

different distances could be expected to yield interesting results.

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APPENDIX

For the evaluation configuration interaction (CI) matrix elements, we follow the method of Pariser¹⁴. In the case of naphthalene excimer, the CI matrix is a 17×17 determinant, while for anthracene excimer, it is 37×37 (because we have a pair of degenerate subadjacent orbitals in anthracene). The evaluation of the various inter- and intra-molecular integrals are as follows :

Integral	Over monomer	Intermolecular
Overlap	Parr and Crawford ¹⁵	Parr and Crawford ¹⁵
Coulomb/ exchange (with ZDO approximation)	Mataga and Nishimoto ¹⁶	Parr's multipole ¹⁷ expansion
core	HMO	
H_{ij}	$E_i \delta_{ij}$	$-10.0 S_{ij}$

Rest of the calculation/procedure is identical with our earlier work¹⁰.

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CHANGES IN SOLUBLE PROTEINS AND ISOENZYMES IN DEVELOPING SORGHUM GRAINS

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ABSTRACT

Electrophoretic patterns of soluble proteins and isoenzymes of peroxidase and esterase showed qualitative and quantitative differences during grain development. The increase in the intensity of protein bands with low electrophoretic mobility at mature stage suggests synthesis of proteins with higher molecular weight.

INTRODUCTION

SOLUBLE proteins are the physiologically active fractions which constitute major bulk of enzymes involved in plant metabolism. Several workers have

observed marked qualitative and quantitative changes in soluble proteins and isoenzyme patterns during grain development of wheat^{1,2}, maize³ and barley⁴. The specificity of enzyme pattern implies a role of

specific enzymes and isoenzymes in development and differentiation⁵. In the present investigation soluble protein and isoenzyme changes in sorghum have been studied during grain development.

MATERIAL AND METHOD

Sorghum (variety CSH-2) was grown at I.A.R.I. farm. The cobs were harvested at 10, 17, 24 and 31 days (mature) after ear emergence. At 10 days, seeds were taken along with husk.

For proteins and esterase extraction, fresh seeds were hand ground at 0–4° in a chilled pestle-mortar with 50 mM Tris-Cl buffer [(pH 7.6) containing 5 mM 2-mercaptoethanol]. For the extraction of peroxidase 2-mercaptoethanol was omitted. The extracted material was centrifuged at $10,000 \times g$ for 20 min. An aliquot of the supernatant (200–250 µg protein) was layered on polyacrylamide gel (10%) and electrophoresis was done using cationic system⁶ for soluble proteins. Isoenzymes were separated by anionic system^{7,8} using 7% gel. The gels were stained for protein in 0.1% amido black and destained by diffusion in 7% acetic acid.

Detection of esterase isoenzymes.—Gels were incubated in 50 mM phosphate buffer (pH 6.0) containing 1 ml of 1% α -naphthyl acetate in 60% acetone and 25 mg fast blue RR at room temperature for 10–30 min.

Detection of peroxidase isoenzymes.—Gels were first incubated for 30 min in the reaction mixture containing 0.5% O-dianisidine-HCl, 1 ml; 0.6 M NaOAc buffer, 3 ml; and H₂O₂, 26 ml. Then the gels were incubated in 0.1 M H₂O₂ until the visible bands developed. The gels were preserved in 7% HOAc.

A control gel for each enzyme was incubated in the mixture without substrate. In such controls no bands were observed. At least two independent extractions were made. The R_f of each band with respect to front formed by the tracking dye was calculated. Densitometer tracings of the gels were obtained on a Joyce-Loebl chromoscan.

RESULTS AND DISCUSSION

Soluble proteins

The soluble protein pattern from the seeds at different stages of grain development is shown in Fig. 1. The number of bands decreased with maturity. Band at R_f 0.04 was present at 17 days stage, while R_f 0.11 band was present only in mature grains. Bands with R_f 0.09 and 0.22 were present only at 10 days stage.

Bands at R_f 0.13, 0.17, 0.25, 0.46, 0.48, 0.57, 0.63, 0.69, 0.74, 0.80, 0.85, 0.91 and 0.95 were common to all stages of grain development. With

the development of the grain, the bands with medium electrophoretic mobility decreased while bands with high electrophoretic mobility showed a slight increase.

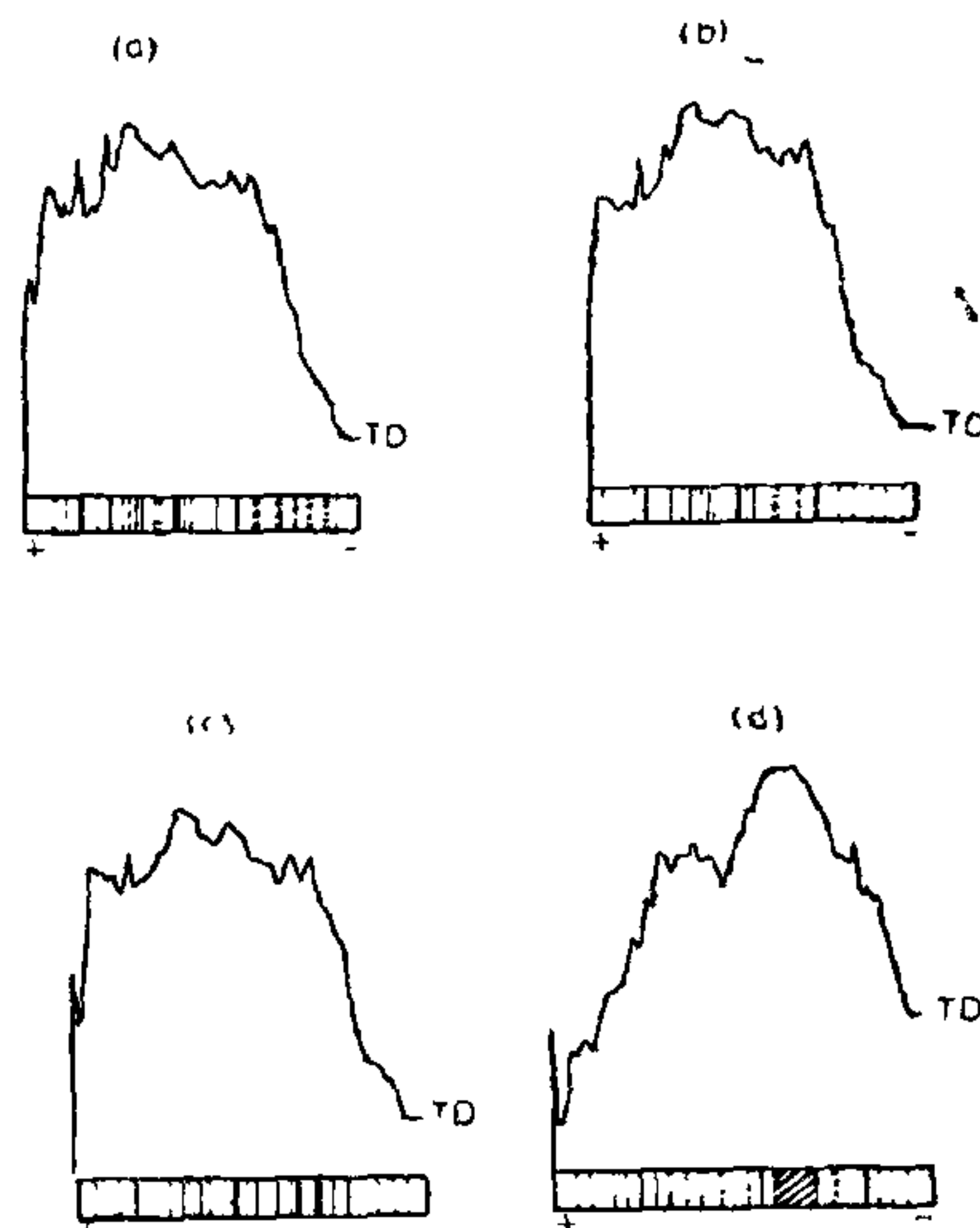


FIG. 1. Soluble protein pattern at (a) 10 days, (b) 17 days, (c) 24 days and (d) 31 days.

Increase in the intensity of low electrophoretic mobility bands was observed at mature stage. This may be due to the synthesis of new high molecular weight protein and/or anionic proteins. It is more likely that new proteins of high molecular weight are synthesized during later stages of maturity. Disappearance of bands with medium electrophoretic mobility (R_f 0.31, 0.33, 0.39 and 0.51) during grain development suggests that these proteins represent metabolic proteins rather than storage proteins. Lodha *et al.*⁹ also observed a decrease in the number of bands during grain development in normal and opaque-2 maize. Many of the bands at all stages of development were common, thereby suggesting that they are stable proteins and may be involved in storage protein biosynthesis.

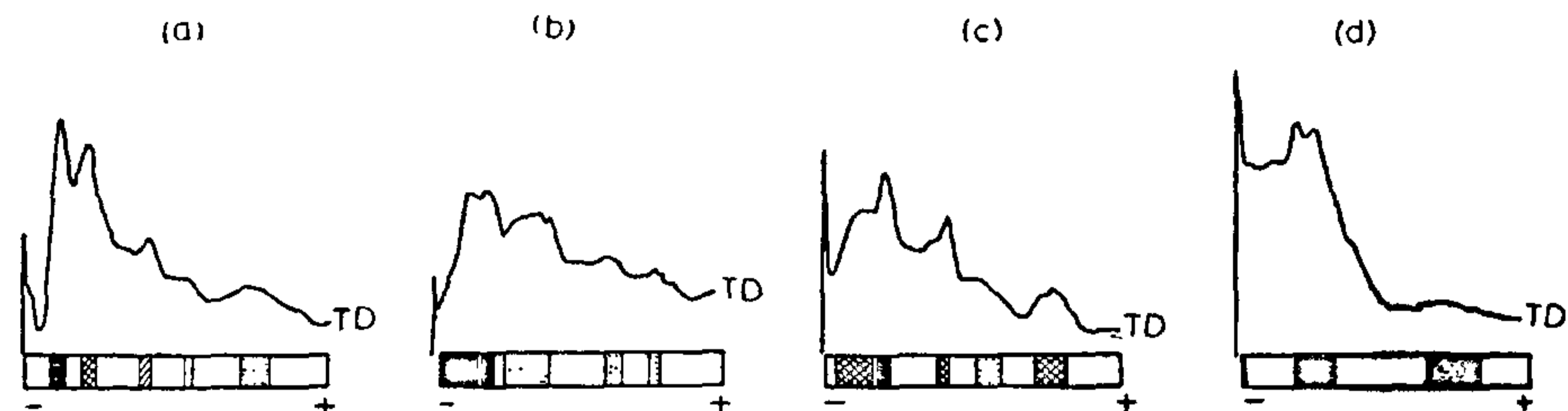
Esterase.—The isoenzyme patterns are shown in Fig. 2. The number of esterase isoenzymes were found to be 7 at 10 days stage and 5 at 31 days stage. The bands at R_f 0.00, 0.45, 0.76 and 0.88 were common to all stages, while band at R_f 0.13 and 0.59 present at 10 and 17 days stage disappeared at later stages and instead the band at R_f 0.18 appeared anew during later stages of maturity.

Peroxidase

Electrophoretic patterns of peroxidase from grains are shown in Fig. 2. Five bands were present at 10, 17 and 24 days stage. The intensities

the plants under certain circumstances. Sorghum grains contain tannins which bind with proteins. The effect of peroxidase on tannin contents is not clear but it is likely that these enzymes can change

PEROXIDASE



ESTERASE

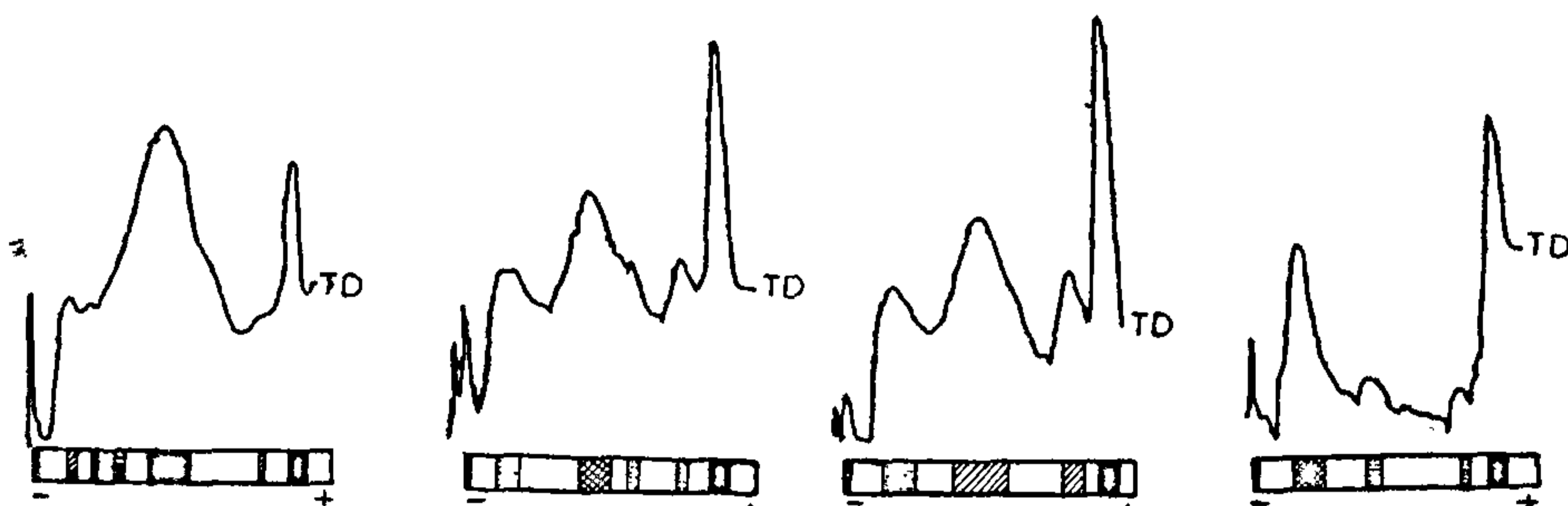


FIG. 2. Peroxidase and esterase isoenzyme pattern at (a) 10 days, (b) 17 days, (c) 24 days and (d) 31 days.

of these bands varied considerably. The bands with R_f 0.39 and 0.76 were lighter at 10 and 17 day stage compared to 24 day stage. The bands with R_f 0.12, 0.39 and 0.55, present at earlier stages, disappeared at 31 days, while bands at R_f 0.00 and 0.27 appeared anew. A total of only four bands were present in mature grains. Disappearance of existing bands and appearance of new bands suggest the shifts of specific isoenzymes¹⁰. The number of peroxidase bands present in sorghum grain are lesser than the number of bands present in maize⁹.

The changes observed in peroxidase during grain development may be due to differential gene activity or due to different cellular environment. The exact physiological role of peroxidase in plant is not known. One of the suggested roles of these enzymes include oxidation of certain toxic phenols and amines which may be deleterious to

the properties of phenolic compounds by oxidation, which might affect protein-tannin complexes.

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