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CHANGES IN POLYAMINE CONTENTS DURING DEVELOPMENT AND GERMINATION OF RICE SEEDS

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Key Word Index—*Oryza sativa*; Gramineae; spermine; spermidine; cadaverine; agmatine; putrescine; polyamines; arginine decarboxylase.

Abstract—In rice seed, polyamine concentration on a fresh weight basis increased for 16 days after fertilization, followed by a gradual decline. Arginine decarboxylase activity also followed the same pattern. On germination, the polyamine concentration was greatest after 24 hr and the arginine decarboxylase showed a peak after 48 hr.

INTRODUCTION

Large amounts of polyamines (PA) are known to be present in seeds of a number of gymnosperms and angiosperms [1] and it was of interest to determine their concentration during the formation and germination of rice seeds [2, 3] since enhanced elaboration of PAs and increased activities of enzymes involved in PA biosynthesis are associated with growth and developmental processes. Recent studies by Smith [4] have indicated that agmatine obtained by decarboxylation of arginine is the major precursor of putrescine and hence there should be a correlation between PA content and arginine decarboxylase (ADC) activity.

In the present work our interest is centred not only on the analysis of PAs and ADC during the early stages of germination as was studied by others [3, 4] but also on the different stages of seed development.

RESULTS AND DISCUSSION

Fig. 1a shows the pattern of the changes in total PA and protein, RNA and DNA in the different stages of rice seed development. At the initial stage, i.e. just after pollination, total PA content is low but it begins to increase and by the 16th day of development (milky stage), it has increased 4-fold. The rise in total PA is associated with a rise of RNA (3-fold), DNA (2.5-fold) and protein (3-fold). After this, the PAs, nucleic acids and protein begin to decline and in mature seed the levels are low.

It is now well documented that in a number of biological systems the increased synthesis and accumulation of PAs, preceded by the enhanced activity of the enzymes mediating their formation, are intimately associated with the growth and development [5]. The increase in amine levels and macromolecules is progressively decreased when cell division begins to decline and is gradually reduced as the dying cells of the endosperm become filled with storage material. The increase of RNA content reflects the growth and increasing metabolism of the developing seeds.

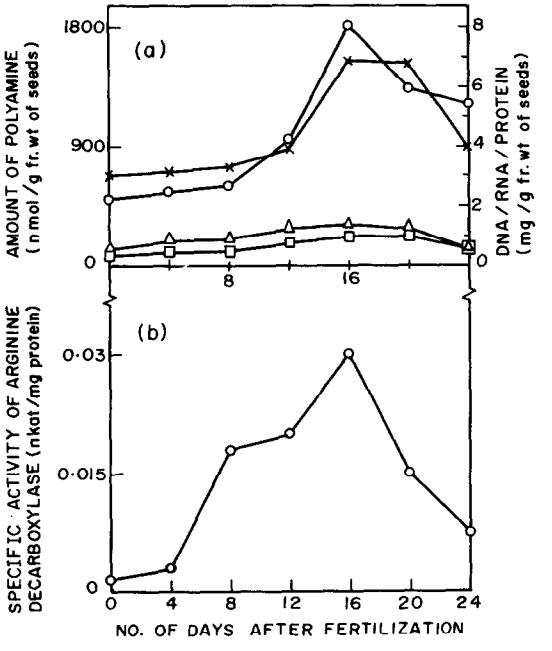

Fig. 1. (a) Changes in total polyamines (PAs) (○), RNA (□), DNA (△) and protein (×). (b) Activity of arginine decarboxylase (○) during the development of rice seeds.

Putrescine, cadaverine, agmatine, spermine and spermidine are identified in rice seeds (Fig. 2). Initially the spermidine content is small compared with agmatine and spermine, but it rises dramatically in the milky stage when it is 38-fold higher than the initial value. Though spermine and agmatine are present in large amounts in the initial stage, their further increment was less pronounced, being of the order of 2- to 3-fold. Enhancement of putrescine (12-fold) is quite pronounced but for cadaverine the value

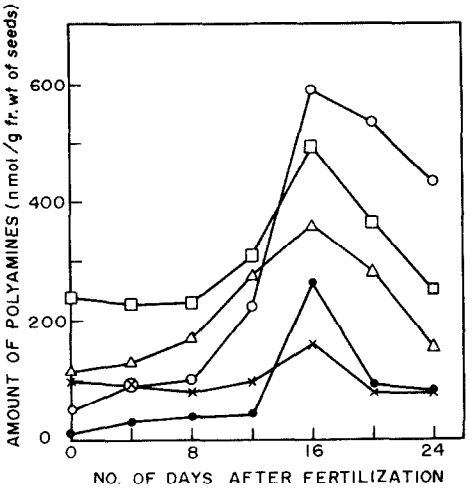

Fig. 2. Changes in polyamine contents during development of rice seeds. PAs were separated in Dowex 50 (200–400 mesh) using a linear HCl gradient (1–6 M) and estimated by the ninhydrin reaction. The values are shown as nmol of polyamines per g fr. wt of rice seeds; spermidine, ●; cadaverine, ×; putrescine, ○; spermine, □; agmatine, △.

is marginal, although initially both of them are present more or less in the same amount. A great increase of spermidine may be due to the conversion of spermine to spermidine. The presence of all these amines in the resting seed to the extent of about 108 μg/g fr. wt raises the question of their significance in the different period of the growth of the embryo. To accomplish this, the levels of amines as well as macromolecules were determined in the

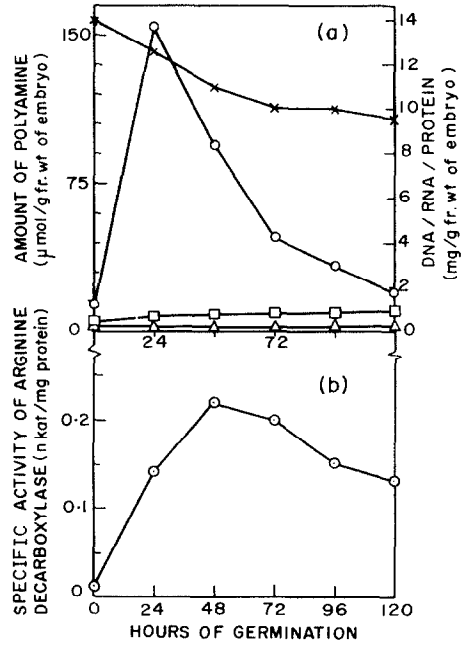

Fig. 3. (a) Changes in total polyamines (○), RNA (□), DNA (△) and protein (×). (b) Activity of arginine decarboxylase (⊙) during germination of rice embryo.

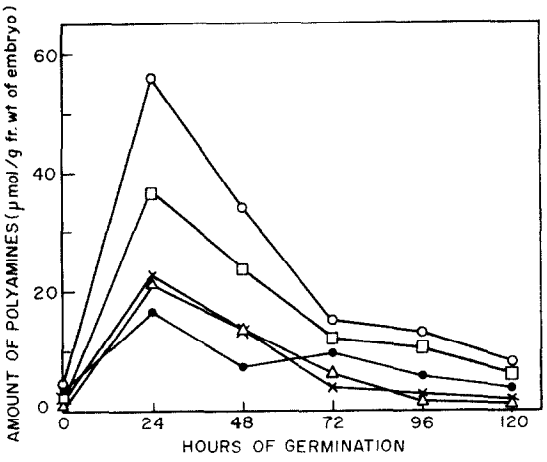

Fig. 4. Changes in polyamine contents during germination of rice embryos. Method of separation and abbreviations as in Fig. 2.

embryonic axis at daily intervals after germination. During the development of the embryo there occurs a progressive increase in the concentrations of the amines, enhancement being greatest with putrescine, spermine and spermidine, followed by agmatine and cadaverine (Fig. 4). The increase in amine levels is progressively reduced after the growth period is enhanced, i.e. around 24–48 hr in agreement with previous observation [6]. Within the first 24 hr of germination a 12-fold rise of total PAs is found, of which putrescine, cadaverine and agmatine attained 14-, 15- and 24-fold respectively, those of spermine increased 14-fold, and spermidine was enhanced by only 4-fold. So the progressive decrease of amines is correlated with the decline in individual amine level. Comparing these data with RNA and protein it is found that protein content declines appreciably, whereas RNA continues to increase until the 5th day of germination. The fall in protein content during the growth of the embryo may be due to proteolytic degradation [7].

Various workers [8, 9] have found large concentrations of putrescine and spermidine in fast growing tumours of plants and animals, which is in conformity with our results. Moreover, appreciable amounts of putrescine in early growth of the embryo have been demonstrated by others [6, 10] although Bagni could detect only a trace of putrescine in cotyledons or the embryo of *Phaseolus vulgaris*. The presence of a large amount of cadaverine was also observed in chick embryo [11] and in seedlings of *L. sativus* [10].

The enhancement in the concentration of the amines may be of considerable significance for the physiology of the development of seed and growth of the embryo. According to Smith [12] in higher plants agmatine, putrescine and the PAs are metabolically connected by a precursor–product relationship with arginine as starting point.

The specific activity of ADC increases with the development of seed and maximum activity (18-fold) occurs at the milky stage, after which the level begins to decrease, and at the resting stage ADC activity is barely detectable. Between 24 and 48 hr after germination, a dramatic rise of 22- to 38-fold of enzyme activity is

observed, after which the level decreases (Fig. 3b). Despite the subsequent decrease in the enzyme activity the decline is not comparable with the loss of PAs.

Results of the present studies show an elevated ADC (18-fold) and putrescine (12-fold) content in the milky stage. Similarly elevation of ADC by 38-fold and putrescine by 15-fold is obtained in the very early stage of the embryo growth. So undoubtedly the high level of ADC and putrescine supports the assumption of Smith [13] for the operation of an arginine → agmatine → putrescine pathway in the growth and development of rice seed. Given the large amount of PAs present within the cells at all stages of the life cycle of rice seed it is probable that a number of cellular processes in the growth phase and in development depend on PAs.

EXPERIMENTAL

Chemicals. Spermine, spermidine, cadaverine, agmatine, putrescine, Dowex 50 (200–400 mesh) and BSA were from Sigma. [^{14}C]arginine (sp. act. 142 mCi/mmol) was from the Bhaba Atomic Research Centre, Trombay, India.

Winter variety of rice (*Oryza sativa* L.) local cv Rupsail was grown during the Kharif season in the field of the Bose Institute, Calcutta. Collection of the developing seeds was started just after pollination and was continued at 4-day intervals up to maturation.

Germination of seeds. After surface-sterilization with 0.1% $HgCl_2$ the seeds were allowed to germinate in dark at 37 ± 1° on moistened filter paper in Petri dishes, and amine levels were determined in the embryonic axis at 0–5 days after germination.

Determination of amines. PA was extracted from different samples according to ref. [10]. Briefly, the pooled $HClO_4$ fraction at pH 8 was extracted with 3 vols of *n*-butanol. The acidified butanol extract after evaporation at 60° was taken up in H_2O. The amine fractions adjusted to pH 5 were applied to Dowex 50 × 2 (200–400 mesh, H^+ form) column. After washing with 2 M NH_4OH and H_2O the column was eluted with a linear HCl gradient (1–6 M). The effluent was collected in 6 ml fractions in an automatic LKB fraction collector. After neutralization, the PA contents of the individual fractions were estimated by the ninhydrin reaction [14].

DNA, RNA and protein. These were estimated by the diphenylamine reaction [15], orcinol reaction [16], and Lowry's method [17] respectively.

Arginine decarboxylase (arginine carboxy-lyase, EC 4.1.1.19) activity was determined as described in ref. [18]. The assay mixture consisted of 160 μmol Tris–HCl buffer (pH 8.5), extract containing 0.5–2 mg protein, 50 μmol pyridoxal phosphate, 2 μmol dithiothreitol in a total vol. of 1 ml and was incubated in a Dubnoff metabolic shaker at 40° for 1 hr. The evolved CO_2 was absorbed in M KOH and the reaction was terminated by adding 4 M H_2SO_4 to the incubation media. Liquid scintillation counts were taken and blank values were obtained by terminating the reaction with acid immediately after extract addition. Protein was determined by solubilization in M NaOH of ref. [17] after precipitation with TCA.

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