CHANGES IN THE ACTIVITIES OF SOME HYDROLASES AND OXIDOREDUCTASES IN IAA-TREATED HYPOCOTYL CUTTINGS OF *PHASEOLUS VULGARIS* L. DURING ADVENTITIOUS ROOT INITIATION AND DEVELOPMENT

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ADVENTITIOUS root formation involves several processes that require different conditions[1,2]. These processes involve either the exertion of mechanical pressure or enzymatic digestion[3,4]. The differentiation of tissues in plant organs is accompanied by changes in the activities of various enzymes[5,6]. Very little is known about biochemical changes in adventitious root primordium initiation and development[7,9]. It is not clear how biochemical differentiation in the rooting zone as a whole specifically relates to primordium initiation. The purpose of this investigation was to obtain insight into enzymatic changes that may occur during adventitious root initiation and development in *Phaseolus vulgaris* hypocotyl cuttings. The enzymes studied were acid phosphatase, α- and β-amylase, β-glucosidase and catechol oxidase.

Apparently healthy and uniform seeds of *P. vulgaris* were selected and soaked for 24 h in distilled water and then spread on moist, acid-washed sand placed in enamel trays. The trays were covered with glass sheets and placed in a BOD incubator at 22±1°C under 18 h light and 6 h dark cycle for germination of the seeds. Twenty-four hours after sowing, the seeds were supplied with complete Hoagland nutrient solution[10]. When the seedlings were 4 days old they were uprooted and cuttings were made by severing the hypocotyls 6 cm below the cotyledons. The cotyledons were excised from the cuttings. The hypocotyl cuttings were placed in plastic tubes (7 × 2.5 cm) containing 20 ml of test solution and only hypocotyl was dipped in the test solution. The hypocotyl cuttings were treated with 0.25 ppm of IAA for the first 24 h; control cuttings were not treated. Samples were taken on days 0, 2, 4, 6, 8 and 10. Each sample consisted of three segments, each of three cm length (lower part), from three hypocotyl cuttings. Fresh weight of the samples was recorded and the tissue was homogenized in 0.1 M phosphate buffer, pH 6.5. The extracts were centrifuged at 3000–4000 *g* for 10 min at 0°C. The supernatants were taken and the final volume was adjusted to 3 ml in each case with buffer. α-Amylase was estimated[11] using starch as the substrate. β-Amylase was assayed[12] using dinitrosalicylic acid reagent. Activity of β-glucosidase was measured using *p*-nitrophenyl-β-glucoside as substrate[13]. Acid phosphatase and catechol oxidase were estimated by adopting the methods of Simola and Soparen[14] and Arnon[15] using *p*-nitrophenyl phosphate and catechol as substrates respectively. Protein was estimated according to Lowry *et al*[16].

The activity of acid phosphatase decreased during the first two days, then showed an increasing trend until day 6, and declined thereafter (figure 1a). The specific activity was higher in the case of IAA-treated hypocotyl cuttings. This enzyme might be playing a two-fold role in adventitious root formation: first, by causing a lysis of the cells which come in the way of the emerging root primordium,

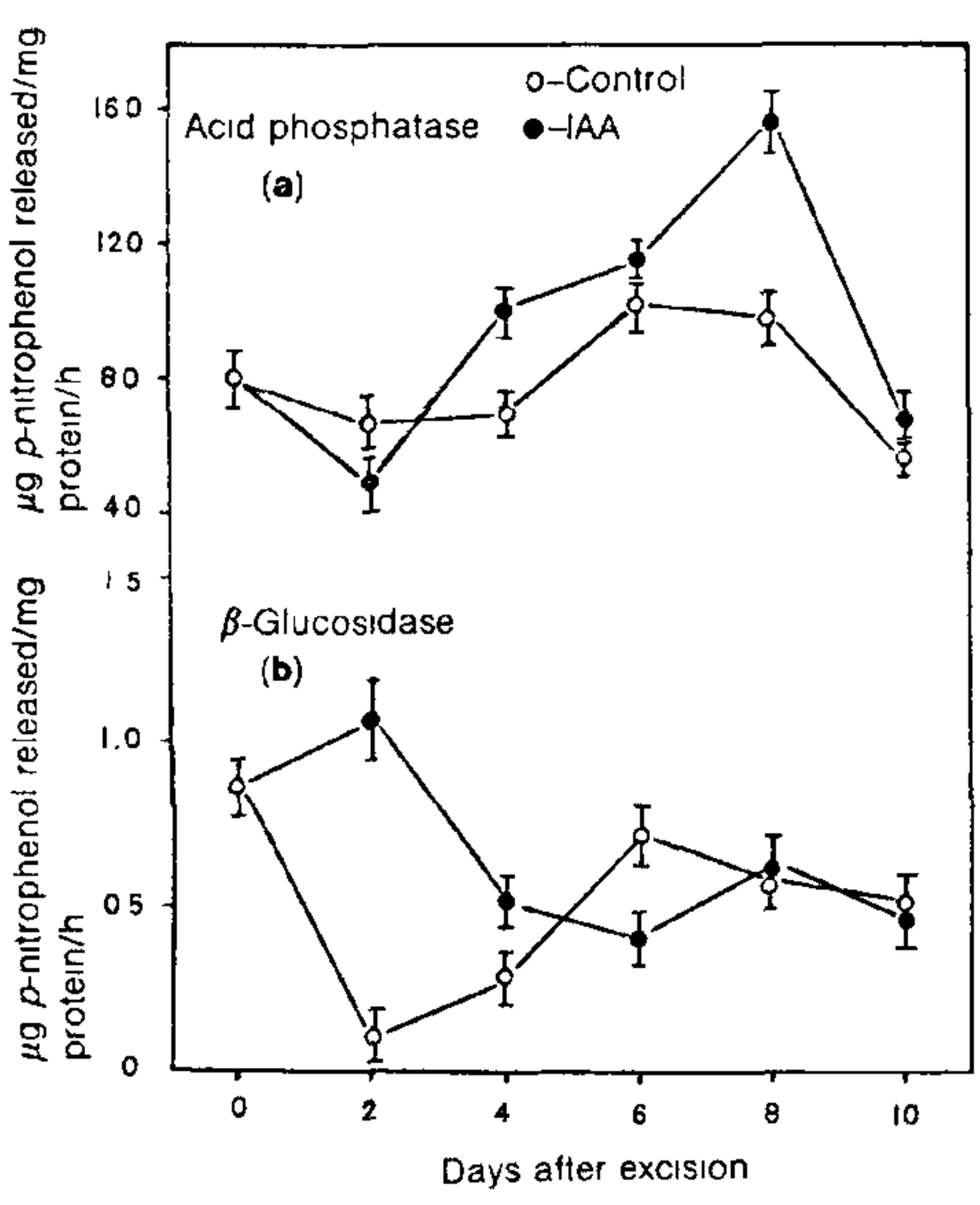

Figure 1. Changes in specific activity of (**a**) acid phosphatase and (**b**) β-glucosidase from extracts of *Phaseolus vulgaris* L. hypocotyl cuttings. Vertical bars are ± SD.

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and secondly, by assisting in the absorption of nutrient material derived from these cells. This enzyme was also reported to be involved in polysaccharide transformation occurring during the critical stages of morphogenesis and to be possibly concerned with autolysis[17]. β-Glucosidase decreased in control cuttings during the first two days, while in IAA-treated cuttings there was an increase in activity (figure 1b). Increase in β-glucosidase activity has been shown to be correlated in time with decrease in cell wall glucan and increase in growth rate of hypocotyls of seedlings of *P. vulgaris*[18]. The decrease in activity of the enzyme from cell-free extracts may reflect transfer of the enzyme from the cytoplasm to the wall fraction. Its roles in lignification[19], sugar uptake[20] and cell elongation[21] have been reported. A sharp increase in the activities of both α- and β-amylase during root initiation and development is evident (figure 2a, b). Activities are lower in the case of IAA-treated hypocotyl cuttings than in controls. It is possible that enhanced amylase activity immediately following excision is simply a wound response that normally accompanies subsequent cell proliferation[22]. The significance of IAA suppression of amylolytic activity in rooting hypocotyls is not clear.

A slight decrease in the activity of catechol oxidase was observed in the first two-day period in IAA-treated cuttings (figure 3). In the controls peak activity was at the six-day stage while in IAA-treated hypocotyls the peak was at the four-day stage. Presumably this temporal shift in the behaviour of catechol oxidase is in some way correlated with the process of root primordium development and emergence. Catechol oxidase has been reported to be increased when mechanical injuries occur in plant tissues[23]. As emerging root primordium may involve a sort of mechanical injury to adjacent cells, the observed increase in activity in catechol oxidase during root primordium emergence stage may have a similar connotation. Catechol oxidase or polyphenol oxidase is required for the synthesis of auxin–phenol complex, also suggested to be auxin synergist[24]. Recently it has been suggested that polyphenol oxidase is apparently not involved in phenolic biosynthesis, but is probably involved in the production of *o*-quinones during pathogen invasion, and its role as 'oxygen buffer' has been postulated[25]. IAA promotes induction of mRNAs[26] and enzyme modification[27]. To what extent enzymatic activity is altered in the root primordium initiating cells is yet to be known, since they comprise only a small fraction of the hypocotylar tissue and any causal

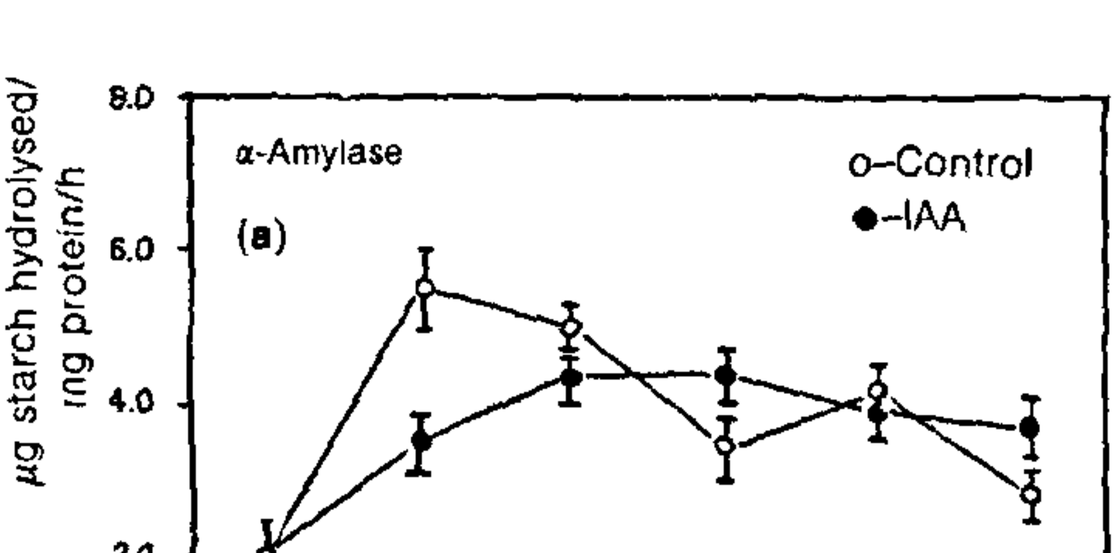

Figure 2. Changes in specific activity of (a) α-amylase and (b) β-amylase from extracts of *Phaseolus vulgaris* L. hypocotyl cuttings. Vertical bars are ± SD.

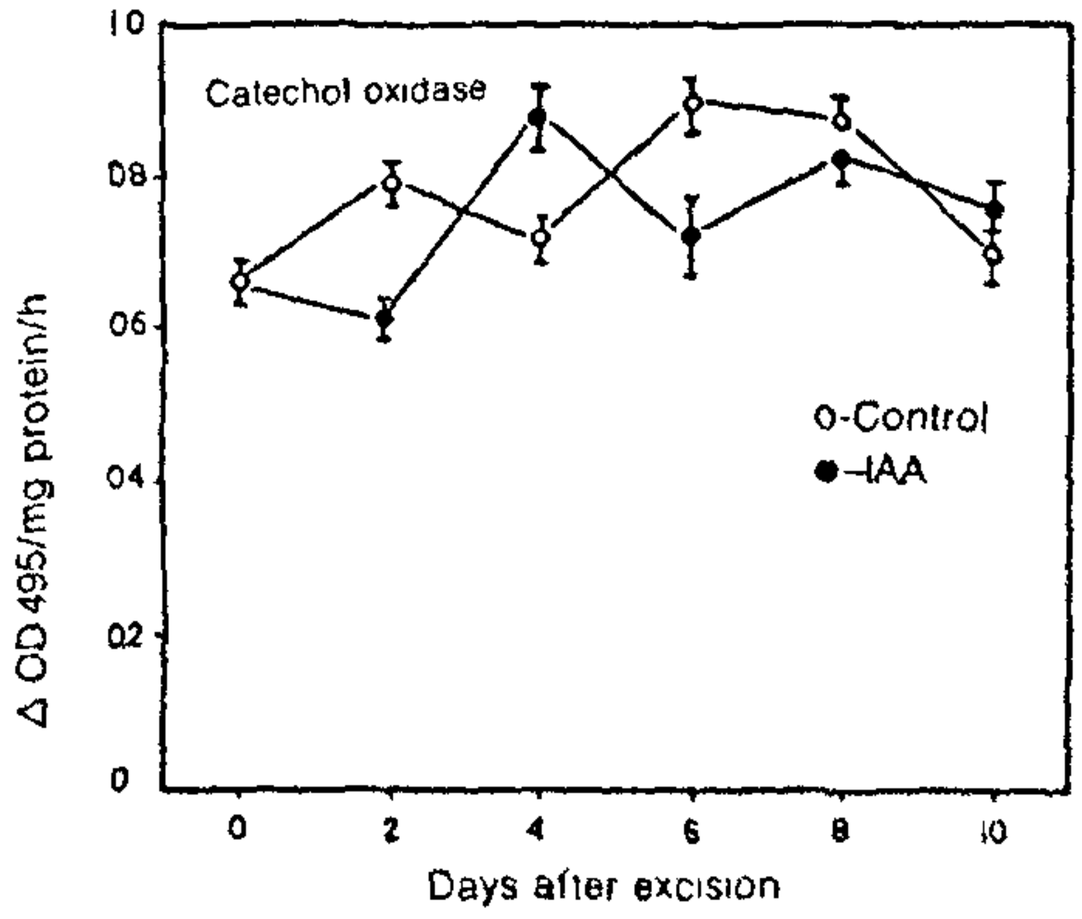

Figure 3. Changes in specific activity of catechol oxidase from extracts of *Phaseolus vulgaris* L. hypocotyl cuttings. Vertical bars are ± SD.

relationship between them and rhizogenesis remains obscure and needs to be studied further.

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ENHANCED CERIUM CONCENTRATION IN MAGNESIUM-DEFICIENT PLANTS

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MONAZITE, found in the coastal belt of tropical countries, is rich in elements like thorium and cerium. We had reported elevated levels of thorium in cardiac tissue samples of patients with endomyocardial fibrosis, a tropical cardiomyopathy, and postulated that the disease may be the 'cardiac expression of an elemental interaction that causes a toxic metal to replace or displace an essential element at the cellular level'[1]. Subsequent studies[2] have shown that the levels of cerium in the cardiac tissue samples are higher than those of thorium in endomyocardial fibrosis. Since the higher tissue levels of thorium and cerium coexist with a relative decrease in the concentration of magnesium, it is possible that the deficiency of magnesium might enhance the levels of these toxic elements. As tuber crops in Kerala have been reported to have high concentrations of monazite elements like thorium[3], an experimental study was undertaken to determine whether the deficiency of magnesium might promote the concentration of cerium in a tuber crop, *Coleus parviflorus*, grown in culture.

Coleus parviflorus was grown in Murashige and Skoog medium containing 1 μM benzyladenine, 1 μM naphthaleneacetic acid and 0.8% agar[4]. Magnesium-deficient medium was obtained from Hi-Media (Bombay, India), and the required amount of magnesium added to it. The normal medium (A) contained 180.8 mg/l $MgSO_4$ (anhydrous) and the

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