BIOLOGIA PLANTARUM (PRAHA)

25 (5) : 321—325, 1983

Changes in Protein Patterns during Growth of *Vigna unguiculata* (L.) WALP. Callus Tissues

K. K. DE and S. C. ROY

Centre of Advanced Study, Department of Botany,
University of Calcutta*

Abstract. Alterations in protein pattern were observed in the callus tissues of *Vigna unguiculata* (L) WALP. up to tenth passage from its initiation. A gradual increase in quantity of protein was found up to sixth passage of culture. Decrease in the quantity of protein after eight months of culture might be correlated with the cytodifferentiation of the tissues. It has been recorded that with the initiation of morphogenesis there is an increase in the number and intensity of protein bands at the anionic end of the polyacrylamide gel.

Disc electrophoretic study of protein pattern of callus tissue is a highly useful biochemical index by which any change in protein metabolism during growth, development and differentiation in tissue culture can be observed. The growth and development phase of meristematic undifferentiated callus tissue are characterised by a series of metabolic states which are the results of succession enzymatic states. Now when these cells pass from the undifferentiated state to the differentiated state the cells acquire and loose some specific biochemical properties in bringing about the functional specialization at the tissue level by the synthesis and or degradation of protein (enzyme) molecule (SCANDALIOS 1974, SCANDALIOS and SORENSON 1977). This specialization results in changes in the relative amount of various proteins within the cells of the callus tissue in polyacrylamide gel which may indicate the nature of the cellular developmental and biochemical properties during the callus culture cycle.

The present paper reports the variation of both quantitative and qualitative protein profile in callus tissue during prolonged culture with respect to the growth, development and tissue differentiation in solid media.

MATERIAL AND METHODS

The callus tissue used in the present experiments was derived from hypocotyl segment (0.8 cm—1 cm) of *Vigna unguiculata* (L.) WALP. cv. 152, cultured in MURASHIGE and SKOOG (1962) medium supplemented with 2,

Received July 30, 1981; accepted December 15, 1982

* *Address:* 35, Ballygunge Circular Road, Calcutta — 700 019, India.

4-D (2 mg^{-1}) and kinetin (0.5 mg^{-1}) and incubated at 25—26 °C with 16 h light (5000 lx) in 50—60% relative humidity. Cultures were maintained in a serial subculture with 28 days passage duration. The material studied was harvested from first to tenth passage of cultured tissues. Samples (10 g) of callus tissue were harvested and homogenised in 3 ml of cold 0.05 M Tris-glycine buffer (pH 8.3). The resulting homogenates were centrifuged in a Sorvall RC-2B centrifuge at 20 000 *g* for 20 min at 0 °C. The clear supernatant after centrifugation was dialysed at 30 min interval against different dilution of 0.05 M Tris-glycine buffer solution, pH 8.3 (water : buffer — 4 : 1, 3 : 2, 2 : 3, 1 : 4) at 4 °C. The volume of protein extract of each passage was 7 ml in each case. Protein estimation was done by the method of LOWRY *et al.* (1951). Depending upon the initial concentration, protein extract was diluted with 2 M sucrose solution to give a final concentration of 1 μg protein 1 μl^{-1} . 150 μg of protein was applied to each gel for electrophoresis. Polyacrylamide disc electrophoresis was performed following the method described by DAVIS (1964). The protein extract was passed through double gels — upper spacer gel (3.33%) and lower separating gel (8%). Electrophoresis was done at 4 °C in the dark with a constant current 4 mA/tube. Gels were stained by 0.25% Coomassie brilliant blue R 250 and destained in a solution consisting of 0.5 : 8.75 : 0.75 methanol : water : glacial acetic acid. Gels after proper destaining were photographed and scanned at 565 nm.

For the evaluation of structural changes of callus tissues in the individual subculture, a small amount of tissue was taken from the various portions of the callus mass and was dehydrated in different grades of alcohol and stained by 1% Saffranin (dissolved in 70% alcohol) and 1% Light green (dissolved in 1 : 3 alcohol : clove oil) following the conventional double staining procedure. Finally the stained callus pieces were transferred to a grease-free slide and a drop of 20% glycerine was added on it. A cover slip was applied and the tissue was squashed exerting uniform pressure.

RESULTS AND DISCUSSION

The histological study of callus tissue of the first to third passage shows a mixed population of small, more rounded, oval and a few elongated cells with dense cytoplasm (Fig. 1A). From fourth to sixth passage the cells become more elongated with thick walls (Fig. 1B). After the eighth passage callus tissue shows maximum xylogenesis (60—70%) with tracheary elements (Fig. 1C) having continuous spiral deposition of secondary wall materials. The developing tracheary elements are oval in shap and developed tracheary elements are elongated with tapering ends.

The estimation of buffer soluble protein from each passage of culture exhibits a wide range of variation in quantity. From the first to sixth passage, the quantity of protein gradually increased from 0.740 mg g^{-1} to 1.417 mg g^{-1} of the callus tissue. But from the seventh passage to tenth passage the quantity of protein gradually decreased from 1.401 mg g^{-1} to 0.780 mg g^{-1} of the callus tissue.

The protein extract of each passage when fractionated on polyacrylamide gel and stained with Coomassie brilliant blue R 250 displayed 13—20 protein bands (Fig. 2A—J) which varied in intensity from faint and narrow to intense dark and broad. The intensity of stain in each band was scanned and

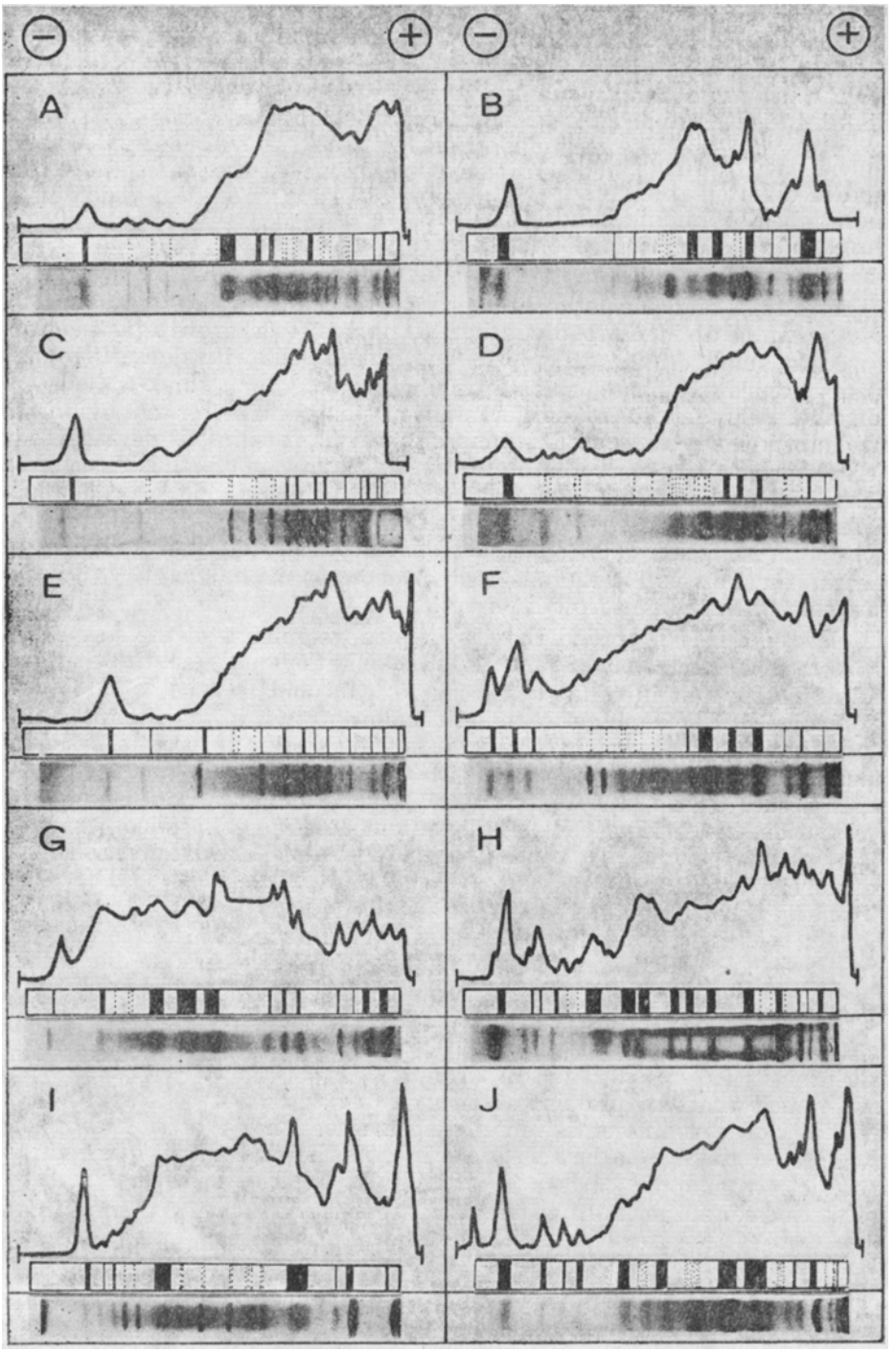

Fig. 2. Changes in protein pattern starting from first passage (A) to tenth passage (J) of culture.

showed maximum scanning peak for the more intense and thick bands (Fig. 2A—J). Some protein bands appear more or less consistently in serially successive culture of tissue and other bands vary in number, position and relative intensity of bands. The consistent appearance of such protein bands indicates that these proteins are either non metabolic or storage. The Rf value of each protein was also measured to confirm the position of a particular band in different passages. It was also found that there was an increase in the number of anionic bands from the 7th passage onwards (Fig. 2G—J). From the seventh passage onwards again cellular differentiation or xylogenesis started in the tissue (Fig. 1C).

The growth and developmental process of callus tissue up to the tenth passage can be divided broadly into three phases — (a) growth phase characterized by small, friable, newly dividing, round cells with dense cytoplasm, (b) developmental phase characterised by slow growth thick-walled, more elongated cells and (c) cytodifferentiation phase characterised by the formation of xylem vessels within comparatively hard callus tissue. There is an increase in quantity of protein up to the sixth passage of callus tissue grown in artificial media containing hormones during which period the cells remain in phases of growth and elongation. After the optimum growth and elongation there is an initiation of xylogenesis from mitotically blocked and elongated cells. During this period there is a slight decrease in the quantity of protein from that of the earlier growth phases.

It has also been recorded that age and development of the tissue is associated with alteration of not only the quantity but also the quality of protein component in callus tissue (HUFFAKER and PETERSON 1974). Polyacrylamide gel electrophoretic studies of callus protein during different growth phases indicate that the dynamic state of fluctuation in protein bands and quantity of protein are associated with the cellular development within the callus tissue. GAHAN and MAPLE (1966) suggested that during xylem differentiation, the autolysis of the cell content occurs as a result of the release of proteolytic enzymes from the lysosomes. On the other hand during the transformation of a living cell into a dead, empty, tracheid, the biosynthesis of some proteins (enzymes) are functionally related to the ultrastructural changes associated with lignin synthesis (WARDROP 1965). Therefore, the decrease in quantity of protein after the sixth passage may be due to autolysis of cell contents and the retardation of protein synthesis during differentiation of cells into xylem vessels. It has been observed that with the initiation of morphogenesis within the tissue, there is an increase in number and intensity of protein bands at the anionic end (Fig. 2G—J). Thus it may be assumed that the proteins at the anionic end may be responsible for initiation of cytodifferentiation of the tissue. The alteration in protein profile from passage to passage both in quantity and quality as indicated in the electrophoretic pattern is an index of the role of several metabolic processes essential for morphogenesis.

Acknowledgements

The authors are grateful to Prof. A. K. Sharma, Ghosh Professor and Programme Coordinator, Centre of Advanced Study, for providing laboratory facilities and his encouragement. The financial assistance by the Council of Scientific and Industrial Research, New Delhi, is also gratefully acknowledged.

REFERENCES

DAVIS, B. J.: Disc electrophoresis II. — Ann. N. Y. Acad. Sci. **121** : 404—427, 1964.

GAHAN, P. B., MAPLE, A. J.: The behaviour of lysosome like particles during cell differentiation. — J. exp. Bot. **17** : 151—155, 1966.

HUFFAKER, R. C., PETERSON, L. W.: Protein turnover in plants and possible means of its regulation. — Annu. Rev. Plant Physiol. **25** : 363—392, 1974.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., RANDALL, R. J.: Protein measurement with folin phenol reagent. — J. biol. Chem. **193** : 265—275, 1951.

MURASHIGE, T., SKOOG, F.: A revised medium for rapid growth and bioassays with tobacco tissue culture. — Physiol. Plant. **15** : 473—479, 1962.

SCANDALIOS, J. G.: Isoenzymes in development and differentiation. — Annu. Rev. Plant Physiol. **25** : 225—258, 1974.

SCANDALIOS, J. G., SORENSON, J. C.: Isoenzymes in plant tissue culture. — In: REINERT, J., BAJAJ, Y. P. S. (ed.): Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture. Pp. 719—730. Springer Verlag, Berlin—Heidelberg—New York 1977.

WARDROP, A. B.: Cellular differentiation in xylem. — In: COTE, W. A., JR. (ed.): Cellular Ultrastructure of Woody Plants. Pp. 61—97. Syracuse University Press, Syracuse 1965.

Fig. 1 at the end of the issue.

BOOK REVIEW

BELITZ, H.-D., GROSCH, W.: LEHRBUCH DER LEBENSMITTELCHEMIE. — Springer Verlag, Berlin—Heidelberg—New York 1982. 880 pp. Gebunden DM 124.—; *cca* US $ 49.60.

Die Autoren dieses Buches, Prof. Dr. H.-D. Belitz, Leiter des Instituts für Lebensmittelchemie der Technischen Universität München und Prof. Dr. W. Grosch, stellvertretender Direktor der Deutschen Forschungsanstalt für Lebensmittelchemie München, behandeln in ihrem Werk das Gesamtgebiet der Lebensmittelchemie. Sie konnten sich dabei auf ihre Vorlesungen an der Technischen Universität München stützen. Die physikalischen und chemischen Eigenschaften von Lebensmittelinhaltstoffen und Lebensmitteln werden ausführlich behandelt, wobei insbesondere Zusammenhänge zwischen Struktur und Eigenschaften deutlich gemacht werden. Da die Lebensmittelchemie stofflich und methodisch ein sehr weites Feld abzudecken hat, muss sie in ganz besonderem Masse den Entwicklungen in der gesamten Chemie und in den Nachbardisziplinen gegenüber offen sein, um diese Entwicklungen für ihre spezifischen Fragestellungen nutzen zu können. Das Werk unterzieht sich dieser Aufgabe mit Bravour. Den heute so besonders aktuellen Themen der Lebensmittelzusatzstoffe und Lebensmittelkontamination sind einige Kapitel gewidmet.

Das Buch kann nicht nur für Lebensmittelchemiker und Ernährungswissenschaftler, sondern auch für Pflanzenbiochemiker, Pflanzenphysiologen und Pflanzenökologen sehr nützlich sein. Es enthält 23 Kapitel, die in zwei thematische Bereiche — Lebensmittelinhaltstoffe und Lebensmittelgruppen — eingeteilt sind. Für Pflanzenbiologen und Pflanzenchemiker sind folgende Kapitel besonders wichtig: Wasser — Aminosäuren, Peptide, Proteine — Enzyme — Lipide — Kohlenhydrate — Aromatstoffe — Vitamine — Mineralstoffe — Zusatzstoffe — Kontamination von Lebensmittels — Getreide und Getreideprodukte — Hülsenfrüchte — Gemüse und Gemüseprodukte — Obst und Obstprodukte — Zucker, Zuckeralkohole und Honig — Kaffee, Tee, Kakao — Gewürze.

Jedes Kapitel enthält Literaturhinweise, die zur Vertiefung des Stoffes dienen. Einen wichtigen Teil des Buches stellen die 345 Abbildungen und 458 Tabellen dar.

J. LUŠTINEC (*Praha*)

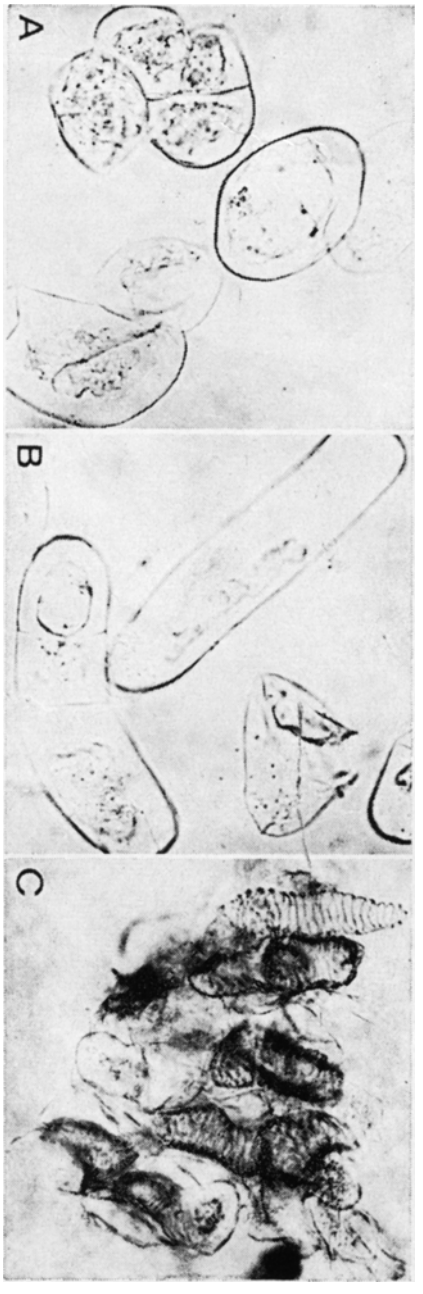

Fig. 1. Morphological changes in cells up to 3rd passage (A), from *4th—6th* passages (B) and after 8th passage (C) of culture.