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CHANGES IN SOLUBLE PROTEIN AND ISOENZYMES IN NORMAL AND OPAQUE-2 *ZEA MAYS* ENDOSPERM DURING GRAIN DEVELOPMENT

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Abstract—Electrophoretic patterns of soluble proteins, pH 5 enzyme fraction, peroxidase, glutamic dehydrogenase, leucine aminopeptidase in developing endosperm of normal and opaque-2 were studied. Multiple forms were found for all the enzymes studied. The GDH pattern showed considerable differences in normal and opaque-2 maize; the soluble protein pattern also differed, both qualitatively and quantitatively. The leucine-aminopeptidase pattern was identical and the peroxidase pattern showed slight differences.

INTRODUCTION

DEPRESSED zein synthesis in opaque-2 is mainly responsible for higher lysine and tryptophan content compared to normal maize.[1–4] Diverse metabolic changes brought about by the opaque-2 single recessive gene include higher ribonuclease activity,[2,5,6] altered nucleic acid and protein metabolism[7] and leucine and lysine incorporation *in vivo*[8] and *in vitro*[9,10] during grain development. In addition, differences in soluble protein and isoenzymes[11] and acetate metabolism[12] during seed germination in normal and opaque-2 maize have been observed. In the present investigation, earlier studies[7,10,11] have been extended to the soluble protein and isoenzyme differences in endosperm of normal and its opaque-2 counterpart during grain development.

RESULTS

Soluble proteins

The changes in soluble protein spectrum of endosperm of normal and opaque-2 maize during grain development are shown in Fig. 1. In general the protein spectrum was found

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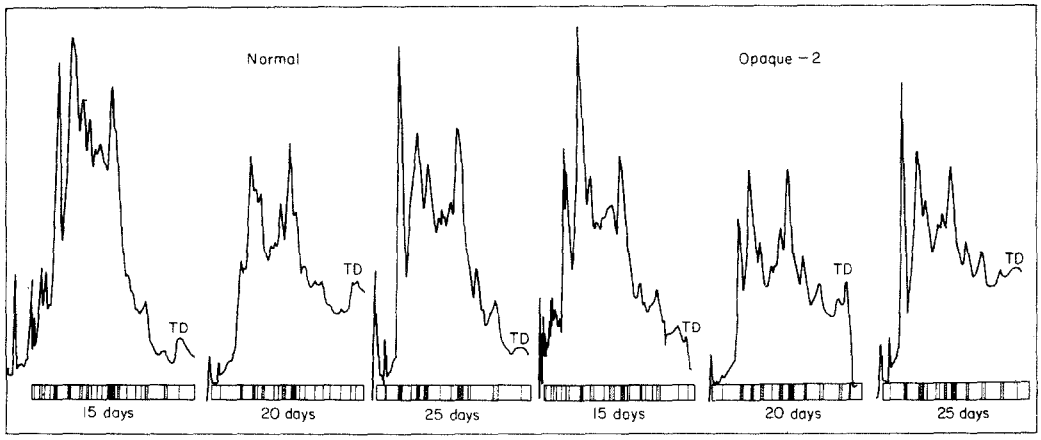

FIG. 1. DENSITOGRAMS OF SOLUBLE PROTEIN PATTERN IN NORMAL AND OPAQUE-2 ENDOSPERM AT DIFFERENT DAYS AFTER POLLINATION.

to be similar, except for bands with R_p 0·32 and 0·47 which were absent in opaque-2 and a band with R_p 0·80 which was absent in normal. During grain development the protein pattern changed considerably. The bands with low electrophoretic mobility ($R_p < 0·20$) disappeared 20–25 days after pollination. There was also a change in the relative intensity of medium and high electrophoretic mobility bands. The number of bands decreased with grain development. At 15 days, 20 and 19 bands were present and at 20 days post-pollination 16 and 11 bands were present in normal and opaque-2 respectively. At 25 days post-pollination, the number of bands decreased to 12 in normal whereas in opaque-2 it remained at 11.

pH 5 *enzyme pattern*

Electrophoresis of pH 5 enzyme fraction from normal and opaque-2 endosperm was done. The R_p values of different bands along with their intensities are shown in Table 1. In general the electrophoretic pattern was similar in normal and opaque-2, except for the bands with R_p 0·08 and 0·17 which were absent in opaque-2 at 15 days post-pollination. The bands at 0·27 and 0·29 were most intense. The band at 0·37 appeared *de novo* in both normal and opaque-2 at 25 days post-pollination.

TABLE 1. ELECTROPHORETIC PATTERN OF pH 5 ENZYME FRACTION FROM NORMAL AND OPAQUE-2 ENDOSPERM OF *Zea mays*

	Days after pollination, intensity					
	15		20		25	
R_p	Normal	Opaque-2	Normal	Opaque-2	Normal	Opaque-2
0·01	+ + + +	+ + + +	—	+ + +	+	+
0·08	+	—	+	+	+	+
0·17	+	—	+ + + +	+ + + +	+ +	+ +
0·27	+ + +	+ + +	+ + + +	+ + + +	+ + +	+ + + +
0·29	+ +	+ +	+ + +	+	+ + +	+
0·37	—	—	—		+ +	+

Peroxidase

Electrophoretic patterns of peroxidase from the endosperm of normal and opaque-2 at 15, 20 and 25 days post-pollination are shown in Fig. 2. The isoenzyme pattern was more or less similar in normal and opaque-2 endosperm at all the stages, except for a band at R_p 0·14 which was absent at 15 and 20 days post-pollination and for another band at R_p 0·21 which was absent at 20 and 25 days post-pollination in normal endosperm. A total of seven bands were present in normal and six bands were present in opaque-2 endosperm at 15 days post-pollination. During grain development the number of peroxidase bands in normal endosperm decreased while in opaque-2 endosperm it remained the same. The band with R_p 0·68 had maximum intensity in both normal and opaque-2 at all the stages.

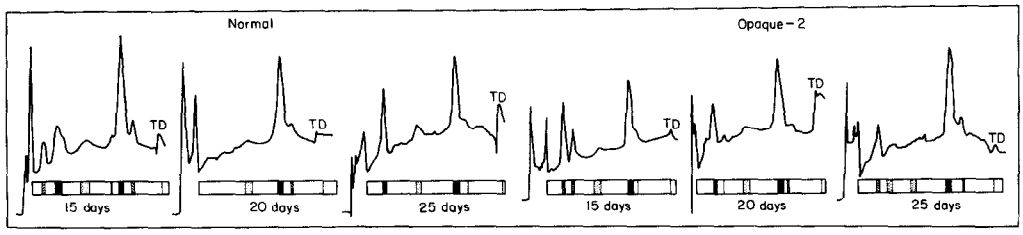

FIG. 2. DENSITOGRAMS OF PEROXIDASE ISOENZYMES IN NORMAL AND OPAQUE-2 ENDOSPERM AT DIFFERENT DAYS AFTER POLLINATION.

Leucine-amino peptidase

The isoenzyme pattern was similar in both normal and opaque-2 endosperm at all the stages examined. Only two bands with R_p 0·44 and 0·53 were present; of these, the second was the most intense. No changes were observed during grain development.

Glutamate dehydrogenase

The glutamate dehydrogenase patterns from normal and opaque-2 endosperm showed considerable differences during grain development (Table 2). Though the number of isoenzymes were similar, there were qualitative differences. The enzyme band at R_p 0·32 was intense in normal endosperm and this band was absent in opaque-2 endosperm. Instead a band with R_p 0·37 was very intense in opaque-2. This band was missing in normal endosperm at 15 and 20 days post-pollination but appeared at 25 days post-pollination. The

TABLE 2. GLUTAMATE DEHYDROGENASE ISOENZYMES IN DEVELOPING NORMAL AND OPAQUE-2 ENDOSPERM OF *Zea mays*

	Days after pollination, intensity					
	15		20		25	
R_p	Normal	Opaque-2	Normal	Opaque-2	Normal	Opaque-2
0·18	+	—	++	—	—	—
0·20	+	—	++	—	+	—
0·22	—	+	+	+	+	+
0·24	—	+	—	+	—	+
0·25	—	+	—	+	—	+
0·27	+++	+++	+	+++	Faint	+++
0·32	+++++	—	+++	—	+++	—
0·34	++	—	—	—	—	—
0·37	—	+++++	—	+++++	+++++	+++++

bands at R_p 0·22, 0·24 and 0·25 in opaque-2 and at 0·18, 0·20 and 0·22 in normal were very sharp and narrow. The band at 0·27 was intense during early endosperm development in normal.

DISCUSSION

Ever since the discovery in maize of opaque-2, a single recessive gene, by Mertz *et al.*,[1] diverse metabolic changes have been noticed in the endosperm of this plant. Differences in proteins of normal and opaque-2 endosperm based on their solubility have been investigated by many workers[1-4] and electrophoretically by Kadam *et al.*[11] In the present isoenzyme investigation, a major difference was noticed with respect to GDH and minor differences with respect to peroxidase. During grain development the GDH pattern changed considerably. Characteristic differences in GDH isoenzyme pattern in normal and opaque-2 during germination have been observed by Kadam *et al.*[11] The exact physiological role of different GDH isoenzymes is not yet clear but, on the basis of the results obtained by Sodek and Wilson[8] and Nagpal and Mehta,[12] it appears likely that these may be involved in the synthesis of glutamic acid and in maintaining the glutamic acid:aspartic acid ratio. Leucine-amino peptidase and peroxidase pattern did not change much. The soluble protein pattern showed differences at early grain development from normal and opaque-2. During grain development also the protein pattern changed considerably with the disappearence of slow bands and change in relative intensity of medium and fast running bands.

EXPERIMENTAL

One of the high combining, well adapted inbred line Fla 3H 94-f available in the maize programme, and its opaque version were used for the present study. The opaque-2 version was obtained after 3 backcrosses. The opaque and normal lines were grown at the IARI, New Delhi during the monsoon season. Individual plants were self-pollinated. The self-pollinated ears were harvested at the specified dates after pollination. The harvested ears were immediately chilled, and kernels were dissected, pericarp and embryo were removed and endosperm was collected. The endosperms were ground for 5 min in mortar and pestle and for 5 sec 2 × in a Waring blender using buffer I (0·4 M sucrose; 0·1 M Tris, pH 7·8; 50 mM KCl; 10 mM $MgCl_2$; 4 mM β-mercaptoethanol) (1:2, w/v) in the presence of 0·8 mg/ml bentonite prepared according to Hadziyev *et al.*[13] The homogenate was filtered through eight layers of cheese cloth, the filtrate centrifuged for 30 min at 30 000 *g* to remove nuclei and mitochondria. The supernatant obtained was layered over 2 ml 1 M sucrose and centrifuged for 40 min at 60 000 rpm in Ti 75 rotor of Beckman L2-75B ultracentrifuge. The upper two third portion of the supernatant was collected.

Preparation of pH 5 *enzyme fraction.* pH 5 enzyme fraction was prepared and purified as described earlier.[10]

Soluble proteins. The supernatant obtained after removel of pH 5·00 enzyme fraction was adjusted to pH 7·60 and used for electrophoresis of soluble proteins and isoenzymes. All the operations, unless otherwise stated, were performed at 4°. Polyacrylamide gel electrophoresis for soluble proteins and isoenzymes was done as described by Kadam *et al.*[11]

Peroxidase. Gels were first incubated in the reaction mixture consisting of 0·5% *O*-dianisidine in 1 M HCl, 1 ml; 0·6 M NaOAc buffer, 3 ml and H_2O, 26 ml for 0·5 hr. Then the gels were incubated in 0·1 M H_2O_2 until the visible bands developed. The gels were preserved in 7% HOAc.

Glutamic dehydrogenase. Bands were developed after electrophoresis according to the method used by Kadam *et al.*[11]

L-*Leucine aminopeptidase.* Gels were incubated at 37° until visible bands developed in the reaction mixture containing L-leucyl β-naphthylamide HCl, 10 mg; Black-K salt, 20 mg; 0·2 M Tris-Maleate buffer, pH 3·3, 50 ml; 0·2 M NaOH buffer, pH > 14, 20 ml and distl. H_2O 30 ml.

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 for all materials examined. For each group of enzymes triplicate runs were made. The relative migration (R_p) 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.

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