2. Peroxidase isozymes in the developing endosperm of maize.

Zymograms of peroxidase isozymes from five different stages, i.e., 10, 15, 20, 25 and 30 day old developing endosperms, of opaque-2 mutant and normal Indian inbred maize (CM 201) were developed at pH 8.5 in polyacrylamide gel. The bands of zymograms were divided into three zones, a cathodal zone with slow mobility, a central zone with intermediate mobility, and an anodal zone with faster mobility. Bands within each zone were numbered in increasing order of mobility.

The opaque-2 and the normal differed widely in the number of peroxidase bands in the 10 day and 30 day old endosperms, but only by a single band in the 15 day, 20 day and 25 day old endosperms.

The 25 day old endosperm of opaque-2 exhibited the maximum number of twelve bands, five cathodal (C_1-C_5), two intermediate (I_1 and I_2), and five anodal (A_1-A_5); whereas the normal showed eleven bands with only the A_3 band of the anodal zone missing.

The 10 day old endosperm of opaque-2 showed seven bands C_2, C_3, C_4, I_1, I_2, A_1 and A_2 while the normal showed only four bands (C_2, C_4, I_1 and I_2). The 30 day old endosperm of normal showed eight bands (C_1 to C_4, I_1, I_2, A_1 and A_2) but opaque-2 showed only six, the bands I_1 and A_2 being absent.

Differences in the intensity of the bands were also observed in terms of the maturity and the type of endosperm, especially in the C-zone.

The developing opaque-2 and normal endosperms show significant qualitative differences only in the C-zone, which suggests that this zone might control the phenotypic difference and possibly the high lysine and tryptophan content in the opaque-2 mutant.

Table 1

The peroxidase isozyme pattern in normal (N) and opaque-2 (o_2) endosperm

Bands	10 day		15 day		20 day		25 day		30 day	
	o_2	N	o_2	N	o_2	N	o_2	N	o_2	N
A. Cathodal zone										
C_1	-	-	-	-	-	-	+	+	+	+
C_2	+	+	+	+	+	+	+	+	+	+
C_3	+	-	+	+	+	+	+	+	+	+
C_4	+	+	+	+	+	+	+	+	+	+
C_5	-	-	+	+	+	+	+	+	-	-
B. Central zone										
I_1	+	+	+	+	+	+	+	+	+	+
I_2	+	+	+	+	+	+	+	+	-	+
C. Anodal zone										
A_1	+	-	+	+	+	+	+	+	+	+
A_2	+	-	+	+	+	+	+	+	-	+
A_3	-	-	+	-	+	-	+	-	-	-
A_4	-	-	+	+	+	+	+	+	-	-
A_5	-	-	+	+	+	+	+	+	-	-

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3. Induction of mutations with hydrazine.

Homozygous multiple dominant seed with Bm_2, Lg_1, A_1, Su_1, Pr, Y_1 Gl_1, Wx and G_1 markers was treated with 0.04 M and 0.08 M of hydrazine ($NH_2NH_2 \cdot H_2O$) at pH 8.5. One thousand seeds for each treatment were taken. The seeds were presoaked for 24 hours in water. The treatment duration was 24 hours.

In the case of 0.04 M hydrazine treatment, the following eleven seedling mutations were observed out of 512 plants in the M_1 generation (M_1 = the seedlings raised from treated seeds).