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Role of an acid phosphatase isoenzyme in callus tissue during cytodifferentiation

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Summary. Both histological and isoenzyme patterns of acid phosphatase were observed in callus tissue of *Vigna unquiculate* (L.) Walp. up to the tenth passage from its initiation. It was observed that when undifferentiated cells begin to transform into a differentiated condition in the form of tracheids, and xylem vessels, a new acid phosphatase band appears at the anionic end of the polyacrylamide gel. The transformation of living cells into a dead, empty tracheid during cellular differentiation and the biosynthesis of the acid phosphatase enzyme are functionally related to the autolysis of the cell contents and lignin synthesis.

Key words: Acid phosphatase – Callus tissue – Differentiation

Introduction

In vitro growth of meristematic, undifferentiated callus tissue and cellular differentiation into the form of tracheids, xylem vessels etc. are charaterised by a succession of metabolic states which are the result of a succession of enzymic states (Brown and Robinson 1955). Cells changing from the undifferentiated condition through some phasic developmental processess acquire and lose certain biochemical properties while bringing about the morphological as well as functional specialization at the tissue level, by continuous synthesis and or degradation of protein (enzyme) molecules (Scandalios 1974). Disc electrophoresis on acrylamide gels is potentially the most useful tool for locating any qualitative changes in protein metabolism at the cellular or tissue level and thus provides a sensitive index of the basic changes occurring in the tissue. The growing callus tissues, like the growing root and shoot of the intact plant, are the most convenient experimental material for the study of growth and development of cells from their meristematic to a differentiated state. Both quantitiative and qualitative variations in the protein profile of callus tissue derived from the hypocotyl of *Vigna unquiculate* (L.) Walp. during long term subculture, with respect to growth, development and the nature of tissue differentiation, has been observed. In the present paper, the isoenzymes of an acid phosphatase, an orthophosphoric monoester phosphohydrolase, was investigated electrophoretically in every passage of undifferentiated and differentiated callus tissue of *Vigna unquiculate* (L.) Walp.

Materials and methods

The material used was *Vigna unquiculata* (L.) Walp., var. 152, an agricultural strain supplied by the Oil and Pulse Research Station, Berhampore (W.B.). The hypocotyl segments (0.5 cm–1 cm) of aseptically germinated seedlings were used as explant. The callus tissue was initiated and maintained in MS medium supplemented with 2,4-D(2 mg/l) and kinetin (0.5 mg/l). The callus tissue was subcultured into fresh medium at 28 day interval.

For the histological study, a small amount of callus tissue was sectioned from the various portions of the callus mass, dehydrated in different grades of alcohol and stained by Safranin and light green.

For the extraction of enzyme, 10 gm of callus tissue from each passage (28 days) of the culture was harvested fresh and homogenised in 3 ml of cold 0.05 M Tris-Glycine buffer (pH 8.3) using a chilled (0–4 °C) mortar and pestle in an ice tub. The resulting homogenates were centrifuged in a Sorval RC-2B centrifuge at 0 °C for 30 min. The clear supernatant was dialysed at 4 °C for 1 h in a 1 : 1 dilution of Tris-Glycine buffer (pH 8.3). The final volume of enzyme extract from each passage was adjusted to 7 ml. The protein concentration in the extract was estimated before electrophoresis by the method of Lowry et al. (1951). To avoid loss of protein after dialysis, the supernatant was used directly as the enzyme extract. Polyacrylamide gel electrophoresis was performed essentially as described by Davis (1964). 150 µg protein was loaded onto

each gel. Electrophoresis was done at 4 °C in the dark. The current was regulated to 4 mA/tube for the desired time (90–120 min). At the end of the electrophoresis, the gel cylinders were removed and acid phosphatase was detected by incubating the gels for 3 h in a solution containing α-naphthyl acid phosphate (1 mg/ml), fast blue RR (1 mg/ml), NaCl (20 mg/ml), polyvinyl-pyrrolidone (5 mg/ml) and a few drops of 10% $MgCl_2$ in 0.1 M acetate buffer, pH 5.0. The buffer was changed every 30 min to lower the pH of the gel from above 8 to 5. After the appearance of the bands, gels were stored in 7% acetic acid. Gels were photographed immediately and diagrammed. The position of the isoenzyme band in the gel was expressed as relative mobilities (Rf) by measuring the distance migrated by the particular band relative to that of bromophenol blue used as tracking dye during electrophoresis. Gel scanning was done using a Carl Zeiss gel scanner with an automatic recorder.

Results and discussion

The histological study of callus tissue from the first to third passage shows a mixed population of small, more rounded, oval and few elongated cells with dense cytoplasm (Fig. 1 A). With an increase in passage number, cells become more elongated with a thick wall (Fig. 1 B) and the calli are friable. By the tenth passage, callus tissue shows maximum xylogenesis (60–70%) with tracheary elements having a continuous spiral deposition of secondary wall materials. The developing tracheary elements are oval in shape and developed tracheary elements are elongated with tapering ends (Fig. 1 C).

The isoenzyme bands of acid phosphatase with their scanning diagrams are shown in Figs. 2 and 3. Enzyme activity was evaluated by noting the changes in number of isoenzyme bands, staining intensity and the maximum scanning peak of each band. In this investigation the bands are arbitarily labelled as AP_1, AP_2, AP_3 and so on.

The enzyme extract from callus tissue of the first to the sixth passage was resolved into three isoenzymes, namely AP_1, AP_2 and AP_3 (Fig. 2) having Rf values of 0.22, 0.22 and 0.63, respectively. But from the seventh to the tenth passage an additional band, i.e., AP_4, appears in the gel, (Fig. 3) having an Rf value of 0.80. Thus, the isoenzyme of acid phosphatase shows a striking change in the electrophoretic pattern from the seventh to the tenth passages. From the first to the sixth passages of culture, the isoenzyme patterns are found to be remarkably similar with respect to Rf values of individual bands, staining intensity and scanning peaks. Again, from the seventh to the tenth passages of culture, the isoenzyme patterns are also alike in all respects. A photograph isoenzyme patterns from the first to the sixth passages and a photograph of the same from the seventh to the tenth passages are depicted in Figs. 2 and 3, respectively. Isoenzyme AP_1 is present in material taken from every passage. The intensity of AP_2 successively decreases. Increased intensity of AP_1 and

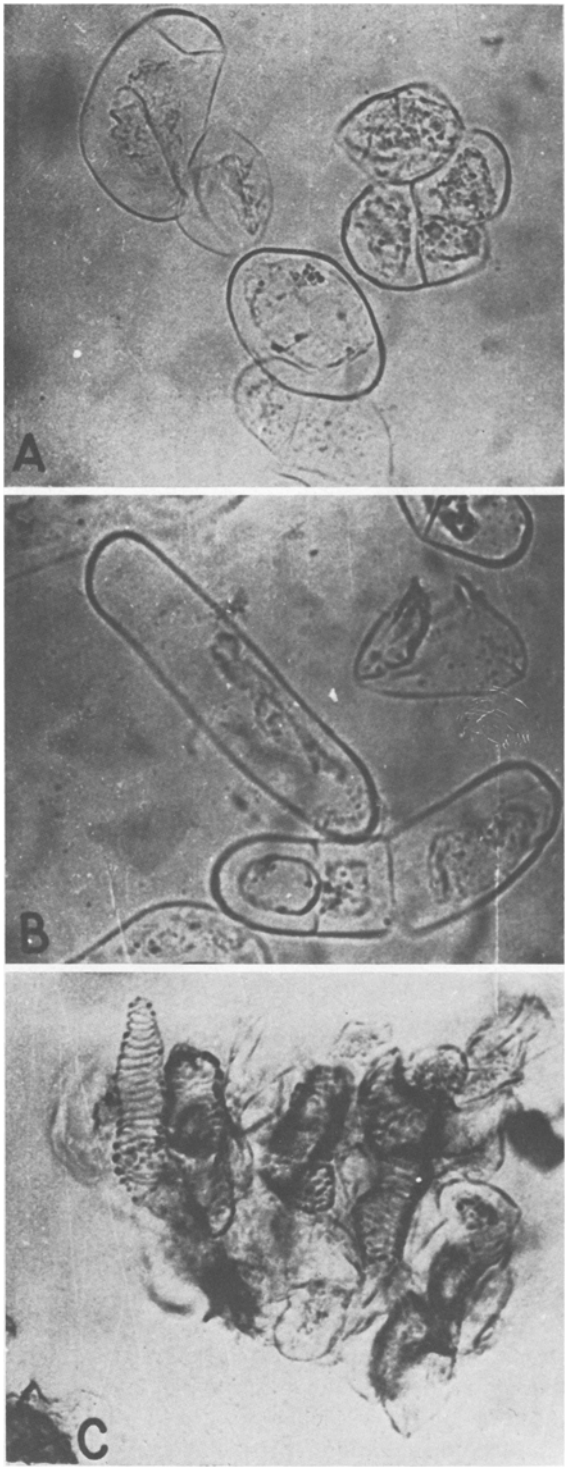

Fig. 1. Morphological changes in cell derived from callus tissue, up to 3 passages (**A**), after 3 passages (**B**) and after 8 passages (**C**) of culture. × 1,500

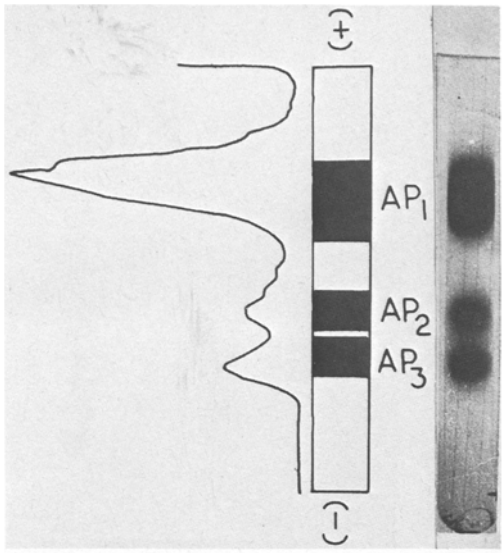

Fig. 2. Electrophoretic pattern and scanning of acid phosphatase isozymes up to sixth passage of culture showing three bands

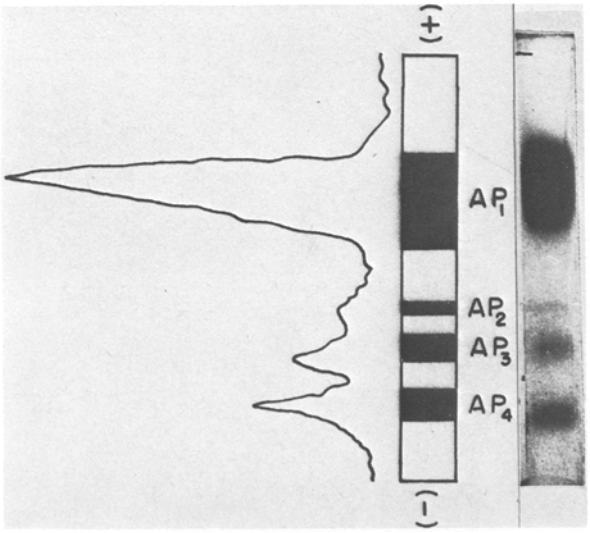

Fig. 3. Electrophoretic pattern and scanning of acid phosphatase isoenzymes from the seventh passage onwards showing an additional band (AP_4) at the anionic end

the appearance of anionic AP_4 indicates the increase of acid phosphatase activity and the synthesis of an additional multiple molecular form of acid phosphatase during the cytodifferentiation of the callus tissue.

The growth, development and cytodifferentiation phases of callus tissue are the outcome of a complex interaction of exogenous and endogenous levels of auxin and cytokinin. The growth phase, or cell division, in the tissue is found to be essential for xylem differentiation. Torrey and Fosket (1970) noted that mitosis in a particular environment is necessary for the induction of xylogenesis. In the present investigation, it has been noted that, up to the sixth passage of culture, the callus tissues remain in the growth and elongation phase. From the seventh passage onwards, there is an initiation of xylogenesis from mitotically blocked and elongated cells. Since callus tissues are composed of both differentiated and undifferentiated cells, the phenomenon of cytodifferentiation might lie in the degree to which autophagic activity in the cell has progressed. Autophagic activity in turn is closely linked with certain hydrolytic enzymes (Gahan and Maple 1966).

Acid phosphatase, an enzyme, is commonly present in the cell and has been detected in association with the cell wall (Arnison and Boll 1978), dictyosomes and plastids (Bornman 1974). In a review, Bornman (1974) mentioned that the presence of acid phosphatase is indicative of autolysis of the protoplast during cytoquiescence and cytosenescence of the cell. Therefore, the increased staining intensity of AP_1 and the appearance of AP_4, indicating the increased activity of acid phosphatase during the seventh to the tenth passages of the culture, may be associated with autolysis of the cell contents during xylogenesis. It is known that acid phosphatase causes autolysis of protoplasts during xylem differentiation. Arnison and Boll (1974) showed that cell wall bound isoenzymes of acid phosphatase were cathodic and, therefore, the anionic isoenzymes were organelle bound. But it is not definitely known whether cathodic or anionic isoenzymes are responsible for the autolysis of cell contents during xylogenesis. Bornman (1974) suggested that organelle bound isoenzymes of acid phosphatase were at least partial contributing factors to its own destruction during xylogenesis.

The appearance of an anionic AP_4 band clearly related with xylogenesis also shows the importance of phosphate metabolism during cytodifferentiation. Lignin synthesis is functionally related to the secondary wall formation of the developing xylem and lignin synthesis is also dependent on phosphate metabolism. Hence, the cathodic acid phosphatase, i.e. AP_1, may be associated with lignin biosynthesis for spiral deposition of secondary wall materials of the developing tracheary elements in callus tissue whereas the anionic band viz., AP_4, may be responsible for autolysis of cells.

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