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Abstract. Changes in levels of macromolecular constituents and activities of some hydrolases and oxido-reductases have been studied in the developing embryos of okra, *Abelmoschus esculentus*. The hydrolases include acid and alkaline phosphatases, acylesterase, α- and β-glucosidases. Oxido-reductases studied are malate dehydrogenase, peroxidase and IAA-oxidase.

Keywords. *Abelmoschus esculentus*; phosphatases; acylesterase; glucosidases; peroxidase; malate dehydrogenase; IAA-oxidase.

1. Introduction

Okra (*Abelmoschus esculentus* (L.) Moench) is an important vegetable and grown extensively in India. The mature seeds of this plant have not been exploited for human nutrition and recently okra seeds were suggested as a new protein source since amino acid composition in them is similar to that of soybeans (Karakoltsidis and Constantinides 1975). Seed development has been studied (Chandra and Bhatnagar 1975, 1976) but no information is available regarding its physiological and biochemical aspects. The present study was undertaken to determine the activity profiles of some enzymes during embryogeny of okra and to correlate the significance of these enzymes with the basic morphogenetic changes.

2. Materials and methods

Plants of okra, were raised in the field during summer of 1978. Individual flowers were tagged at anthesis everyday during the flowering period and fruits of different ages were collected at desired intervals. For biochemical analysis and weight determination, embryos were dissected out. Fresh weight was determined and then embryos were dried to constant weight at 80°C. Materials for other analyses were frozen and stored at −20°C. Total nucleic acid determination was carried out following the technique of Ishikawa and Usami (1976), starch by that of Clegg (1964), soluble sugars by the anthrone method (Loewus 1952) and reducing sugars by the method proposed by Nelson (1944).
In order to find out a practical method for extracting several enzymes different buffers were tried. Ultimately, Tris-maleate (pH 7.0, 100 mM, containing 1 mM dithiothreitol) was used. After homogenisation of a known number/weight of embryos at 0°C in a mortar and pestle, the homogenate was centrifuged at 20000 g for 30 min at 0°C and the supernatant was used for enzyme assays.

2.1. Malate dehydrogenase

The method employed was a modification of that of Antonn and Roberts (1975). The assay mixture contained 0.2 ml, 30 mM L-malate; 0.2 ml, 1.5 mM NAD and enzyme extract 0.1 ml. The volume was made up to 3 ml with glycine-NaOH buffer (0.1 M, pH 10.0) and the reaction was initiated by adding L-malate and increase in absorbance at 340 nm recorded with Gilford 250 spectrophotometer.

2.2. IAA-oxidase

The incubation mixture according to Nanda et al (1975) contained 0.5 ml enzyme extract, 0.5 ml IAA (1 mM IAA prepared in 5 mM MnCl₂, 1 ml of 0.5 mM DCP and 0.5 ml of 0.067 M phosphate buffer (pH 6.5). The mixture was incubated for 30 min at 30°C in dark. Then 3 ml of Salkowski’s reagent (Gordon and Weber 1951) was added to this and the pink colour developed was measured at 530 nm.

2.3. Peroxidase

The reaction mixture according to Shannon et al (1966) for peroxidase comprised 3.3 ml phosphate buffer (0.1 M, pH 6.5), 0.2 ml enzyme extract and 0.025 ml o-dianisidine (100 mg in 5 ml methanol). The reaction was initiated by the addition of 0.2 ml of 0.6% H₂O₂. The change in absorbance at 430 nm was recorded.

2.4. Acid and alkaline phosphatases

The assay mixture of acid and alkaline phosphatases contained p-nitrophenyl phosphate 5 mM, in acetate buffer (0.05 M, pH 5.0) or Tris-HCl (0.04 M, pH 8.0) and extract 0.2 ml in a total volume of 1 ml. The incubation was carried out for 30 min at 32°C. The reaction was terminated by the addition of 2 ml 0.1 N NaOH and absorbance was recorded at 410 nm (Murray and Collier 1977).

2.5. Esterase

The colorimetric assay employed was based on coupling of 1-naphthol released by hydrolysis of 1-naphthyl acetate with tetrazotised-o-dianisidine and was modified from that of Amador and Wacker (1965).

2.6. α- and β-glucosidase

α- and β-glucosidase activity was assayed by using p-nitrophenyl-α-glucoside and p-nitrophenyl-β-glucoside (See McCreight et al 1976).
2.7. Estimation of proteins

Proteins were extracted by precipitation with 10% TCA and then solubilisation with 1N NaOH. Protein content was estimated by Lowry et al (1951).

3. Results and discussion

The present studies reveal significant changes in the activities of various hydrolases and oxido-reductases. Of the hydrolases, glycosidases were believed to play a direct role in cell wall modification and enlargement (Murray and Bandurski 1975). Increased β-glycosidase activity was correlated with growth rate of hypocotyls in Phaseolus vulgaris (Nevins 1970). The fresh and dry weight increases in embryos are given in figure 1. The embryo growth rate and fresh weight of developing embryos increase from 8th day and more markedly after the 10th day. The activity of β-glucosidase increases from 10th day (figure 2) and comparatively declines on 12th day, thus indicating that highest enzyme concentration does not coincide with the cotyledon growth. In contrast, α-glucosidase activity showed almost a linear increase (figure 2). The physiological significance of α- and β-glucosidase determined with p-nitrophenyl glycosides as an artificial substrate is uncertain.

The activity of acid phosphatase increases linearly with cotyledon growth but the alkaline phosphatase decreases after 16th day (figure 3). It was proposed that acid phosphatase could be important in the regulation of carbohydrate levels in the developing seed (Turner and Turner 1975; Murray and Collier 1977). Maximum level of soluble sugars and starch was observed on 14th day and 12th day after anthesis. The level of soluble proteins, DNA and RNA increases linearly upto 12th or 14th day (table 1). The increased activity

![Graph](image)

**Figure 1.** Changes in fresh and dry weight in the developing embryos of okra.
Figure 2. Activity of glucosidases (α, β) in the developing okra embryos.

Figure 3. Developing embryos showing alkaline and acid phosphatases.

Figure 4. Acylesterase activity pattern in the developing okra embryo.
of unspecific acylesterase is also detected in the developing embryos by using naphthylacetate as a substrate (figure 4). Such esterases seem to be relatively specific in attacking esters of short-chain carbonic acids in comparison to long-chain fatty acids (Norgaard and Montgomery 1968).

The oxido-reductases studied include malate dehydrogenase, peroxidase and indole acetic acid-oxidase. Peroxidase has long been known to catalyse the oxidation of indole acetic acid (Galston et al 1968; Stonier et al 1979). The peroxidase increases after 8th day and then becomes variable (figure 5). Similarly, the IAA-oxidase reaches a peak level at 12th day declining afterwards (figure 6). Both peroxidase and IAA-oxidase are known to regulate growth and development by altering the endogenous level of indole-3-acetic acid. Earlier
studies have also shown that isozymes of peroxidase can act as IAA-oxidase. The varying levels of peroxidase and IAA-oxidase on 12th day as observed in present studies is difficult to interpret. Gove and Hoyle (1975) observed the complicated relationship of the numerous multiple forms of this enzyme system. Based on the isoelectric-focusing studies they could not find a unique isozyme which has only one type of activity, i.e., peroxidase and IAA-oxidase. Whether differences in the present studies in the activity profiles of peroxidase and IAA-oxidase are due to appearance of such unique isozyme is not clear.

The activity of malate dehydrogenase increased sharply from 10th to 17th day after anthesis (figure 7). This enzyme is known to be present in both mitochondrial and cytoplasmic fraction. Mitochondrial enzyme is concerned with respiratory processes while cytoplasmic fraction has various roles, of which dark
CO₂ fixation is most significant. It is possible that malate dehydrogenase activity observed presently might be involved in refixing CO₂ in the okra seeds and fruits by C₄-pathway. Further work to elaborate the role of this enzyme in carbon economy of the developing okra cotyledons is in progress.

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