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CHANGES IN POLYAMINE CONTENTS DURING ROOT AND NODULE GROWTH OF *PHASEOLUS MUNGO*

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Key Word Index—*Phaseolus mungo*; Leguminosae; mung bean; polyamines; spermine; spermidine; putrescine; arginine decarboxylase.

Abstract—In *Phaseolus mungo* seeds, polyamine content increased during early germination, being maximum after 24 hr; and the arginine decarboxylase showed a peak after 18 hr. During nodule initiation and growth two peaks of polyamine contents were noted—the first being 2 weeks after nodule initiation and a second one after 5 weeks. Arginine decarboxylase activity also followed the same pattern. In the roots the polyamine concentration as well as arginine decarboxylase increased up to week 2 after sowing followed by a gradual decrease. Estimation of RNA, DNA and protein contents showed a pattern similar to that of the polyamines.

INTRODUCTION

The significance of the aliphatic diamines and polyamines, mainly putrescine, spermidine and spermine, in physiological and biochemical processes has been recognized and studied extensively during the last 30 years. Polyamines occur in micro-organisms [1, 2], higher plants [3, 4] and animals [5]. In higher plants, polyamines have been analysed during germination, development and in culture in order to elucidate their functions [6–8]. However, the precise role of polyamines in cell metabolism is yet to be determined. It has been reported [9, 10] that certain bacteria can utilize the polyamines, putrescine and spermidine as sole nitrogen source. In higher plant culture, Bagni [11] showed that the diamine, putrescine, can be directly used as the nitrogen source, thereby substituting for inorganic nitrogen. The empirical usefulness of leguminous plants as green fertilizers, in maintaining world wide agricultural productivity, is based mostly on their symbiotic nitrogen fixation in the root nodules. Nodulation and nitrogen fixation have been studied extensively [12, 13]. However, there is no report except that of Smith [14] on the levels of polyamines in developing nodules and roots.

In our present work we report the results of some primary studies concerning the presence and changes in the level of polyamines during early germination and development of nodules and roots in the leguminous plant, *Phaseolus mungo*. Recent reports [15–17] suggest that either arginine decarboxylase (ADC) or ornithine decarboxylase (ODC) can act as the regulatory enzyme for polyamine biosynthesis in plants. However, the level of ADC, being a common and widely reported key enzyme in higher plants [18], was estimated to correlate with polyamine levels.

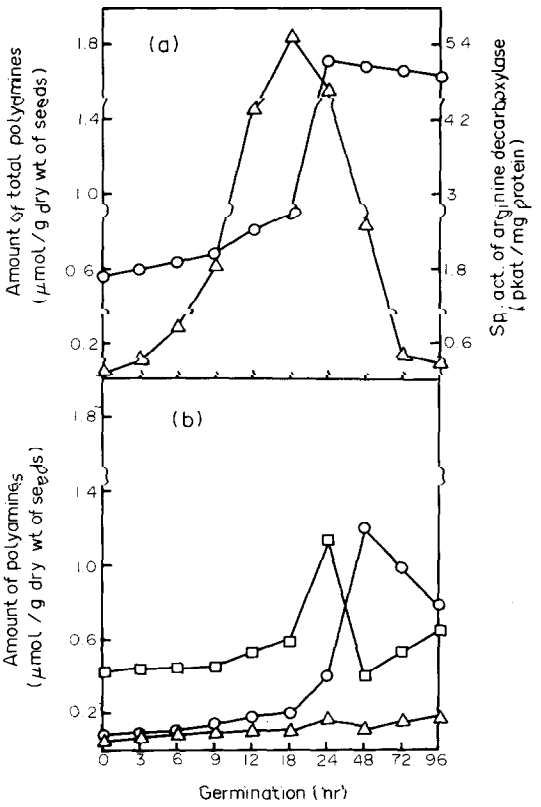

Fig. 1. (a) Changes in total polyamines (PAS) (○) and arginine decarboxylase activity (△). (b) Changes in polyamine contents, putrescine (○), spermidine (△) and spermine (□) during early germination of *P. mungo*.

RESULTS AND DISCUSSION

Changes in polyamine contents as well as the activity of ADC have been estimated during early germination of *Phaseolus mungo*. Figure 1(a) shows that the total polyamine contents gradually increase reaching a maximum (three-fold) after 24 hr of germination, and then gradually decline. Similarly, the activity of ADC attains the peak

value (39.8-fold) after 18 hr germination. It has been reported previously [19, 20] that enzymes associated with polyamine biosynthesis increase in activity during growth together with the increase in polyamine level. From Fig. 1(b) it is clear that individual polyamines, mainly putrescine, spermidine and spermine, also show a change in their level during germination. Initially, i.e. at 0 hr germination, the level of putrescine and spermidine is low compared to that of spermine. Spermine and spermidine show a 2.7- and 2.5-fold increase after 24 hr, but putrescine attains its peak (by 14.8-fold) after 48 hr germination. The content of putrescine and spermine increases significantly in comparison to the spermidine level.

As shown in Table 1 the levels of macromolecules, namely protein, RNA and DNA are positively correlated with the changes in polyamine content during germination. Protein and RNA reach their peak (1.6- and 2.3-fold, respectively) after 18 hr, but the maximum DNA content (3.6-fold) is recorded with total polyamines 24 hr after germination followed by a decline, suggesting that DNA synthesis is much more related to polyamines, which supports previous findings [4, 7]. The fall in protein content during the growth of the embryo may be due to proteolytic degradation [21].

The presence of large amounts of putrescine and spermidine has been reported in fast growing tumors of plants and animals [22, 23]. Although Bagni [6] detected only a trace of putrescine in *Phaseolus vulgaris*, its presence in considerable amounts has been recorded by others [4, 24].

Previous reports [25, 26] about the presence of appreciable amounts of growth regulating substances in nitrogen fixing root nodules led us to investigate the polyamine level in the nodules of *Phaseolus mungo*, as the polyamines have been recognized to have growth regulating activity [27]. Sampling of nodules was done as described in the Experimental (Table 2).

Figure 2(a) shows the level of total polyamines and ADC activity during growth and development of nodules. The total polyamine content increases gradually from the time of nodule initiation, i.e. from week 3 from the time of sowing the seeds, maximum level (2.2-fold) being recorded in the 2-week-old nodules followed by a gradual decline. A second peak, though much smaller than the first one, is noticed in 5-week-old nodules. The activity of ADC also follows the same pattern. A similar decrease in enzyme activity with ageing is also reported [28]. Interestingly, the second peak of polyamines was obtained at the start of flowering which is similar to the increase in cytokinin activity in nodules during bud break [26].

Putrescine, spermidine and spermine increased gradually during growth (Fig. 2b). Putrescine, spermidine and spermine showed a 4.5-, 1.6- and 2.1-fold increase, respectively, when the nodules were 2-weeks-old. Putrescine and spermine showed a second peak in 5-week-old nodules, but the spermidine declined steadily. In the nodules too, the initial putrescine level was low in comparison to spermidine and spermine, but the enhancement of the former was greater (4.5-fold).

Nucleic acids and protein content (Table 2) also gradually increased during nodule growth, the protein and RNA content being maximum in the 2-week-old nodules which then decreased gradually. The peak of DNA, however, was obtained in 5-week-old nodules. Bond [29] suggested that in larger and older nodules the decrease in activity may be due to the increasing number of infected cells.

To determine the relation between root growth and nodule development, the former's polyamine contents were also measured. Figure 3(a) shows the level of total polyamines and ADC activity during growth and development of roots. The results indicate a gradual increase in the level of polyamines, reaching a peak (2.7-fold) 2 weeks after sowing. With the initiation of nodule development the content of the polyamine in roots gradually declined. The ADC activity also showed a similar pattern, being maximum (2.3-fold) after 2 weeks. Another peak of polyamines as well as ADC activity was also obtained in the roots in week 4.

During root development (Fig. 3b) there was only a small amount of spermidine in the initial stage compared to that of putrescine and spermine. However, in week 4, the spermidine content increased by 5.12-fold, whereas putrescine and spermine showed respective peak values

Table 1. Changes in the level of protein, RNA and DNA content during early germination of *Phaseolus mungo*

Germination time (hr)	Protein (mg/g dry wt)	RNA (mg/g dry wt)	DNA (mg/g dry wt)
0	31	3.3	0.3
3	35	4	0.3
6	36	4.4	0.3
9	38	5	0.4
12	40	6	0.5
18	48	8	0.6
24	38	6.6	0.9
48	34	6.4	0.8
72	24	6	0.8
96	18	1.2	0.8

Table 2. Changes in the level of protein, RNA and DNA content during growth of nodules in *P. mungo*

Age of the nodules (weeks)	Average No. of nodules per 100 mg	Protein (mg/g dry wt)	RNA (mg/g dry wt)	DNA (mg/g dry wt)
1	275	94	32	12
2	190	162	36	12
3	85	128	22	10
4	40	119	24	13
5	16	115	25	15
6	16	98	15	8

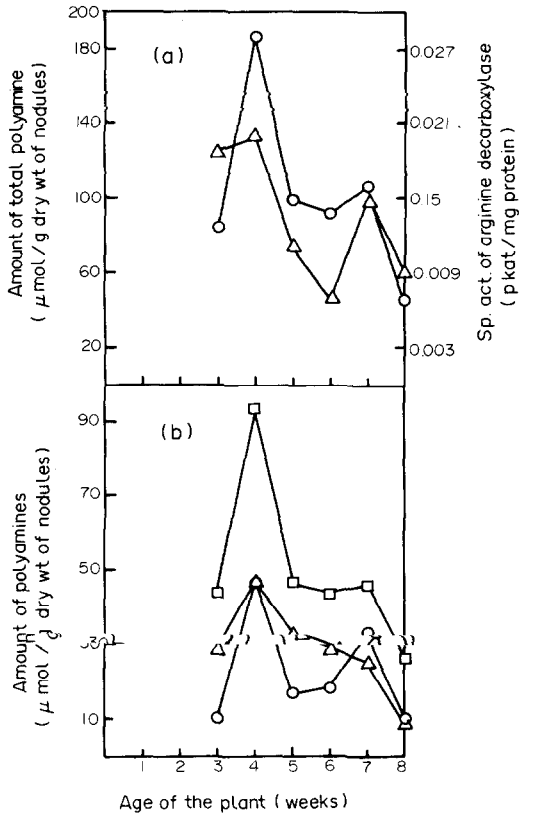

Fig. 2. (a) Changes in total polyamines (PAS) (○) and arginine decarboxylase activity (△). (b) Changes in polyamine contents putrescine (○), spermidine (△) and spermine (□) during growth of nodules in *P. mungo*.

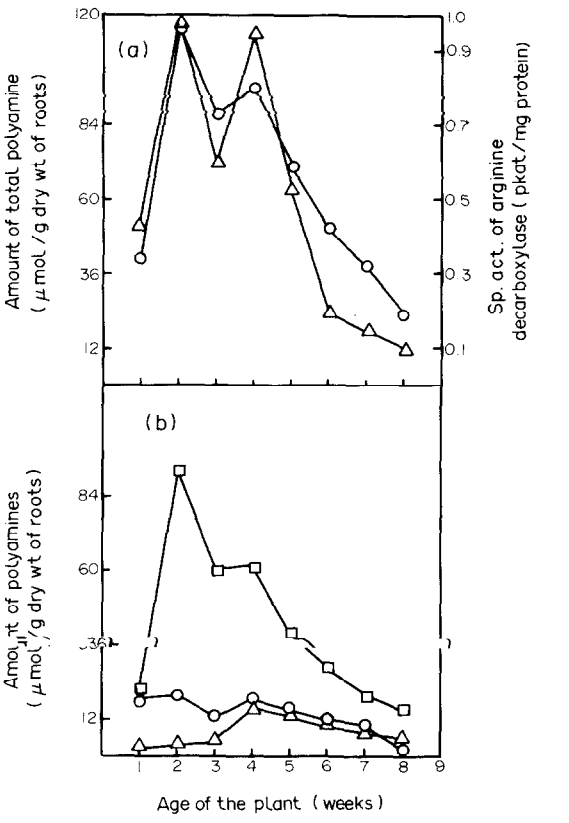

Fig. 3. (a) Changes in total polyamines (PAS) (○) and arginine decarboxylase activity (△). (b) Changes in polyamine contents, putrescine (○), spermidine (△) and spermine (□) during root growth of *P. mungo*.

(1.03- and 4.7-fold) in week 2. With nodule initiation the levels of all these three amines gradually decreased. Perhaps these polyamines are utilized in cellular differentiation during nodule growth.

DNA, RNA and protein content in roots gradually increased up to week 2 followed by a decrease in their contents (Table 3). In brief, our study showing distinct correlations of polyamines with nucleic acids and protein in different stages of root and nodule development supports the formulated fact that polyamines have a positive role in nucleic acids and protein synthesis.

The presence of appreciable amounts of amines, may be of considerable physiological significance in the development of nodule and root, as well as in the growth of embryo. However, only Smith [14] has reported the presence of polyamines in roots and nodules of pea, although Bagni *et al.* [11] have shown that in higher plants, polyamines may act as a nitrogen source. Nodules are the nitrogen fixing apparatus, which have high sink activity [30].

Table 3. Changes in the level of protein, RNA and DNA content during root growth in *P. mungo*

Age of the roots (weeks)	Protein (mg/g dry wt)	RNA (mg/g dry wt)	DNA
1	42	13	4.2
2	52	21	6
3	28	13	3.8
4	20	12.4	3
5	15	7	2
6	11	8	2.2
7	18	7.3	3
8	18	5	1

EXPERIMENTAL

Chemicals. Putrescine, spermidine, spermine and BSA were purchased from Sigma. L[U-^{14}C]Arginine (sp. act. 228 mCi/mmol) was from the Bhaba Atomic Research Centre.

Plant material. Seeds of *Phaseolus mungo* were grown during the winter in the field at the Bose Institute, Calcutta. Collection of the nodules was started from their time of initiation, i.e. 3 weeks from the day of seed sowing and continued up to 8 weeks. Sampling of nodules was made according to their size and number. Roots were sampled from week 1 and continued up to week 8 after sowing.

Germination of seeds. The seeds after surface sterilization with 0.1% $HgCl_2$ were germinated in the dark at 37° ± 1° on moistened filter paper in Petri dishes. The germinated seeds were collected at various times for analysis.

Determination of amines. Polyamines extracted with 5% TCA were dansylated and separated by Si gel TLC by the procedure of

ref. [31] using EtOH–cyclohexane (2:3) as solvent. After removal from the solvent mixture, the plates were immediately sprayed with $(C_2H_4OH)_3N$–*iso*-PrOH (1:4). The spots were marked under the UV-light with reference to the standard used. The dansylated polyamines were eluted with Me_2CO and the fluorescence intensities were measured in a spectrofluorometer, with the excitation and emission wavelengths at 360 and 506 nm, respectively.

Enzyme activity. Arginine decarboxylase (arginine carboxylyase, EC 4.1.1.19) in the tissue extract was estimated by measuring the release of $^{14}CO_2$ from L-[^{14}C]arginine under the conditions reported in ref. [7].

Protein, RNA and DNA. These were estimated by the method of ref. [32], the orcinol reaction [33] and the diphenylamine reaction [34], respectively.

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