

## ISOZYMES OF SUPEROXIDE DISMUTASE FROM GROUNDNUT (*ARACHIS HYPOGAEA*) SEEDLINGS

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### ABSTRACT

Five isoenzymes of superoxide dismutase (SOD) were identified in groundnut seedlings *Arachis hypogea* and the maximum activity was observed in 6-day-old seedlings. SOD II, III, IV and V were sensitive to cyanide and SOD I was sensitive to chloroform-ethanol mixture. By using inhibitors isoenzymes II, III, IV and V were found to be Cu-Zn and isoenzyme I contains Mn. Mn enzyme is present in mitochondria whereas the Cu-Zn enzymes are distributed in chloroplast.

### INTRODUCTION

It appears that the superoxide radical is a common intermediate in the biological reduction of oxygen and that superoxide dismutase (SOD) is the primary defence against the potentially deleterious reactivities of this radical<sup>1</sup>. The three classes of SOD, namely Cu/Zn, Mn and Fe containing enzymes have been isolated and characterized from several sources<sup>2</sup>. Among germinating seeds, bajra<sup>3</sup>, mung bean<sup>4</sup> and wheat germ<sup>5</sup> contain three isozymes of SOD whereas green pea<sup>6</sup> contains only two isozymes. In the present communication we report the nature and distribution of isozymes of SOD in groundnut seedlings.

### MATERIALS AND METHODS

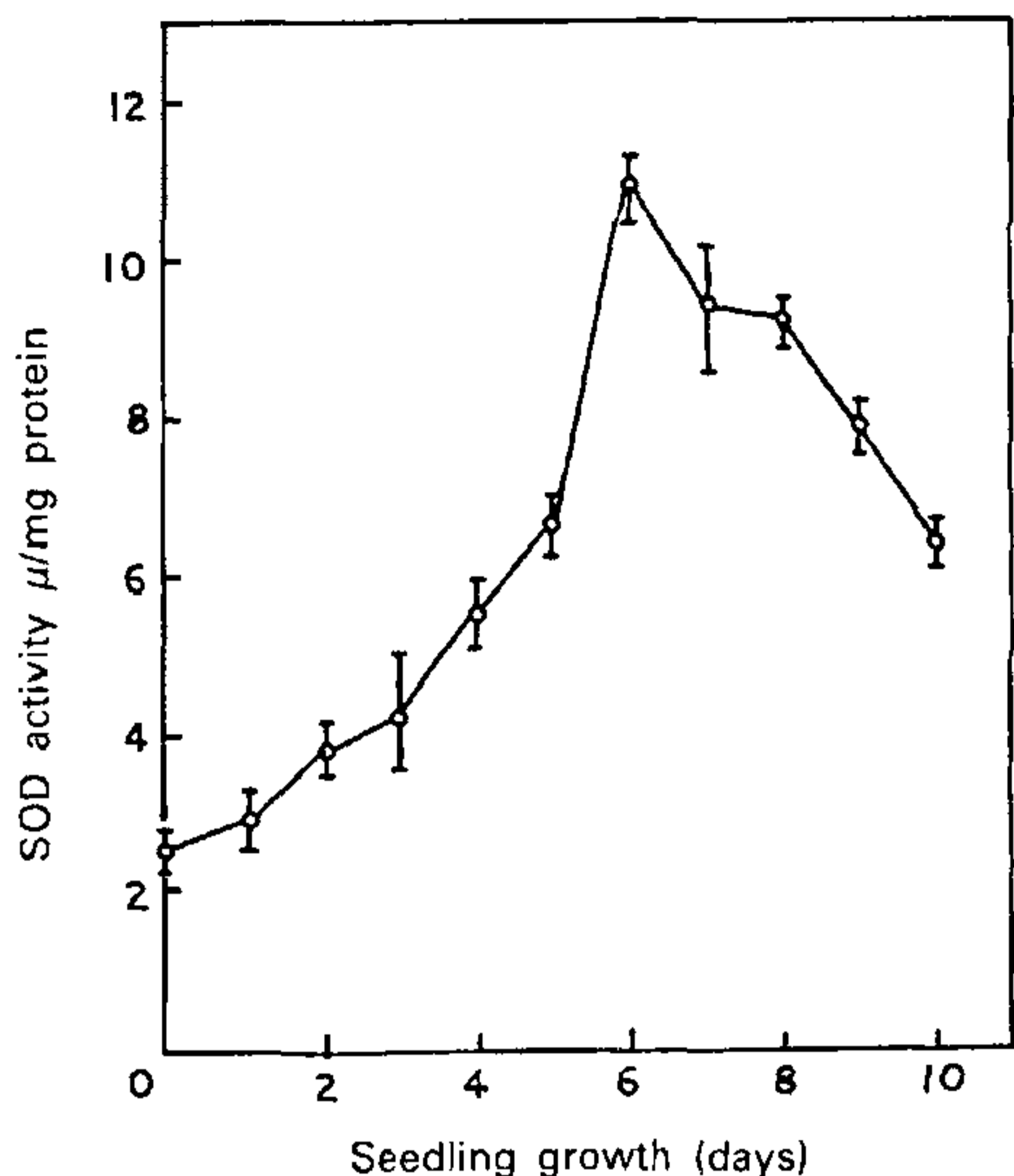
Groundnut J. L. 24 var. (*Arachis hypogea*) seeds were procured from the Agricultural College, Tirupati. Healthy seeds were sterilized with 0.1% HgCl<sub>2</sub>, washed with tapwater and allowed to germinate at 37°C in petri dishes for ten days. Starting from zero hour to ten days (at 24 h intervals) the powder was prepared from 2 g of seedlings with chilled acetone. Each time 500 mg of this powder was used to extract the enzyme with 10 ml of 0.02 M sodium phosphate buffer of pH 7.6. The enzyme extracts were subjected to ammonium sulphate precipitation (0–70%) followed by dialysis against 0.01 M phosphate buffer pH 7.6 for 2 days. The protein content was estimated by the method of Lowry *et al*<sup>7</sup> using BSA as standard. The enzyme was assayed by the method of epinephrine auto-oxidation at pH 9.8 using a double beam UV spectrophotometer (Shimadzu 180) as described by Misra and Fridovich<sup>8</sup>. Polyacrylamide gel (7.5%) was electrophoresed according to Davis<sup>9</sup> and the isozymes of SOD were located in the gels by using

the photochemical method<sup>10</sup>. Isozyme pattern was represented from top to bottom in polyacrylamide gels. Mitochondria were isolated from six-day (dark grown) seedlings by the method of Bonner<sup>11</sup>. Chloroplasts were isolated from 15-day-old leaves as described by Walker<sup>12</sup>. The mitochondria and chloroplasts were lysed, centrifuged and the supernatants were subjected to polyacrylamide gel electrophoresis and activity staining to get the isozymic profile. All the operations were done at 4°C.

### RESULTS

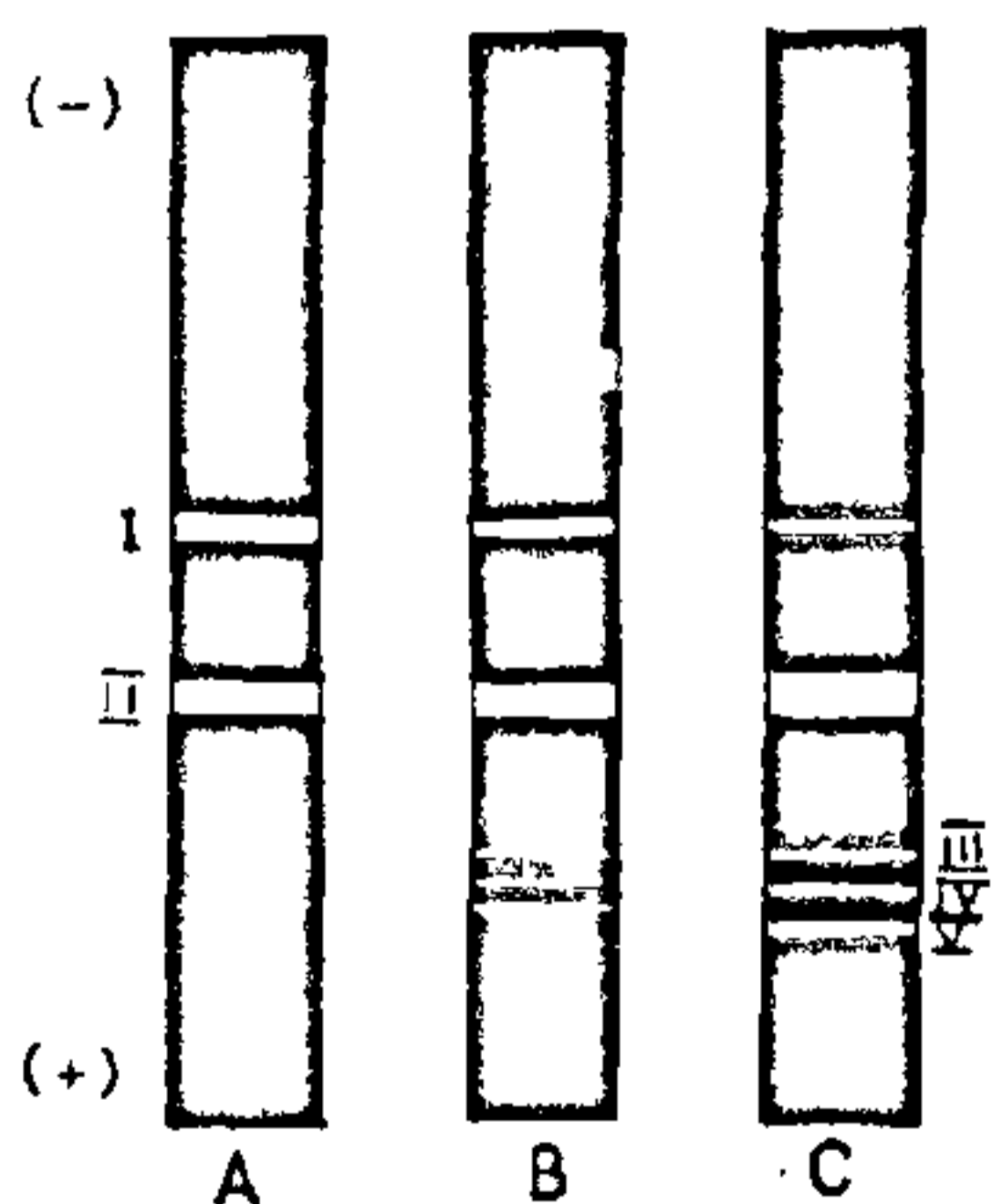
The specific activity of SOD in the dry seed and in different stages of seedling growth is shown in figure 1. Polyacrylamide gel electrophoresis and activity staining reveal that dry seed contains only two isozymes i.e. SOD I and II (figure 2), and the formation of three other isozymes (SOD III, IV and V) with increased activity was observed with the growth of the seedling. The five distinct isozymes of SOD had maximum activity in 6-day-old seedlings (figure 2). However the activity of the enzyme decreased after 6 days of germination but the electrophoretic pattern of the isozymes remained unaltered. Isozymes II, III, IV and V were present in the chloroplast whereas SOD I was localized in mitochondria (figure 3).

To identify the metal ions present in the isozymes, 6-day-old seedlings were subjected to inhibitor studies. The enzyme extract was incubated separately with 20 mM H<sub>2</sub>O<sub>2</sub> or 3:5 v/v mixture of chloroform and ethanol at room temperature (28°C ± 2) for 2 h with proper controls and all the samples were subjected to polyacrylamide gel electrophoresis and activity staining. One of the control gels was stained for activity in the presence of 2 mM cyanide and all others were stained in the usual way to localize the

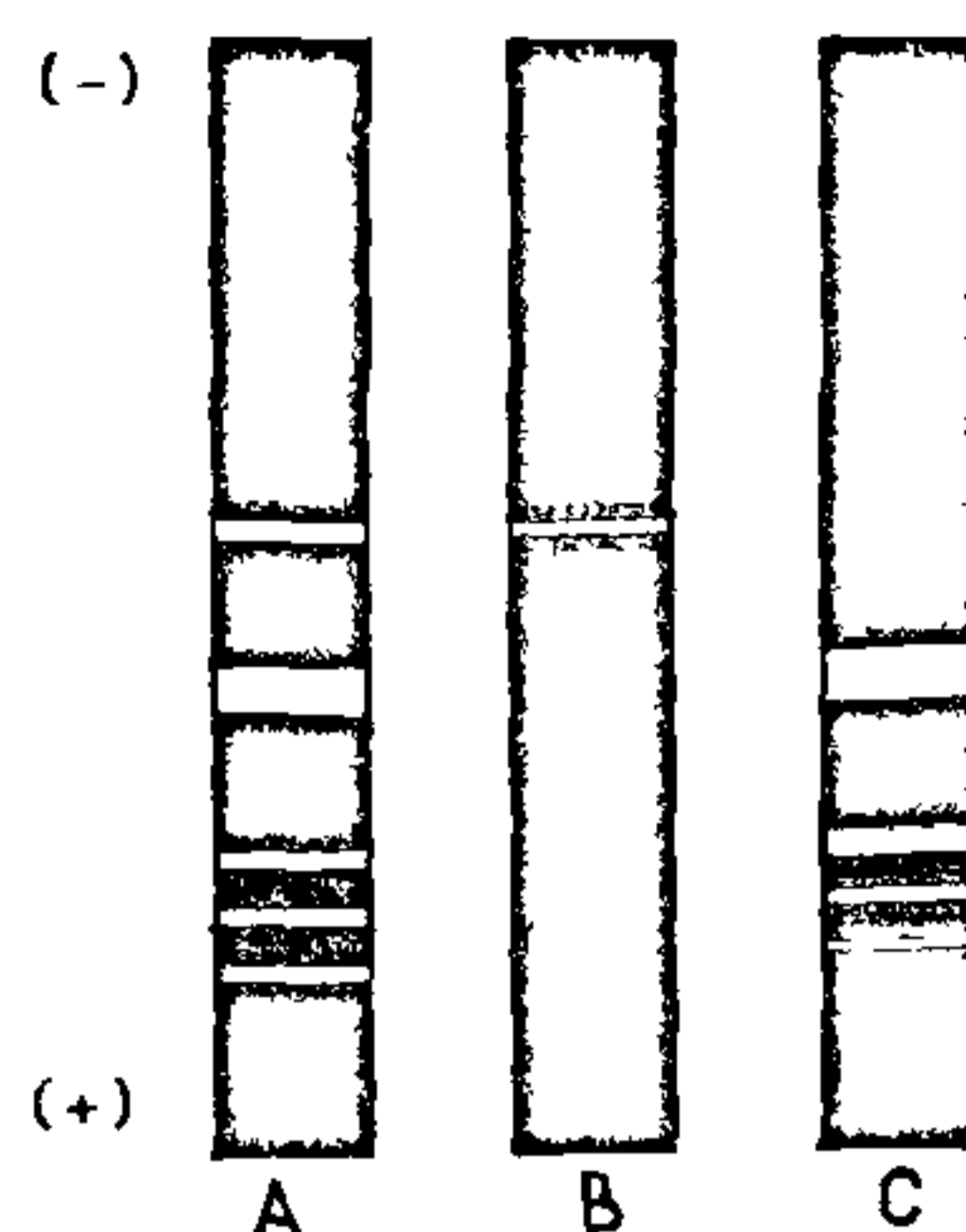


**Figure 1.** Superoxide dismutase activity in the acetone powder extracts of zero to 10-day-old seedlings of groundnut.

isozymes in the polyacrylamide gels. SOD II, III, IV and V were sensitive to cyanide. However, SOD I was sensitive to chloroform-ethanol mixture treatment, and none of the enzymes were inhibited by hydrogen peroxide (figure 4).



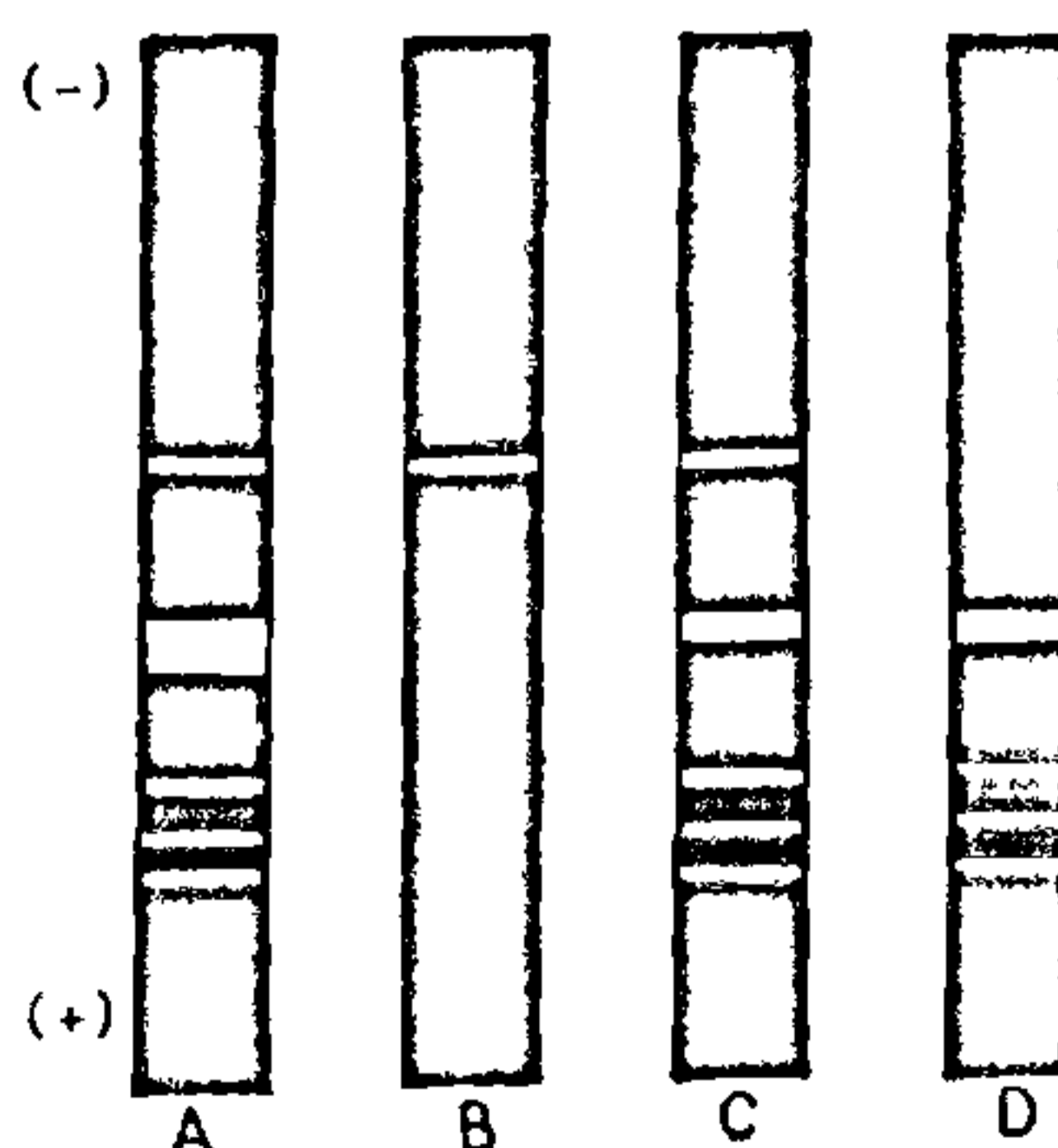
**Figure 2A-C.** Polyacrylamide gel electrophoresis and activity staining for isozymes of SOD at three different stages of germination. Each time about 600 µg of protein were applied on to the gel. A. Buffer extract from dry seed showing SOD I and II; B. Buffer extract from 4-day-old seedlings showing the formation of SOD III, IV and V; C. Buffer extract from 6-day-old seedlings showing maximum activity of all the five isozymes.



**Figure 3A-C.** Intracellular localization studies. A. Buffer extract from 6-day-old seedlings showing all the five isozymes; B. Isolated mitochondria showing SOD I activity; C. Isolated chloroplasts showing SOD II, III, IV and V activities.

### DISCUSSION

Dry seed contains only two isozymes with low enzyme activity. Five distinct isozymes with increased activity appear with the seedling growth. This may be due to the development of chloroplast where the molecular O<sub>2</sub> univalently photoreduce to generate O<sub>2</sub> radicals through autooxidation of an electron acceptor in photosystem I<sup>13</sup>. Photo production of superoxide appears to be indispensable for the prevention of over-reduction of electron carriers in the cyclic electron transport<sup>14</sup>. Scavenging of



**Figure 4A-D.** Inhibition studies. A. Buffer extract from 6-day-old seedlings showing all the five isozymes; B. Same as in (A) but stained for activity in the presence of 2 mM cyanide; C. Buffer extract after incubation with 20 mM H<sub>2</sub>O<sub>2</sub> for 2 h at room temperature before electrophoresis; D. Buffer extract treated with chloroform-ethanol mixture before electrophoresis.

superoxide radical and hydrogen peroxide is essential for chloroplasts to maintain their ability to fix carbon dioxide, because several enzymes in the carbon dioxide reduction cycle are sensitive to active oxygen<sup>15</sup>. The formation of SOD III, IV and V isozymes and high activity of SOD II from the fourth day of germination suggest that these enzymes may be associated with chloroplast and further confirmed by localization of these enzymes by polyacrylamide gel electrophoresis from the isolated chloroplast.

Cyanide at 2 mM concentration completely inhibits Cu-Zn superoxide dismutases<sup>5</sup>. The susceptibility of SOD II, III, IV and V towards cyanide suggests that these four are Cu-Zn enzymes. The cyanide insensitive SOD II, was not affected by hydrogen peroxide but was inactivated by chloroform-ethanol mixture treatment indicating that it is a Mn enzyme<sup>16</sup>. The low activity of SOD I (figure 2) could be attributed to the sensitivity of this enzyme towards organic solvent-acetone<sup>17</sup>. The intracellular localization of Mn SOD in mitochondria agrees with earlier studies on bajra<sup>3</sup> and mung bean<sup>4</sup>.

#### ACKNOWLEDGEMENTS

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## ANNOUNCEMENTS

### NATIONAL SYMPOSIUM ON 'NEW TRENDS IN BIOTECHNOLOGY'

The above symposium will be held at Trivandrum during June 1988. For details please contact: C. Balagopalan, Organizing Secretary, C/o

Fermentation Section, Regional Research Laboratory, Trivandrum 695 017.

### THIRD WORLD DEVELOPMENT CENTRE, NEW DELHI — WORKSHOP

The workshop on "Implementation of reservation directives for SC/ST employees in Universities and Educational Institutions" will be held during April-9, 1988 at New Delhi. Details can be had from Shri

K. J. Iyer, Programme Officer, Office Complex, First Round Circle, B-97, Kalkaji, New Delhi 110 019.