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CHANGES IN ISOENZYMES IN EMBRYO AND ENDOSPERM OF NORMAL AND OPAQUE-2 *ZEA MAYS* DURING IMBIBITION

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Key Word Index—*Zea mays*; Gramineae; isoenzymes; normal and opaque maize; changes on imbibition.

Abstract—Electrophoretic patterns of soluble proteins, peroxidase, esterase, alcohol dehydrogenase (ADH) and glutamic dehydrogenase (GDH) from embryos and endosperm of normal and opaque-2 maize were studied after different periods of imbibition. The soluble protein pattern from endosperm of normal and opaque-2 differed both qualitatively as well as quantitatively. The embryo protein patterns were identical. Multiple forms (isoenzymes) were found for all the enzymes studied. The enzyme patterns changed during imbibition. Peroxidase and GDH patterns from embryos of normal and opaque-2 showed considerable differences during imbibition. Esterase and ADH pattern from embryo and endosperm of normal and opaque-2 showed few differences.

INTRODUCTION

ISOENZYMES can be used as markers in studying developmental processes. The gap between gene (DNA) and gene product (protein) is relatively small in comparison to secondary morphological characters which are also subject to more genetic-environment interplay in their expression. Ever since marked increases in lysine and tryptophan content in the presence of a simple recessive gene opaque-2 were found by Mertz *et al.*[1] attention has been paid to the biochemical differences in opaque-2 and normal maize. The higher lysine and tryptophan content in opaque-2 compared to normal maize is a direct consequence of depressed zein synthesis in opaque-2 compared to normal.[1,2] Recently Sodek and Wilson[3] studied the lysine incorporation and Mehta *et al.*[4] the nucleic acid and protein metabolism and observed considerable differences between opaque-2 and normal. The present study deals with soluble protein and isoenzyme differences in embryos and endosperm of normal maize and the opaque-2 counterpart after different periods of imbibition.

RESULTS

Soluble Proteins

The changes in soluble protein spectrum of endosperm and embryo of normal and opaque-2 maize during imbibition are shown in Figs. 1 and 2. The proteins of normal and opaque-2 embryos showed almost identical patterns whereas the endosperm proteins showed considerable qualitative and quantitative differences. During imbibition in the embryo, protein bands with low electrophoretic mobility were replaced by new bands with higher electrophoretic mobility. In contrast, in the endosperm low mobility protein bands disappeared, the rate of disappearance being slower in opaque-2 endosperm.

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Peroxidase

Electrophoretic patterns of peroxidase from embryos and endosperm of normal and opaque-2 at 0, 48 and 72 hr imbibition are shown in Fig. 3. The number of peroxidase isoenzymes increase with increase in length of imbibition period. Normal and opaque-2 embryos behaved similarly with respect of number of isoenzymes; however, the intensity of the fast-running bands (R_f, 0·63 and 0·69) in opaque-2 was greater than in normal maize at 72 hr imbibition, and there were marked differences in the isoenzyme patterns after 72 hr imbibition. The bands of high electrophoretic mobility (R_f 0·63 and 0·69) which were observed in embryos were absent in endosperm. The change in peroxidase pattern in endosperm of normal and opaque-2 maize was more or less similar during imbibition.

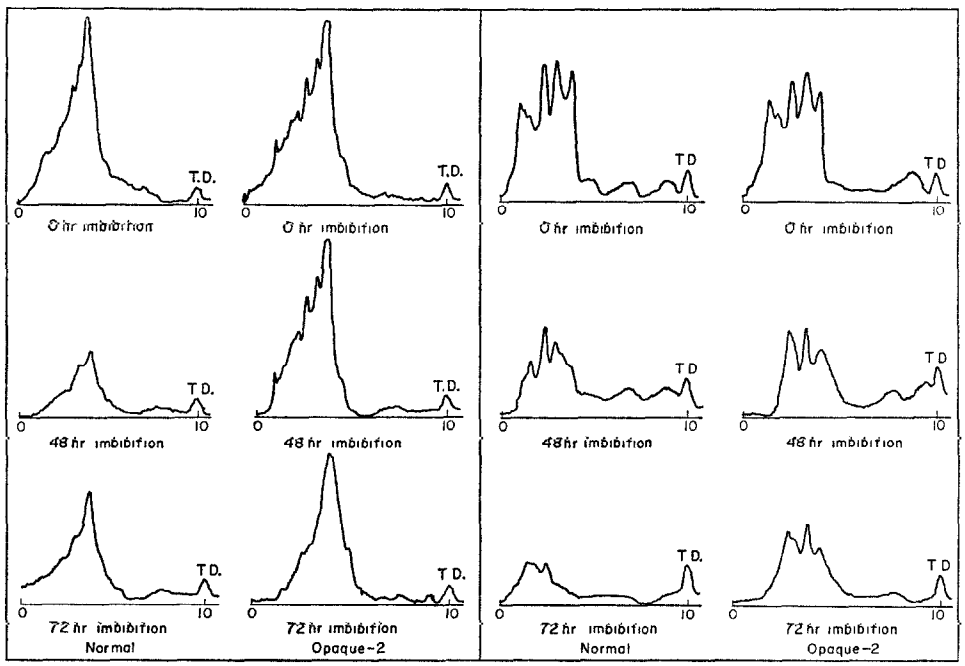

FIG. 1. DENSITOGRAMS OF PROTEIN PATTERNS IN ENDOSPERM OF NORMAL AND OPAQUE-2 MAIZE DURING IMBIBITION.

FIG. 2. DENSITOGRAMS OF PROTEIN PATTERNS IN EMBRYOS OF NORMAL AND OPAQUE-2 MAIZE DURING IMBIBITION.

Esterases

The esterase pattern of normal and opaque-2 maize embryos were found to be similar (Fig. 4). During imbibition the intensity of all the bands except the fast-running band decreased in normal maize whereas in opaque-2 embryo there was increase in the intensity of bands at 72 hr imbibition. The esterase pattern in the endosperm also was similar in normal and opaque-2 maize except for minor bands (Fig. 4). The intensity of the fast band with R_f 0·84 increased with imbibition period in opaque-2 maize endosperm.

Alcohol Dehydrogenase

Three enzyme bands with R_f 0·35, 0·36 and 0·40 were observed in normal and two with R_f 0·40 and 0·42 in opaque-2 embryo from dry seed (Table 1). After 48 hr the activity of

the ADH decreased and bands having R_f 0·35 and 0·36 in normal and R_f 0·42 in opaque-2 disappeared at 72 hr imbibition. The ADH band with R_f 0·40 common in normal and opaque-2 embryos was less intense. The extracts from endosperm did not show any ADH activity.

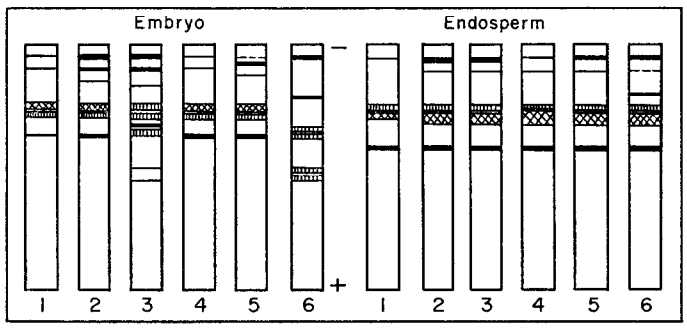

FIG. 3. PEROXIDASE ISOENZYMES IN NORMAL AND OPAQUE-2 MAIZE EMBRYOS AND ENDOSPERM.

Normal. (1) 0 hr; (2) 48 hr; (3) 72 hr; imbibition. Opaque-2. (4) 0 hr; (5) 48 hr; (6) 72 hr; imbibition

Glutamate Dehydrogenase

Glutamate dehydrogenase patterns from normal and opaque-2 embryos showed considerable differences (Table 2). The number of enzyme bands was greater in normal compared to opaque-2 embryo from dry seed. The enzyme band with R_f 0·20 was much more intense in opaque-2 at 72 hr compared to normal .Two additional enzyme bands with R_f 0·02 and *ca.* 0·40 were present in normal embryos. GDH pattern of normal endosperm showed one band whereas two bands were found in opaque-2 (Fig. 5). The activity of GDH from endosperm was considerably lower compared to embryos. During imbibition, there was little change except for the appearance of a faint band R_f 0·33 in normal endosperm, and disappearance of band R_f 0·27 in opaque-2 endosperm at 72 hr.

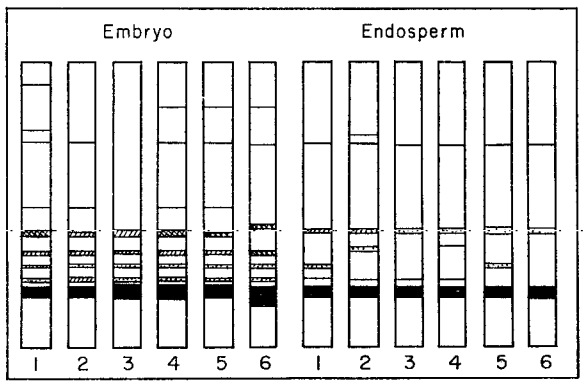

FIG. 4. ESTERASE ISOENZYMES IN EMBRYOS AND ENDOSPERM OF NORMAL AND OPAQUE-2 DURING IMBIBITION (see Fig. 3).

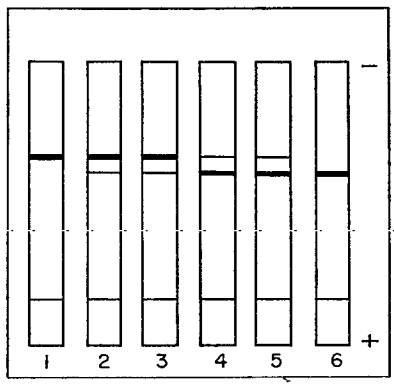

FIG. 5. GLUTAMATE DEHYDROGENASE ISOENYZMES IN NORMAL AND OPAQUE-2 ENDOSPERM DURING IMBIBITION (see Fig. 3).

DISCUSSION

Differences in the solubility of proteins of normal and opaque-2 maize endosperm have been investigated by many workers.[1,2,4] Opaque-2 maize has a considerably lower zein content than normal, but the spectra observed in the present study show considerable qualitative differences for soluble proteins from endosperm of normal and opaque-2 maize. The embryo proteins showed similar patterns. Isoenzyme patterns of peroxidase and glutamate dehydrogenase also showed qualitative or quantitative differences. No differences were observed in esterases.

TABLE 1. ALCOHOL DEHYDROGENASE ISOENZYMES IN NORMAL AND OPAQUE-2 EMBRYO DURING IMBIBITION

Imbibition period (hr)	Normal R_f	Normal Intensity	Opaque-2 R_f	Opaque-2 Intensity
0	0·35	++		
	0·36	+++		
	0 40	++++	0·40	+++
			0 42	Traces
48	0·35	++		
	0 40	+++	0·40	+++
72	0 40	++	0·40	-

During the course of imbibition considerable qualitative and quantitative differences were observed in the tissues of both varieties. The changes in the intensity of protein bands indicate that they are intimately involved in the process of germination. Similar changes in the protein spectra during early development in wheat have been observed.[5,6] The disappearance of existing bands and appearance of new band suggest shifts in activity of specific isoenzymes.[7] The changes observed in peroxidase and GDH isoenzyme pattern in embryo and endosperm of normal and opaque-2 maize during imbibition may be due to differential gene activity during early development or due to different cellular environments.

TABLE 2. GLUTAMATE DEHYDROGENASE ISOENZYMES IN NORMAL AND OPAQUE-2 EMBRYO DURING IMBIBITION

Imbibition period (hr)	Normal R_f	Normal Intensity	Opaque-2 R_f	Opaque-2 Intensity
0	0·02	+		
	0·20	++++	0 20	+++
	0·29	+		
	0·37	++	0·37	Traces
48	0 02	++		
	0·20	+++	0·21	+++
	0 28	+++	0·29	+
	0·38	+	0·38	+
72	0·02	+		
	0·21	++	0·20	++++ +
	0·29	+	0·29	+
	0 38	+++		
	0·40	+		

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The exact physiological role of peroxidase in plants is obscure. It is believed that the interaction between growth hormone IAA and peroxidase may be important in growth regulation.[8] Thus appearance of new bands showing peroxidase activity in embryo extracts following imbibition are of great interest.

The presence of esterase and ADH isoenzymes has also been reported in wheat[6,9] and maize.[10,11] The enzyme patterns of esterase and ADH were more or less similar in the embryos and endosperm of normal and opaque-2 maize but differences were observed during imbibition.

EXPERIMENTAL

The present investigations were carried out with *Zea mays* L. "vijay" (normal) and the opaque-2 cultivar "Rattan". The opaque-2 line was derived following five back crosses. Complete opaque kernels were used to represent the opaque line. Kernels, sterilized with 0·1% $HgCl_2$ for 1–2 min were washed with H_2O and allowed to germinate at 30° in Petri dishes lined with wet filter paper. At different imbibition periods, embryos and endosperm were separated.

Extraction of soluble proteins. Proteins were extracted by hand grinding in a chilled pestle and mortar with 0·05 M acetate buffer (pH 4·5) containing 6 mM β-mercaptoethanol. GDH, ADH, peroxidase, and esterase, were extracted in 0·05 M Tris- HCl buffer (pH 7·4) containing 6 mM β-mercaptoethanol and 5 mM EDTA. All the operations were carried out at 4°. The suspension was centrifuged at 20 000 *g* for 10 min, and the supernatant used for gel electrophoresis.

Separation of proteins. Polyacrylamide gel electrophoresis was used to separate soluble proteins and various isoenzymes. For soluble proteins, a cationic system[12] was used while for the enzymes an anionic system[13,14] was used. Samples containing 200–225 μg protein were layered above the spacer gel. Electrophoresis was at 4° at 3·0 mA current per gel column. Proteins gels were stained in 0·1% amido black in 7% acetic acid for 30 min and destained by diffusing out excess stain in 7% acetic acid. For peroxidase, a saturated solution of benzidine in 25% HOAc was mixed with an equal amount of 1% H_2O_2 and gels incubated at room temp. for 2 min. and photographed immediately. Esterases were detected by incubating gels in 50 ml phosphate buffer (0·05 M, pH 6·0) containing 1 ml of 1% α-naphthylacetate in 60% acetone and 25 mg Fast Blue RR at room temp. for 10–30 min. For ADH gels were incubated at 37° for 20 min in a reaction mixture containing Tris-0·05 M, EtOH, 0·05 ml, Phenazine methosulphate (PMS), 1·0 mg; Nitro-bluetetrazolium (NBT), 10·0 mg; NAD, 40 mg; NaCN (0·002 M), 2·0 ml in a final vol. of 20 ml at pH 8·0.

For GDH, gels were incubated at 37° until visible bands developed in the reaction mixture containing sodium glutamate, 100 mg; NAD, 12·0 mg; NBT, 10·0 mg; PMS, 1·0 mg; $MgCl_2$, 8·0; 0·05 M phosphate buffer to 20 ml vol. and pH 6·7. A control gel for each enzyme was incubated in the mixture without substrate. For dehydrogenases two control gels were incubated one in reaction mixture omitting NAD and the other one without the substrate. In such controls no bands were observed. At least two independent extractions were made for all the material examined. For each group of enzymes triplicate runs were made. The relative migration (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. The photographs were taken using a Phoretophot Assembly obtained from Canalco, U.S.A.

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