TISSUE DIFFERENTIATION AND BUFFER-SOLUBLE PROTEIN PATTERNS IN WHEAT AND TRITICALE

S. BANDYOPADHYAY, J. L. DAS and P. D. GHOSH
Department of Botany, University of Kalyani, Kalyani 741 235, India.

ABSTRACT

When buffer-soluble proteins of normal leaf, leaf derived callus and redifferentiated callus tissues of comparable ages of wheat and triticale were fractionated using the gel electrophoretic technique, remarkable variations in the number of protein bands, their position and intensities were observed. When the leaf tissues redifferentiated into callus in the presence of 2, 4-dichlorophenoxyacetic acid (2, 4-D), while the total number of bands decreased, some new characteristic bands appeared. The latter, however, disappeared with the appearance of some new characteristic bands when the calli were redifferentiated into plantlets in the absence of 2,4-D. The omission of 2,4-D also resulted in the 'reappearance' of some bands originally found in the leaf tissues showing a tendency towards 'restoration of normalcy' in the redifferentiated tissues. It is therefore evident that 2,4-D induces morphogenetic changes through its effect on gene regulation. The persistence of a single band, though differing in R_m values, in all the three types of tissues in both the materials studied was also observed.

INTRODUCTION

THE recent advancement in tissue culture technique has opened up new vistas facilitating the study of tissue differentiation *in vitro* of various plant species. This has resulted in considerable progress in the study of various biochemical changes associated with cellular differentiation. Separation of proteins or multiple molecular forms of enzymes (i.e. isozymes), by polyacrylamide gel electrophoresis has emerged as a tool for providing valuable information relating to problems central to modern biological thoughts, such as tissue differentiation[1]. In the present paper buffer-soluble protein patterns obtained through gel electrophoresis of leaf, leaf callus and redifferentiated callus tissues of two cereal species (wheat and triticale) have been discussed.

MATERIALS AND METHODS

Tissue culture: Common bread wheat (*T. aestivum* L.) cv. sonalika and triticale (x *Triticosecale* Wittmack) var. Rosner were used as source materials. Callus cultures from leaf tissues were induced by culturing young primary leaf segments (4–6 mm) on MS[2] nutrient agar medium containing 3% sucrose. The media were supplemented with 2, 4-D at 2 mg/l. Redifferentiation of callus tissues into plantlets were obtained by transferring the calli to the same medium devoid of any growth regulator (i.e. 2, 4-D). The mode of regeneration was found via somatic embryoid formation in the case of wheat and via organogenetic pathway in the case of triticale.

Extraction of proteins: Tissues weighing about 10 g were homogenized in a pre-chilled mortar and pestle in an ice tub with 0.2 M sodium phosphate buffer (pH 6.8). The homogenate was squeezed through four layers of cheese cloth and the filtrate centrifuged in a ultracentrifuge (Beckman L5–50B) at 20,000 *g* for 20 min at 4°C. The supernatant was brought to 80% saturation with $(NH_4)_2SO_4$ and kept overnight at 4°C. The precipitate was collected by centrifugation at 10,000 *g* for 20 min and was suspended in 1 ml of 0.2 M phosphate buffer, dialyzed against 0.02 M phosphate buffer (pH 6.8) with repeated changes for 24 hr at 4°C. The dialysate was then centrifuged at 10,000 *g* for 30 min and the clear supernatant served as the protein preparation.

Gel electrophoresis of proteins: Fractionation of proteins by polyacrylamide gel electrophoresis (PAGE) was achieved following the method of Davis[3] and protein was estimated according to Lowry *et al*[4]. About 150–200 μg of protein was applied on each gel tube. Electrophoresis was carried out at 300 volts with 4 mA current per tube using bromophenol blue as the marker dye and trisglycine (pH 8.3) as the trough buffer. The gel sticks were stained with 0.1% amido black (E. Merck) and destained with 7% acetic acid. The R_m (E_f) values were calculated as the ratio of distance travelled by a particular protein band and the distance travelled by the tracking dye.

RESULTS

Marked changes in the buffer-soluble protein patterns in wheat (*T. aestivum* cv. sonalika) and triticale leaf, leaf callus and redifferentiated callus tissues of comparable ages were revealed by polyacrylamide gel electrophoretic studies. In normal wheat leaf tissues, a total of 11 bands were detected, distributed throughout the gel stick (figure 1a). In the case of callus tissue, the number of bands decreased to 5, of which 4 bands appeared new as revealed by their R_m values (figure 1b, table 1). These bands were characteristic of the tissue and have been termed as 'unique bands' while in the redifferentiated callus tissue the number of bands further decreased to 4. Here only one band (D) was found to be completely new, whereas two other bands (G and K) which were initially found in the leaf tissues 'reappeared' (figure 1c). The disappearance of bands was mainly noted at the anodic end in both normal and redifferentiated callus tissues. Only one band (I) was present in all the three types of tissues studied.

As compared to wheat, in the case of triticale some interesting deviations were observed so far as the buffer-soluble protein patterns were concerned. The total number of bands was 9, 8 and 6 in the leaf, normal and redifferentiated callus tissues respectively, varying greatly in their distribution pattern and intensity (figure 2a–c, table 2). Interestingly, a total of 7 and 4 bands were unique in normal and redifferentiated callus tissues respectively. Unlike wheat, the protein bands were distributed throughout the gel stick in the case of both undifferentiated and redifferentiated callus tissues and the decrease in the number of bands was also not very pronounced. Here again one band (C′) at the cathodic end continued to be present in all the three types of tissues studied. No highly anodic bands could, however, be detected in redifferentiated tissues of both wheat and triticale.

Table 1 *R_m values of buffer-soluble proteins of normal leaf, undifferentiated and differentiated callus tissues of wheat*

	Types of tissues		
Band Nos	Normal leaf (5)**	Callus (30)**	Redifferentiated callus (60)**
A	0.02	–	–
B	–	0.03*	–
C	0.12	–	–
D	–	–	0.14*
E	–	0.18*	–
F	–	0.20*	–
G	0.25	–	0.25
H	–	0.27*	–
I	0.37	0.37	0.37
J	0.40	–	–
K	0.56	–	0.56
L	0.64	–	–
M	0.67	–	–
N	0.76	–	–
O	0.83	–	–
P	0.92	–	–

*Unique bands; **Age of tissues in days.

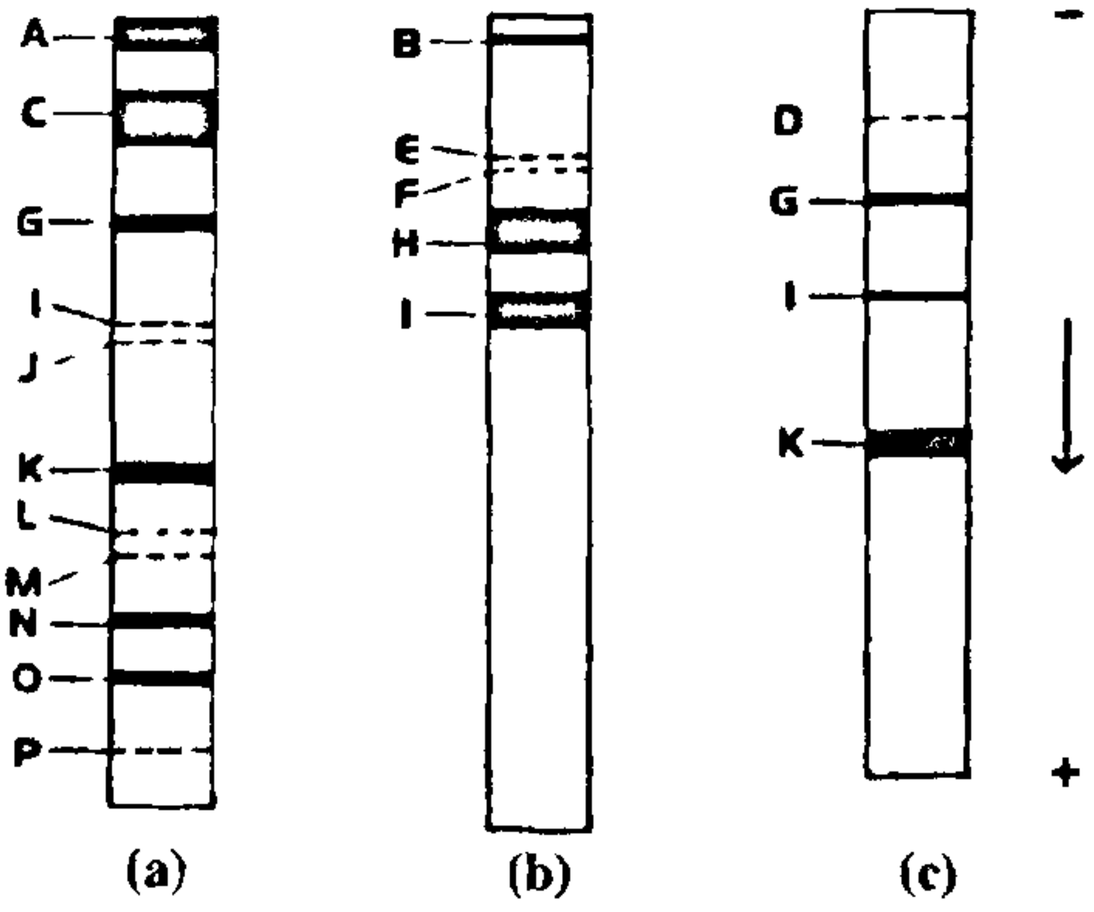

Figure 1. Diagrammatic zymograms of buffer-soluble protein profiles of leaf (**a**), undifferentiated callus (**b**), and redifferentiated callus tissues (**c**) of wheat.

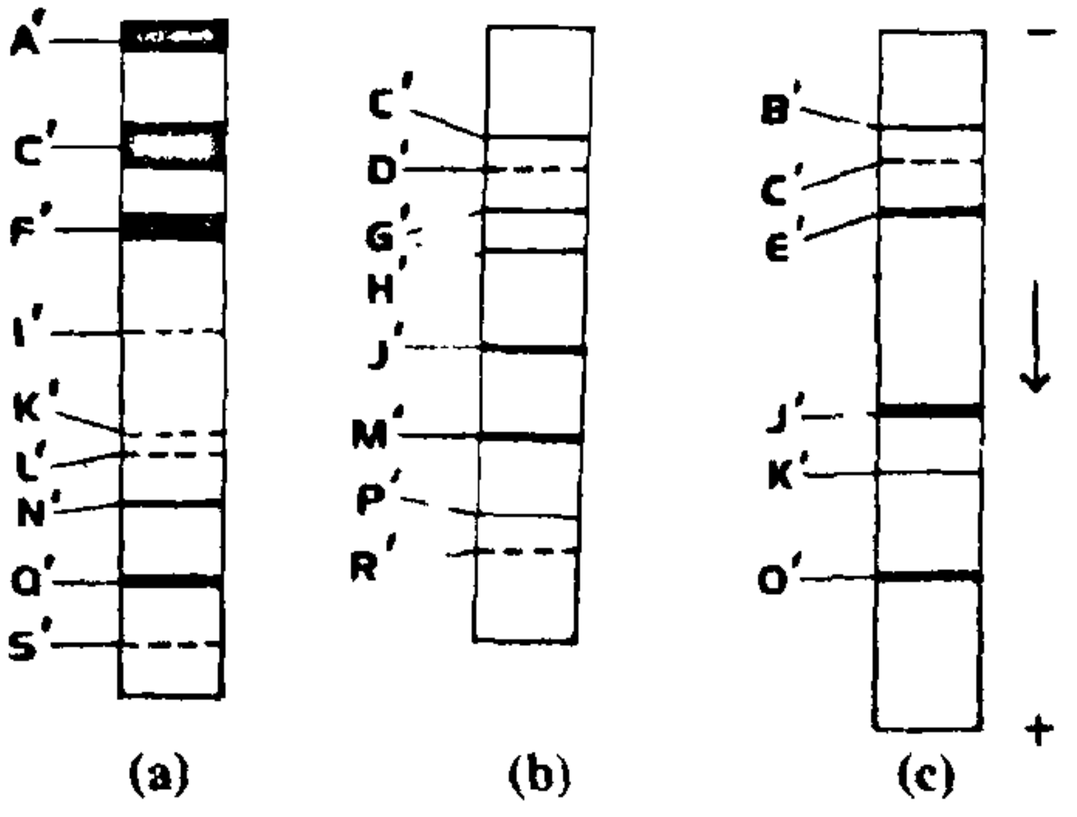

Figure 2. Diagrammatic zymograms of buffer-soluble protein profiles of leaf (**a**), undifferentiated callus (**b**), and redifferentiated callus tissues (**c**) of triticale.

Table 2 *R_m values of buffer-soluble proteins of normal leaf, undifferentiated and differentiated callus tissues of triticale*

	Types of tissues		
Band Nos	Normal leaf (5)**	Callus (30)**	Redifferentiated callus (60)**
A'	0.02	–	–
B'	–	–	0.15*
C'	0.18	0.18	0.18
D'	–	0.23*	–
E'	–	–	0.26*
F'	0.30	–	–
G'	–	0.33*	–
H'	–	0.37*	–
I'	0.46	–	–
J'	–	0.54	0.54
K'	0.62	–	0.62
L'	0.64	–	–
M'	–	0.68*	–
N'	0.71	–	–
O'	–	–	0.76*
P'	–	0.8*	–
Q'	0.83	–	–
R'	–	0.86*	–
S'	0.92	–	–

*Unique bands; **Age of tissues in days.

DISCUSSION

When the normal wheat leaf tissues redifferentiated into callus and finally redifferentiated into plantlets, in the presence and the absence of 2, 4-D, a number of anodic bands disappeared and this might be due to suppression of synthesis of particularly the smaller (low mol. wt.), fast moving and highly negatively charged proteins/enzymes, probably as a response to *in vitro* growth. Some protein bands also might have been present at concentrations below the detection levels. The disappearance of bands was, however, not so much spectacular in the case of triticale. Such decrease in the number of buffer-soluble protein bands during tissue maturation and differentiation was also reported earlier[5]. It has been suggested that the decrease in the number of protein bands may result from a decreased synthesis, aggregation and furthermore hydrolysis of some proteins at the onset of senescence[5].

The presence and persistence of a single band at the cathodic end (band I and C' in wheat and triticale respectively) in normal leaf, normal and redifferentiated callus tissues—representing three different stages in morphogenesis, is quite unique. Though the relative mobilities of these bands were different in wheat and triticale, they may be of particular interest if characterization could be done and biological activities tested.

Variations in proteins and isozymes induced by growth regulators including 2, 4-D during callus culture have been studied in wheat and other plant species[5–10]. That 2, 4-D elicits a response at the transcriptional and translational levels has also been reported[9,11]. The present investigation reveals that a number of characteristic new bands (i.e. unique bands) appeared in the presence of 2, 4-D in the undifferentiated callus tissues. Interestingly, most of these bands disappeared while some new and characteristic bands made their appearance in the calli redifferentiated into plantlets in the absence of 2, 4-D. The reappearance of some bands in the redifferentiated tissues which were originally found in the leaf tissue exhibited a 'tendency towards restoration of normalcy', though it was not so evident in triticale as in wheat. It clearly suggests that 2, 4-D, a synthetic auxin now widely used as the trigger compound for callus induction and growth, has considerable regulatory effects on gene regulation and/or expression. Ono and Nakano[12] made similar observations and suggested that all genetic information necessary for the phenotypic expression is preserved in the genome of callus though their expression is repressed by 2, 4-D. The differences in the genomic constitution and the differential level of response to growth regulator (i.e. 2, 4-D) in wheat and triticale may count much for the variations obtained in their buffer-soluble protein patterns. The possibility of such differences being due to extreme genomic instability of triticale as already reported[13–16] may not also be ruled out.

ACKNOWLEDGEMENTS

Financial assistance from INSA, New Delhi is gratefully acknowledged. Thanks are also due to Prof. K. R. Samaddar for reviewing the manuscript.

22 January 1987; Revised 14 May 1987

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ANNOUNCEMENTS

RHEUMATOID ARTHRITIS — AN UPDATE ON MECHANISMS, MANAGEMENT AND FUTURE DRUG DEVELOPMENT

(22nd/23rd February 1988, Regent Crest Hotel, London)

Rheumatoid arthritis and related inflammatory diseases afflict approximately 5% of the population and are associated with considerable morbidity. However, the mechanisms underlying this disease are poorly understood and our current treatment of such diseases is far from adequate. This meeting will discuss our current understanding of rheumatoid arthritis, its current management and the major areas of basic and clinical research that may hold the key to our future understanding and treatment. The conference should provide a valuable overview of both clinical and basic aspects of this important disease and will be of interest to health professionals, clinicians, scientists and others interested in any aspect of its aetiology and treatment. The meeting will also be of interest to individuals involved in the development of novel therapeutic agents for the future treatment of this and related inflammatory diseases.

For details please contact: Miss Penny Robinson, IBC Technical Services Ltd., Bath House, 56 Holborn Viaduct, London EC1A 2EX.

NEW TREATMENTS FOR ASTHMA

(21st/22nd March 1988, Royal Society of Medicine, London)

Asthma affects over 5% of the population and is becoming more prevalent and more severe, with an increasing mortality, despite an increase in anti-asthma drugs prescribed. There is now a great need for the advancement of new drugs and new approaches to therapy. The aim of this conference is to discuss future developments in our understanding of the mechanisms of this increasingly common disease.

For details please contact: Miss Penny Robinson, IBC Technical Services Ltd., Bath House, 56 Holborn Viaduct, London EC1A 2EX.