1.4		1.1	1.3		1.5		
$(NH_4)_2SO_4$ fraction	[^{14}C]CO_2	1.0	−	5.0	0.8	0.9	1.4	1.3	1.4			1.2		5.9	4.1
			+	4.3	0.5	<0.1	0.2	<0.1	ND					5.5	4.0
		2.5	−	5.4	0.9	1.0									
	[^{14}C]Put	1.0	−	0.8	0.5	0.7	1.2	1.3	<0.1			ND	<0.1	<0.1	
			+	<0.1	<0.1	<0.1	0.1	<0.1							
		2.5	−	1.0	0.6										

were: N^1-acetyl Put (4.3 min); cadaverine (13.9 min); Put (14.6 min); Dap (14.6 min); N^1-acetyl Spd (16.2 min); Agm (17.9 min); Spd (20.4 min); N^1-acetyl Spm (21.4 min), and Spm (22.9 min). The compounds, with retention time values in parentheses, separated on the Whatman SCX 10 HPLC column were: Orn (20.4 min); Lys (22.0 min); Arg (23.8 min); Dap (32.0 min); and Put (35.1 min). Soluble protein was determined only in crude extracts.

RESULTS

The levels of free basic amino acids and polyamines revealed a very broad range depending on the plant family and the species tested (Table I). The highest levels of Arg were found in the legumes and lettuce; they were over 10-fold higher than those in the grasses and the borages. The levels of Orn were much lower than those of Arg in all species except for *H. spathulatum* and *H. indicum*. A very high lysine content was found in corn, pea, and bean leaves.

The total polyamine level was highest in the legumes and lowest in the borages. Great variations in the contents of particular amines were observed depending on the species. Agm was found in all species; it showed the highest concentration in the legumes and the lowest in the borages. At a detectibility threshold of 1 nmol/g fresh weight *N*-acetyl derivatives of Put, Spd, or Spm were not detected in any of the tested species. In the youngest leaves of *H. angiospermum*, *H. spathulatum*, and *H. indicum*, the pyrrolizidine alkaloids amounted to 20 to 34 μmol/g fresh weight and were 60- to 90-fold higher than the total polyamines. The youngest leaves of *H. procumbens* and mature

Table III. *ADC Activities in Crude Extracts in the Presence of 0.1 mM AG*

Plant Species	Arg		DFMA	Activity Based on			
	Labeling	Concn.		$^{14}CO_2$	Thin layer electrophoresis		
					Total	Agm	Put
		mM[a]	1 mM		nmol/h·g fresh wt		
A. sativa	[1-^{14}C]	1.0	−	7237			
			+	24			
	[U-^{14}C]		−	7116	7274	7073	201
			+	27			
		2.5	−	8620	8238	8042	196
H. vulgare	[1-^{14}C]	1.0	−	6346			
			+	28			
	[U-^{14}C]		−	5932	6177	5972	205
			+	29			
		2.5	−	8011	7036	6800	236
Z. mays	[1-^{14}C]	1.0	−	136			
	[U-^{14}C]		−	182	128	49	79
			+	18			
		2.5	−	246	155	60	95
			+	13			
P. sativum cv Alaska	[1-^{14}C]	2.6	−	1390			
	[U-^{14}C]		−	1506	1203	1077	126
			+	<1			
		4.1	−	1484	1227	1095	132
			+	<1			
P. sativum cv Little Marvel	[1-^{14}C]	2.0	−	1102			
	[U-^{14}C]		−	1394	1182	1088	92
			+	13			
		3.5	−	1550	1201	1117	84
Ph. vulgaris	[1-^{14}C]	2.3	−	39			
	[U-^{14}C]		−	40	35	25	10
			+	<1			
		3.1	−	41	34	27	7
L. sativa	[1-^{14}C]	2.5	−	43			
	[U-^{14}C]		−	61	39	8	31
			+	<1			
		4.0	−	66	41	6	35

[a] In Leguminosae and *L. sativa*, the indicated Arg concentrations take into account Arg amounts in the extract aliquots, introduced into the reaction mixtures.

leaves of *H. indicum* revealed much lower pyrrolizidine levels.

Prior to assaying ODC and ADC, crude leaf extracts from all species were tested for possible oxidative degradation of Put and/or Agm as well as for Arg hydrolysis as described previously (6). In the absence of AG, degradation of added [1,4-^{14}C]Put and [U-^{14}C]Agm was observed in extracts from pea (both cultivars), bean, lettuce, *H. angiospermum*, and *H. indicum* leaves. Addition of AG at 0.1 mM to the incubation medium prevented Put losses in all cases; the recovery of added [U-^{14}C]Agm, including the [U-^{14}C]Put derived from it, was about 85 to 92%. AG had no effect on the recovery of labeled Put or Agm in extracts from the grasses, *H. spathulatum* or *H. procumbens* after incubation with labeled Orn or Arg. Significant hydrolysis of Arg, in the absence of added Orn at 20 mM, was observed only in *H. angiospermum* and *H. indicum*; in the presence of Orn, the conversion of Arg into Orn did not exceed 12%.

As indicated by differences between the $^{14}CO_2$- and ^{14}C-Put-based activities for ODC, decarboxylation of Orn not due to ODC occurred in crude extracts from all tested species (Table II). This artifact was very pronounced in *H. indicum*, *H. angiospermum*, oat, and corn extracts even though phosphate rather than Tris·HCl buffer was used. Partial purification with $(NH_4)_2SO_4$ greatly decreased but did not completely eliminate the nonspecific $^{14}CO_2$ release. The Put-based ODC activities, representing values close to V_{max} as indicated by small differences depending on substrate concentration, were very low; they range between traces and 1.5 nmol/h·g fresh weight. No ODC activity could be detected in *H. spathulatum*. In all species, DFMO, the enzyme-activated irreversible ODC inhibitor, did inhibit the enzyme activity although in many cases the inhibition was not apparent when the activities were calculated on the basis of $^{14}CO_2$ release.

Table IV. *ADC Activities in Heliotropium Leaf Extracts Incubated with [U-^{14}C]Arg in the Presence of 0.1 mM AG and 20 mM Orn*

When incubated with [guanido-^{14}C]Arg in the presence of AG and Orn, *H. angiospermum* and *H. indicum* extracts yielded similar results to those found with [U-^{14}C]Arg. These extracts revealed significant losses of added [U-^{14}C]Agm in the absence of AG. In the absence of Orn at 20 mM, the conversion of [U-^{14}C]Arg into Orn—during 30-min incubation—amounted to about 55, 30, and 7% of its radioactivity in extracts from *H. angiospermum*, *H. indicum* (A), and *H. indicum* (B), respectively.

Species	Extract	Arg	DFMA	Activity Based on			
				$^{14}CO_2$	Thin layer electrophoresis		
					Total	Agm	Put
		mM	*1 mM*	*nmol/h·g fresh wt*			
H. angiospermum	Crude	1.0	−	168	6.9	5.8	1.1
			+	171	<0.1		
		2.5	−	277			
	$(NH_4)_2SO_4$	1.0	−	48	7.5	6.0	1.5
	Fraction		+	45	<0.1		
		2.5	−	78	8.1	6.6	1.5
H. spathulatum	Crude	1.0	−	32	18.2	1.0	17.2
			+	3			
		2.5	−	38	21.6	2.1	19.5
			+	2			
H. procumbens	Crude	1.0	−	29	19.4	4.8	14.6
			+	2			
		2.5	−	35	20.2	5.0	15.1
			+	3			
H. indicum (A)	Crude	1.0	−	135	22.2	14.7	7.5
			+	116	<0.1		
	$(NH_4)_2SO_4$		−	49	20.8	13.3	7.5
	Fraction		+	29	<0.1		
		2.5	−	59	21.5	13.1	8.4
H. indicum (B)	Crude	1.0	−	49	5.1	3.0	2.1
			+	41	<0.1		

In contrast to ODC, the ADC assays do not seem to be significantly affected by interfering decarboxylation except for *H. angiospermum* and *H. indicum* (Tables III and IV). The ADC activities varied greatly both within and between plant families. They were highest in oat[4] and barley leaf extracts; but in corn the activity was one-fiftieth as high. Equally large differences were found between pea and bean leaf extracts. *Heliotropium* species exhibited the lowest ADC levels; pH-dependent differences in the ADC activities were about 25 to 30% when tested at pH between 7.0 and 8.5. Taking into account partial losses of Agm, due to its oxidative degradation during incubation, the true ADC activities might have been 10 to 15% higher in the species in which this artifact occurred. In the grasses, *Heliotropium*, and lettuce the ADC activity was precipitated with $(NH_4)_2SO_4$ at 25 to 70% saturation whereas in the legumes it was found mainly in the 0 to 25% saturation fraction.

Under the incubation conditions a partial conversion of Agm into Put was observed; it ranged between 2% (oat, barley) and 90% (lettuce, *H. spathulatum*) of total Agm produced. This conversion was apparently responsible for the significant differences in the $^{14}CO_2$-based activities between extracts incubated with [1-^{14}C]Arg and [U-^{14}C]Arg, especially in the species with a high degree of Agm conversion, which also showed a relatively low ADC activity. Negative results were obtained in a preliminary search for putrescine synthase in extracts from bean, lettuce, and *H. spathulatum* leaves using [U-^{14}C]Agm in the presence of added Orn and Mg (16). This enzyme, found in *Lathyrus sativus* (15), is involved in one of the two known pathways leading from Agm to Put in higher plants (13). DFMA inhibited the ADC activity in the leaf extracts from all species.

DISCUSSION

The results presented here indicate that the interfering $^{14}CO_2$ release may be a rather widespread artifact in ODC assays, its extent being dependent on the species as well as on the leaf age, as indicated by *H. indicum*. The interference of the nonspecific Arg decarboxylation in ADC assays seems to occur rather rarely; it was apparent here only in *Heliotropium* and only in two of four species. Thus, if the conventional method based on $^{14}CO_2$ determination may be burdened with great errors in the case of ODC assays, it seems more reliable in the case of ADC assays, especially if [1-^{14}C] and not [U-^{14}C]Arg is used. The latter may introduce errors, especially in cases when a large portion of newly formed Agm is converted into Put. However, the possible interference of nonspecific decarboxylation as well as of arginase

[4] In the present experiments, the oat ADC showed a 4-fold higher activity than in previous studies (6), in phosphate as well as Tris·HCl buffer. The seeds were taken from the same batch in both cases; they were stored for about 6 months at room temperature. In the previous study, the seedlings were grown in the greenhouse; in this study, they were grown on a well-illuminated windowsill.

deserves monitoring in ADC assays.

Since Put formation seems to be the most reliable method of assaying the activity of crude or partially purified ODC, monitoring the possible interference of amine oxidases during incubation becomes of great importance. These enzymes are widespread in plants (13) and apparently are the principal catabolizers of amines. The search for N^1-acetylated polyamines, known to be involved in polyamine catabolism in animals (11), gave negative results in all plant species tested here.

In all 10 species belonging to four different families the ODC activities, extracted with 0.2 M phosphate buffer from very young leaves, were very low or negligible, compared to ADC activities. Recently, an ODC activity tightly bound to chromatin has been reported for germinated barley seeds; this activity being four times higher than that of the cytosolic ODC (10). If present in the tested species and at similar proportions, the chromatin ODC would not affect the dominance of ADC. Lately a pelletable form of ODC in oat leaves has been reported (17); however, its activity has been presented in cpm of $^{14}CO_2$ and no proof was given that the $^{14}CO_2$ release was due to specific Orn decarboxylation. Unfortunately, in many instances ODC and/or ADC activities have been reported only in terms of cpm or percentages, which makes it impossible to compare activities between studies.

The *in vitro* results presented here seem to support the notion, dominant in the 1970s (12), that in higher plants ADC is mainly responsible for Put biosynthesis. However, physiological effects of ODC inhibitors in some species (2, 8) indicate that ODC may play a role. Unfortunately, it is difficult to assess the enzyme activities in these cases since they were determined only on the basis of the $^{14}CO_2$ released. An extension of comparative studies on ODC and ADC to various plant families, in particular Solanaceae—with all the necessary precautions for avoiding artifacts—seems to be of importance.

The lack of correlation between the level of ADC and the level of total polyamines in the species tested here should be noted. Even if the ADC dominance *in vivo* is similar to that *in vitro* and if its activity is not limited by substrate concentration or other factors, the rate of Put formation from Agm may depend on the activity of enzymes involved in the latter process as well as on other known (14) and unknown pathways into which Agm may be channeled in a given species.

In *Heliotropium* the formation of the alkaloid's pyrrolizidine moiety seems to be a very important metabolic channel for Put. The youngest organs of pyrrolizidine alkaloid-bearing plants are known to exhibit the highest alkaloid levels (5, 7). No correlation was found between the ADC or ODC activities *in vitro* and the alkaloid levels in the youngest leaves of the *Heliotropium* species tested here. Moreover, if the enzyme activities *in vitro* reflect the activities *in vivo* and even if the total Agm and Put formed is converted into pyrrolizidines without any catabolism, the period needed for Put production would be many times longer than the age of the analyzed leaves. Thus, one may suppose that either: (a) the *in vivo* ADC or ODC activities are much higher than the activities *in vitro*; or (b) the very high levels of pyrrolizidines in the youngest organs are due not so much to biosynthesis *in situ* as to an inlfux from old organs. Most of the alkaloids occur in plants as water soluble *N*-oxides. Sharp decreases with leaf senescence in the relative content of alkaloids were shown to be due to export rather than to degradation (7). The approximately 5-fold differences in ADC activities per fresh weight between the youngest and mature leaves, found here with *H. indicum* and previously with *H. angiospermum* (6), do not indicate a significant decline in the enzyme activity per cell, especially when the 5- to 10-fold differences in the leaf size are taken into account.

Both labeled Orn and Arg gave rise to the alkaloid's pyrrolizidine moiety when fed to *Senecio magnificus* plants (3), thus indicating that ODC as well ADC may be involved in Put formation which leads to pyrrolizidine synthesis. However, the possibility that citrulline (1, 9) and not Arg or Orn may be the main Put precursor at least in *Heliotropium* should not be eliminated. Studies not only *in vivo* but also *in situ* using inhibitors of enzymes possibly involved in Put biosynthesis are needed to clarify the problem.

Acknowledgments—We are grateful to Dr. M. W. Frohlich for supplying us with plants of *Heliotropium* species. We also thank S. E. Kelly for her effective assistance during the experiments, and S. M. Treadway for skillful typing of the manuscript.

LITERATURE CITED

1. Akamatsu N, M Oguchi, Y Yajima, M Ohno 1978 Formation of N-carbamyl-putrescine from citrulline in *Escherichia coli*. J Bacteriol 133: 409–410
2. Altman A, R Friedman, N Levin 1983 Alternative metabolic pathways for polyamine biosynthesis in plant development. *In* U Bachrach, A Kaye, R Chayen, eds, Advances in Polyamine Research. Raven Press, New York, pp 395–408
3. Bale NM, DHG Crout 1975 Determination of the relative rates of incorporation of arginine and ornithine into retronecine during pyrrolizine alkaloid biosynthesis. Phytochemistry 14: 2617–2622
4. Birecka H, MW Frohlich, LM Glickman 1983 Free and esterified necines in *Heliotropium* species from Mexico and Texas. Phytochemistry 22: 1167–1171
5. Birecka H, TE DiNolfo, WB Martin, MW Frohlich 1984 Polyamines and leaf senescence in pyrrolizidine alkaloid-bearing *Heliotropium* plants. Phytochemistry 23: 991–997
6. Birecka H, AJ Bitonti, PP McCann 1985 Assaying ornithine and arginine decarboxylases in some plant species. Plant Physiology 79: 509–514
7. Catalfamo JL, WB Martin, H Birecka 1982 Accumulation of alkaloids and their necines in *Heliotropium curassavicum*, *H. spathulatum* and *H. indicum*. Phytochemistry 21: 2669–2675
8. Cohen E, S Arad, YM Heimer, Y Mizrahi 1982 Participation of ornithine decarboxylase in early stages of tomato fruit development. Plant Physiol 70: 540–543
9. Crocomo OJ, LC Basso, OG Brasil 1970 Formation of N-carbamyl-putrescine from citrulline in *Sesamum*. Phytochemistry 9: 1487–1489
10. Panagiotidis CA, JG Georgatsos, DA Kyriakidis 1982 Superinduction of cytosolic and chromatin-bound ornithine decarboxylase activities of germinating barley seeds by actinomycin D. FEBS Lett 146: 193–196
11. Pegg AE, PP McCann 1982 Polyamine metabolism and function. Am J Physiol 243: C212–C221
12. 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, Elmsford, NY, pp 27–81
13. Smith TA 1981 Amines. *In* PK Stumpf, EE Conn, eds, The Biochemistry of Plants, Vol 7. Academic Press, New York, pp 249–268
14. Smith TA, J Negrel, CR Bird 1983 The cinnamic acid amides of the di- and polyamines. *In* U Bachrach, A Kaye, R Chayen, eds, Advances in Polyamine Research, Vol 4. Raven Press, New York, pp 347–370
15. Srivenugopal KS, PR Adiga 1981 Enzymatic conversion of agmatine to putrescine in *lathyrus sativus* seedlings. J Biol Chem 256: 9532–9541
16. Srivenugopal KS, PR Adiga 1983 Putrescine synthase from *Lathyrus sativus* (grass pea) seedlings. Methods Enzymol 94: 335–339
17. Young ND, AW Galston 1984 Physiological control of arginine decarboxylase activity in K-deficient oat shoots. Plant Physiol 76: 331–335