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CHANGES IN POLYAMINE CONCENTRATION DURING SEED GERMINATION

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Key Word Index—*Phaseolus*; *Pisum*; Leguminosae; *Tragopogon*; Compositae; *Triticum*; *Zea*; *Gramineae*; grain development; cadaverine, putrescine; spermidine; spermine; polyamines; automatic ion exchange chromatography.

Abstract—*Phaseolus mungo* seeds 0 to 10 days after germination contained putrescine, spermidine, spermine, cadaverine, agmatine and tyramine. The rate of biosynthesis of total polyamines, proteins and RNA in the developing seeds follows similar profiles, reaching maxima 3 hr from germination. Putrescine, cadaverine, spermidine, spermine and agmatine were the major amines found in *Pisum sativum* 0–7 days after germination. RNA and proteins seem to follow the same pattern as polyamines during the first 12 hr in the developing pea seeds. RNA reaches a peak at 15 hr and polyamines and proteins peak 24 hr after germination. A rise to total polyamine concentration was also observed in seeds of *Tragopogon porrifolius*, *Zea mays* and *Triticum aestivum* 2–12 hr after germination.

INTRODUCTION

The significance of polyamines in biochemical and physiological processes is at present recognised, but their precise role in cell metabolism is not yet established. In recent years the enormous amount of literature that has appeared on di- and polyamines shows the increasing interest in these compounds which occur in animals, microorganisms and plants [1–4]. Putrescine, spermidine and spermine have received much attention and evidence has been obtained for their implication in the control of nucleic acid metabolism and protein synthesis [5–8].

In plants, the changes in the concentration of polyamines during growth have been studied by various authors [8–11]. In the present work, our interest was centered on the possibility of changes in the concentration of polyamines during very early stages of germination. Seeds, therefore, were germinated and collected for analysis at intervals of 2 or 3 hr during the first day and at longer intervals (6, 12 or 24 hr) during a week. The polyamines were analysed by a modification of the method we recently developed [12]. The separation is carried out by automatic ion exchange column chromatography using fluorimetric quantification.

RESULTS AND DISCUSSION

Analytical methods

Two different extraction procedures were employed for sample preparations and compared, before automated chromatographic determinations of polyamine. The first procedure consists of crushing the seeds in a solution of 5% TCA in 0.05 N HCl followed by centrifugation. The supernatants were submitted to analysis after ether extraction. In the second procedure a water–EtOH solution of $HClO_4$ was employed for extraction; after centrifugation the supernatants were neutralized with KOH, to remove $HClO_4$, and then passed through an anionic exchange resin (OH^-) to retain the anionic compounds present in the extracts. The eluate was concentrated and used for analysis of polyamines. Both methods gave similar qualitative and quantitative results. Subsequently, the first method being faster and simpler, was adopted for the present study.

The automatic ion exchange chromatographic method previously described [12] was improved to give a total analysis time of 100 min. The method separates crude extracts equally well. Prior purification of samples is therefore avoided and the risk of losing the polyamines during the purification step was reduced. Compared to previously described methods by others [i.e. 13, 14] ours has the principal advantage of separating a larger number of related amino compounds in a single chromatographic analysis, in a shorter time with higher sensitivity (detection limit: pmol level). The use of an integrator and employing the internal standard in the samples contributes to an easier and reproducible quantification. The results given in the present text are an average of duplicate analysis. The difference observed between them was never higher than 3%. The method is now a routine procedure in our laboratory and its reproducibility is similar to that of automatic amino acid analysis.

Polyamine changes during seed germination

The results presented here were obtained with seeds grown in water in the absence of nutrients. This represents therefore, only the changes produced due to seed reserves. All results are presented on a fr. wt basis.

Fig. 1 shows the changes in putrescine, spermine and spermidine concentration during germination of *Phaseolus mungo* seeds. Putrescine is initially present in very small amounts as compared to spermine and spermidine but it increases slowly during the first 33 hr and thereafter with a rather higher rate. After the 10th day there

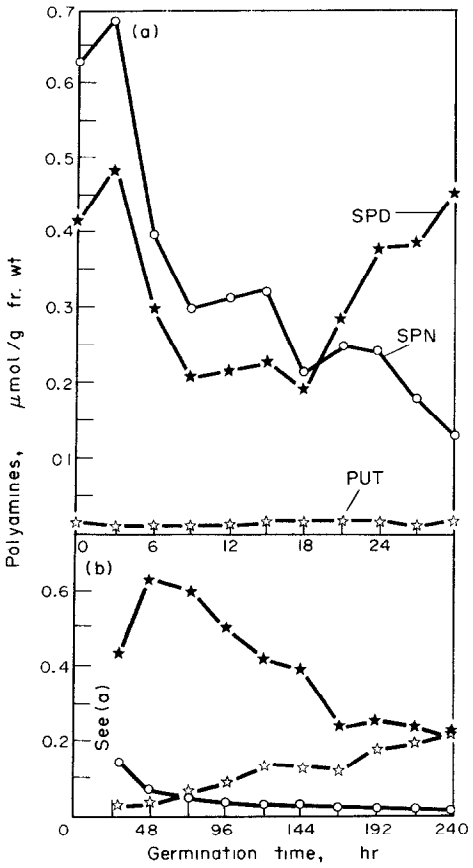

Fig. 1. Changes in concentration of different polamines during germination of *Phaseolus mungo* seeds. ★★ SPD (spermidine); ○○ SPN (spermine) and ☆☆ PUT (putrescine). In (a) changes from 0 to 27 hr; in (b) changes from 27 to 240 hr.

is *ca* 15 times the amount present at 33 hr. Spermine, unlike putrescine, is the main polyamine in the beginning and like spermidine follows the same path as total polyamines, RNA and proteins each having a maxima 3 hr from germination. Both start decreasing thereafter: spermine keeps on declining slowly except for two small peaks at 15 and 21 hr from germination while spermidine start increasing again from 18 hr with a maximum at 51 hr followed by a gradual decrease.

Fig. 2 shows the pattern of changes in total polyamines, protein and RNA in the developing *Phaseolus mungo* seeds. All three increase immediately after the grains are germinated and reach the maximum in 3 hr followed by a steep fall. RNA reaches its first minimum at 6 hr while proteins and polyamines reach it in 12 hr. Thereafter there are small changes in concentration and finally after the 4th day all three decrease very slowly with no remarkable changes. Cadaverine was present from the 4th day; agmatine and tyramine were detected in the samples on the 8th day. All these compounds represented 2–5% of total polyamines.

In the case of *Pisum sativum*, besides putrescine, spermidine and spermine, another diamine, cadaverine, is present in relatively large amounts. As shown in Fig. 3 putrescine is present in small amounts at the beginning but it increases sharply after the first day with a maxima at 72 hr. At the 7th day there is *ca* 8 times the amount present at 24 hr. Cadaverine is present during the first 24 hr at a slightly higher concentration than putrescine. It decreases in the initial hr of germination and then peaks at 9, 24 and 72 hr. Spermidine is the main polyamine at the beginning and like cadaverine seems to follow the same path as total polyamines, RNA and proteins. Spermidine peaks sharply at 24 hr. Spermine is present in very small amounts and decreases slowly during the period of germination.

Fig. 4 shows the pattern of changes in total polyamines, proteins and RNA in the developing pea seeds. A sharp decline in the concentration of all three is observed during the first 6 hr of germination. Then polyamines increase at 9 hr (mainly due to cadaverine: 6 times the amount present at 6 hr). After 9 hr there is twice the amount of RNA as at 6 hr while proteins continue to decrease; 3 hr later proteins increase while RNA and polyamines decrease again. RNA reaches a maximum value at 15 hr, after which it declines. Proteins and polyamines are at their lowest values at 15 hr but they start to rise again to reach their maximum value at 24 hr accompanied by a small rise in RNA. The rise in total polyamines at this point is mainly due to an increase in cadaverine and spermidine (6 and 1.5 times respectively the amount observed at 15 hr). Then, RNA, polyamines

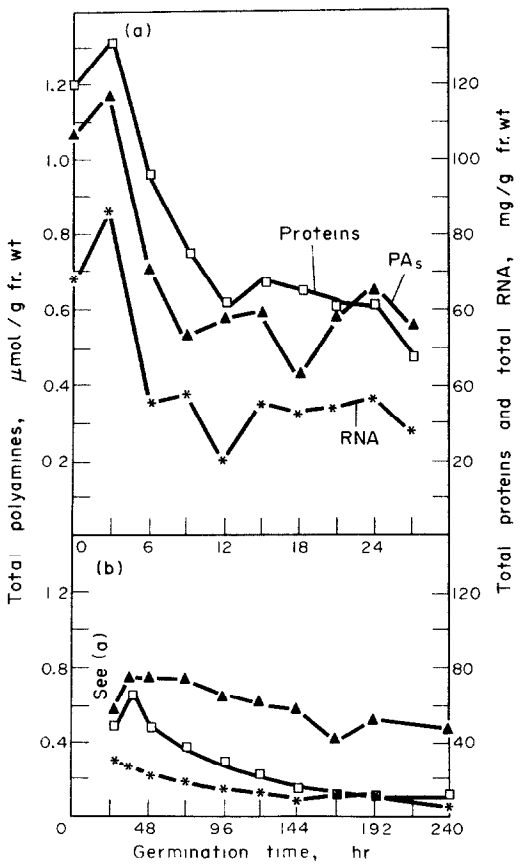

Fig. 2. Changes in total polyamines, proteins and RNA during germination of *Phaseolus mungo* seeds. ▲▲ PAs (polyamines); □□ (proteins) and ✳✳ (RNA). In (a) changes from 0 to 27 hr; in (b) changes from 27 to 240 hr

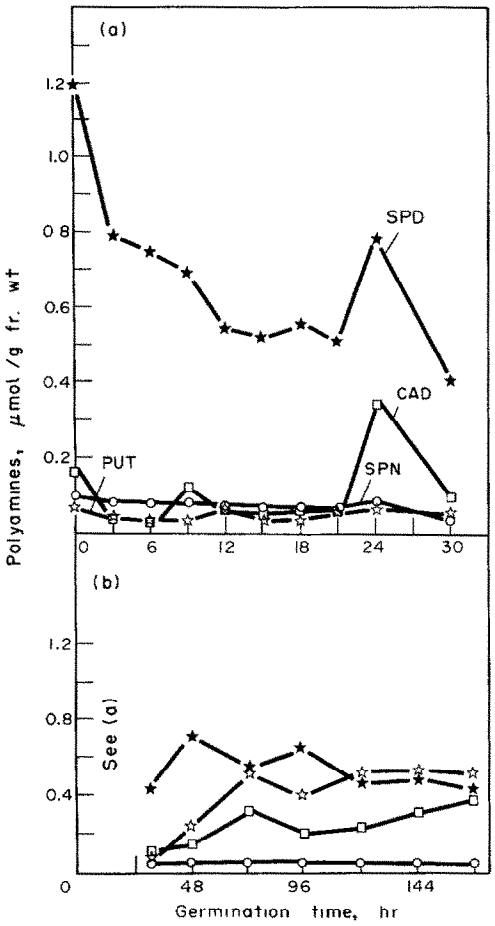

Fig. 3. Changes in concentration of different polyamines during germination of *Pisum sativum* seeds. ★★ SPD (spermidine); □□ CAD (cadaverine); ☆☆ PUT (putrescine) and ○○ SPN (spermine). In (a) changes from 0 to 30 hr; in (b) changes from 30 to 168 hr.

Table 1. Time course analysis of polyamine concentration (pmol × 10^2/g fr. wt) during seed germination of *Zea mays*

Age (hr)	Putrescine	Spermidine	Spermine
0	319	n.d.	n.d.
2	445	1670	217
4	453	1780	241
6	431	1700	208
8	339	1390	163
12	475	1910	257
24	346	1140	175
30	367	1200	173
53	522	1240	128
73	414	611	43
97	701	823	81
120	921	824	69
144	1640	1070	68

n.d. = not detected.

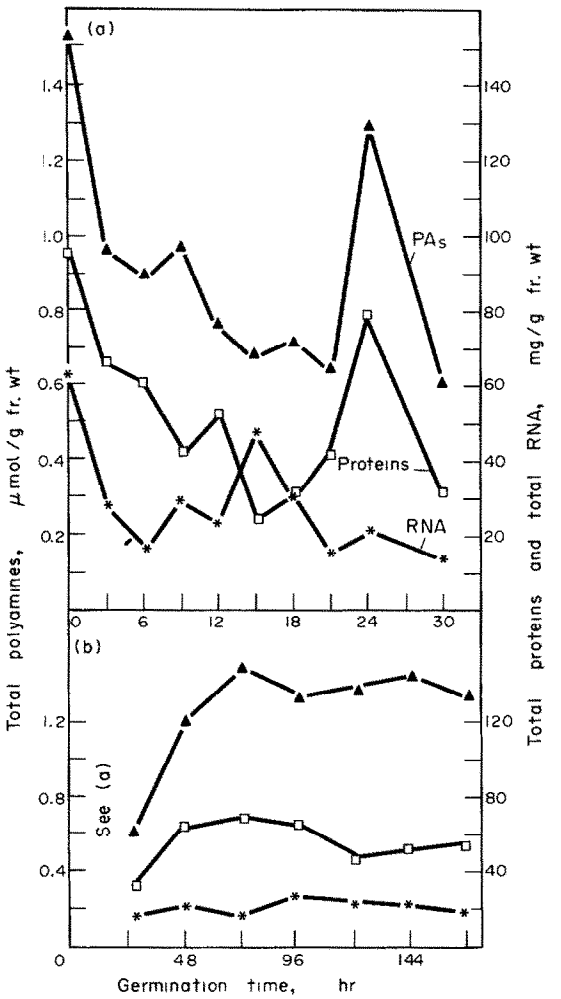

Fig. 4. Changes in total polyamines, proteins and RNA during germination of *Pisum sativum* seeds. ▲▲ PAs (polyamines); □□ (proteins) and * * (RNA). In (a) changes from 0 to 30 hr; in (b) changes from 30 to 168 hr.

and proteins decline appreciably, reaching a minimum at 30 hr. After that, at 48 hr all three increase about 2 times and while RNA and proteins, up to the 7th day, do not present remarkable changes, polyamines continue to increase till 72 hr and then remain almost at the same level till the 7th day. Agmatine was present after the first day in very small amounts (less than 5% of the total polyamines).

The early rise of polyamines observed with *Phaseolus mungo* and *Pisum sativum* seeds was also found during germination of seeds of *Zea mays*, *Phaseolus vulgaris*, *Tragopogon porrifolius* and *Triticum aestivum*. The results of the analysis of polyamines during germination of these seeds are shown in Tables 1, 2, 3 and 4. A rise of total polyamine concentration is observed initially (between 2 and 12 hr) and in general one polyamine compound is present in higher concentration than the others. So in *Phaseolus mungo* and *P. vulgaris*: spermine; in *Zea mays*, *Tragopogon porrifolius* and *Triticum aestivum*: spermidine. *P. sativum* differs from the others in having spermidine and cadaverine in higher concentrations.

In the seedlings analysed, small amounts (between 1 to 5% of total polyamines) of the following amines were

Table 2. Time course analysis of polyamine concentration (pmol × 10^2/g fr. wt) during seed germination of *Phaseolus vulgaris*

Age (hr)	Putrescine	Spermidine	Spermine
0	31	192	283
2	171	357	1600
4	91	295	1210
6	55	256	1030
8	53	331	1290
12	74	245	1030
24	73	217	1020
30	24	224	755
53	119	353	979
73	127	265	813
97	161	281	655
120	344	475	1010
144	503	517	561

Table 3. Time course analysis of polyamine concentration (pmol × 10^2/g fr. wt) during seed germination of *Tragopogon porrifolius* (salsify)

Age (hr)	Putrescine	Spermidine	Spermine
0	129	987	117
2	134	1290	129
4	135	1600	156
6	45	489	17
8	154	1270	259
12	155	2160	226
24	85	793	105
30	308	2140	108
53	312	1470	154
73	199	647	45
97	178	807	84
120	143	957	107
144	215	1210	103

Table 4. Time course analysis of polyamine concentration (pmol × 10^2/g fr. wt) during seed germination of *Triticum aestivum*

Age (hr)	Putrescine	Spermidine	Spermine	Cadaverine	Agamtine
0	425	128	n.d.	n.d.	n.d.
2	257	1870	222	n d.	n.d.
4	263	2170	303	n.d.	n.d.
6	424	2380	276	n.d.	n d.
8	287	2160	312	n.d.	n d.
12	309	2120	309	n.d.	n.d.
24	323	1920	305	n.d.	n.d.
30	472	1960	261	n.d.	n.d.
53	489	1930	272	27	35
73	408	1100	168	21	73
97	1090	1520	215	43	91
120	926	1300	189	52	95
144	1210	1500	196	73	92

n.d. = not detected.

found, cadaverine in seedlings aged 144 hr of *Phaseolus vulgaris*, in seedlings aged 97, 120 and 144 hr of *Zea mays* and in seedlings aged 8, 73, 97 and 144 hr of *Tragopogon porrifolius*. Agmatine was present in seedlings aged 144 hr of *Tragopogon porrifolius* and histamine in dry seeds and seedlings aged 30, 97 and 144 hr of *Triticum aestivum*. A product with the same retention time as that of hexamethylene diamine was found in different samples of *P. mungo*, *P. sativum*, *T. porrifolius* and *T. aestivum*. Further studies are in progress to verify its identity.

The coincidence of the most rapid increase in the content of a particular polyamine with the maximal rates of RNA and protein synthesis in fast growing cells during development has already been observed in other biological systems [15–18]. This could be explained by the stimulatory effect of polyamines on DNA-dependent RNA polymerase activity [18–20], mRNA synthesis [20], rate of amino acid incorporation [21] and peptide elongation [22]. Polyamines could also play a role in a mechanism for the protection of RNA against RNase activity [20, 23].

EXPERIMENTAL

Seeds (1–3 g) were germinated in 9 cm Petri dishes on filter paper for up to 4 days. Plants 5 days and older were grown in 250 ml beakers. H_2O was given initially (10 ml) and after the first 24 hr added when necessary. The plants were grown at 22°, 70% relative humidity and under illumination of combined incandescent and fluorescent light (Lumiere du Jour de Luxe, 125 W), 10^5 ergs/cm^2/sec (9 hr/day).

Extraction. Seedlings were collected, blotted dry with filter paper, weighed (fr. wt) and extracted twice in a pestle and mortar with 5–7 vol of 5% TCA in 0.05 N HCl. After centrifugation the supernatants were pooled and extracted with Et_2O to remove TCA. Aliquots were employed directly for polyamine analysis, after addition of 4-aza heptamethylene diamine, Fluka (375 pmol) which served as internal standard.

Proteins and nuclei acids. The residue obtained by centrifugation after TCA/HCl extraction was treated successively with EtOH, EtOH-$CHCl_3$ (3:1), EtOH-Et_2O (3:1) and Et_2O and employed for protein [24] and RNA [25] determinations.

Amines. An amino acid analyser, Liquimat-Labotron, equipped with a fluorimeter, Labotron FFM-31 (Kontron, Velizy-Villacoublay) using a 50 µl flow cell, was employed. The automated ion exchange chromagraphic method previously described [12] was improved (100 min total analysis instead of 120 min) by replacing the Durrum DC 6A resin by DC 4A (8.5 cm high) and slightly modifying the pH of the two buffers (5.48 for the first; 5.69 for the second).

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