CHROMOSOMAL LOCATION OF TWO MITOCHONDRIAL MALATE DEHYDROGENASE STRUCTURAL GENES IN *ZEA MAYS* USING TRISOMICS AND *B-A* TRANSLOCATIONS[1]

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ABSTRACT

Maize mitochondrial malate dehydrogenase is coded by four genetic loci, *Mdh1*, *Mdh2*, *Mdh3* and *Mdh4*. Two of the four loci have been located on the long arm of chromosome *6*, using trisomic analysis and *B-A* translocations.

MULTIPLE forms of mitochondrial malate dehydrogenase (m-MDH) and soluble malate dehydrogenase (s-MDH) are found in maize (YANG and SCANDALIOS 1974, 1975). Each of the compartmentalized forms of malate dehydrogenase (L-Malate:NAD$^+$ oxidoreductase; EC 1.1.1.37; MDH) has a unique function in metabolism (LEHNINGER 1975). Soluble MDH is considered to be involved in the cytoplasmic side of the malate shuttle, transporting NADH equivalents across the mitochondrial membrane in the form of malate. The other half of the malate shuttle is performed by m-MDH. Mitochondrial MDH is also an essential enzyme of the tricarboxylic acid cycle. Although m-MDH can be detected only in the mitochondria, the enzyme is under nuclear control and is synthesized on cytoplasmic ribosomes (LONGO and SCANDALIOS 1969). The transcription, translation and processing of m-MDH is probably genetically controlled and therefore represents an important system for gene regulation studies. However, the chromosomal locations of the m-MDH structural genes are needed before studies of gene regulation can be attempted.

In this paper, we demonstrate the chromosomal location of two structural genes for mitochondrial malate dehydrogenase (*Mdh1*, *Mdh2*), using trisomics and *B-A* translocations. In addition, this paper illustrates that *B-A* translocations are extremely powerful for determining the chromosomal location of isozyme genes.

MATERIALS AND METHODS

Genetic stocks: The trisomic stocks used in this study were obtained from the Maize Genetics Cooperation Stock Center. The *B-A* translocation stocks were obtained from the Maize Genetics

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Cooperation Stock Center, D. S. ROBERTSON, Iowa State University, and J. B. BECKETT, University of Missouri. Inbred line SD10 was obtained from D. B. SHANK, South Dakota State University. The inbred lines W59 and D10 have been maintained by our laboratory.

Root tip procedure and pollen analysis: Seeds imbibed for 24 hr were placed on moist germination paper and grown in the dark for four days at 23°. After four days, the roots were collected and pretreated for four hr in 3 mM 8-hydroxyquinoline. Following pretreatment, the root tips were fixed in a 3:1 ethanol:glacial acetic acid solution. Fixed roots were stained by the standard Feulgen technique. All trisomic plants and the vast majority of *B-A* translocation plants were verified by root tip counts. Percent pollen abortion was determined for all *B-A* translocation plants, using a pocket microscope. Plants with 25% pollen abortion or greater were crossed to inbred lines.

Electrophoresis and staining procedure: A piece of scutellum was cut from both sides of the embryo from the 24-hr imbibed trisomic and *B-A* translocation seeds; care was taken to ensure that the embryo was not injured.

The pieces of scutella were ground in 0.025 M glycylglycine buffer, pH 7.4. The extract was applied to a 5 × 7 mm piece of Whatman 3 mm filter paper and this was inserted into a vertical slot cut into 12% starch gels. Horizontal starch gel electrophoresis and specific staining for MDH were conducted as described previously (SCANDALIOS 1969).

Genetic control of malate dehydrogenase: For the mapping study presented in this paper, the nomenclature and the model for the genetic control of m-MDH published by YANG, SORENSON and SCANDALIOS (1977) is followed.

The model postulates two diallelic sets of duplicated loci (YANG, SORENSON and SCANDALIOS 1977). The duplicated sets of loci and the isozyme products that each code for are shown in Table 1 and Figure 1. In this model, *Mdh1* is closely linked to *Mdh2*, while *Mdh3* is closely linked to *Mdh4*. Furthermore *Mdh1* and *Mdh2* are not linked to *Mdh3*, *Mdh4*. *Mdh1* codes for the isozymes MDH1 or MDH2, while *Mdh2* codes for the isozymes MDH3 or MDH5. *Mdh3* codes for MDH1 or MDH3a (YANG and SCANDALIOS, unpublished), while *Mdh4* codes for MDH3 or MDH7. The isozyme MDH4 is a hybrid product of MDH3 and MDH5. MDH6 is a hybrid between MDH5 and MDH7 (YANG and SCANDALIOS, unpublished). The isozyme MDH5 can be formed as a hybrid between MDH3 and MDH7 (phenotype C, Figure 1). However, the isozyme MDH3a does not form detectable hybrids. This is exemplified by the discovery of an inbred line in our laboratory that expresses only mitochondrial MDH3a and MDH7 (phenotype E, Figure 1). In this line, no hybrid is seen between MDH3a and MDH7.

Trisomic analysis: All homozygous trisomics were crossed as females to an inbred stock homozygous for another isozyme variant. Trisomic F_1 plants were cytologically selected and self pollinated or backcrossed to the inbred line.

F_2 progeny of self crosses for the chromosomes other than the one that carries the MDH gene set (noncritical trisomics) would be expected to show a 1:2:1 MDH segregation ratio. Trisomic plants for the chromosome that carries the MDH isozyme gene set (critical trisomic) should segregate in a 6:11:1 ratio with 50% transmission of the trisomic. As the transmission of the trisomic becomes less than 50%, the ratio becomes closer to 1:2:1.

Backcross progeny of noncritical trisomics would be expected to show a 1:1 segregation

TABLE 1

Genetic control of m-MDH in Zea mays

Gene set	Genotype	Mitochondrial isozymes expressed
Mdh1–Mdh2	*Mdh1–m1*, *Mdh2–m3*	MDH1, MDH3
	Mdh1–m2, *Mdh2–m5*	MDH2, MDH5
Mdh3–Mdh4	*Mdh3–m1*, *Mdh4–m3*	MDH1, MDH3
	Mdh3–m3a, *Mdh4–m7*	MDH3a, MDH7

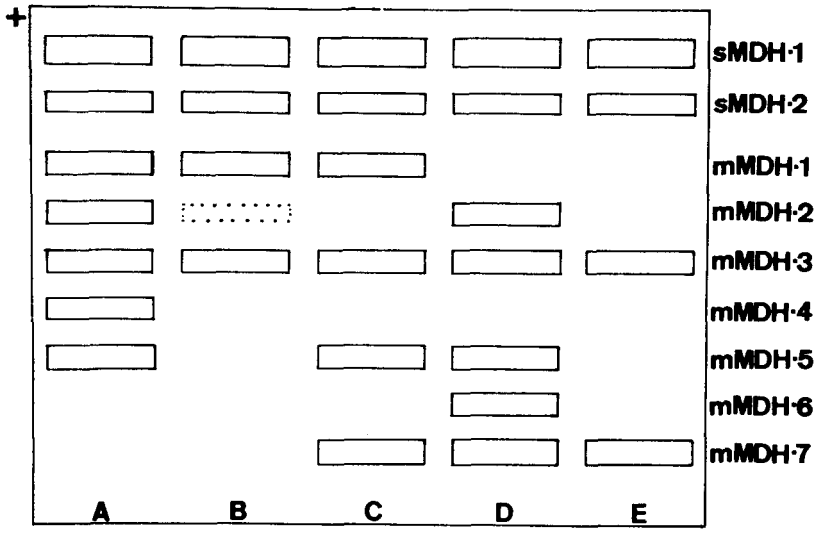

FIGURE 1.—Zymogram phenotypes and genotypes of common MDH-phenotypes in *Zea mays*.

Phenotype	Genotype			
	Mdh1	*Mdh2*	*Mdh3*	*Mdh4*
A	*-m2*	*-m5*	*-m1*	*-m3*
B	*-m1*	*-m3*	*-m1*	*-m3*
C	*-m1*	*-m3*	*-m3a*	*-m7*
D	*-m2*	*-m5*	*-m3a*	*-m7*
E	*-m0 (null)*	*-m0*	*-m3a*	*-m7*

ratio, while the critical trisomic would show a 5:1 ratio with 50% transmission of the trisomic.

B-A *translocation mapping*: ROMAN (1947) X-irradiated pollen from a maize line carrying *B* chromosomes in addition to the normal set of *A* chromosomes. Two types of translocated chromosomes resulted: B^A chromosomes and A^B chromosomes. B^A chromosomes have the centromere of the original *B* chromosome, while A^B chromosomes have the centromere of the original *A* chromosome. Roman observed that the B^A chromosome exhibited nondisjunction in the second microspore division in some male plants carrying the *B-A* translocation. Nondisjunction occurs in 50 to 95% of the pollen (CARLSON 1978).

Since nondisjunction occurs at the second microspore division, the resulting hypoploid and hyperploid sperm nuclei will fertilize the egg or the polar nuclei. As a result, when the scutella and embryo are deficient for a chromosome arm, the endosperm will have two doses of the same arm, and *vice versa*. When a *B-A* translocation line is crossed as a male to an inbred line carrying a different isozyme variant, seeds resulting from nondisjunction of the critical *B-A* translocation chromosome will exhibit zymogram phenotypes in the scutellum different from those in the endosperm. A nondisjunction seed will exhibit only the maternal isozyme variant in the endosperm or scutellum, depending on which received the hypoploid sperm nucleus (Figure 2).

Since nondisjunction gametes of the critical *B-A* translocation chromosome can result in one phenotype in the scutellum and another in the endosperm, the use of *B-A* translocations to locate isozyme structural genes provides both elegant and conclusive proof of their chromosomal locations. Recent reviews on *B* chromosome behavior and *B-A* translocations have been published (BECKETT 1978; CARLSON 1978).

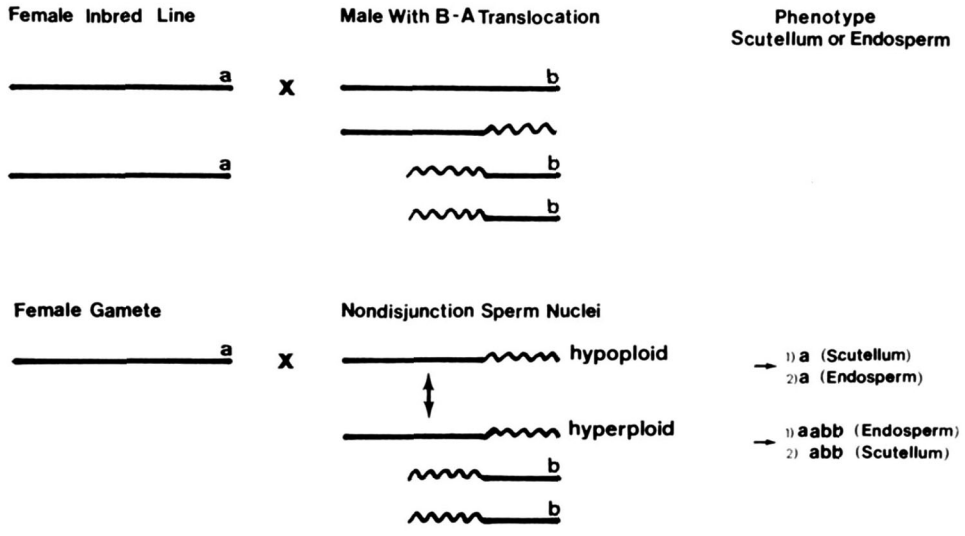

FIGURE 2.—Nondisjunction gametes in *B-A* translocations produce two different sperm nuclei. (1) When the hypoploid sperm nucleus fertilizes the egg cell, the scutellum will exhibit phenotype *a* and the endosperm will exhibit phenotype *aabb*. (2) When the hyperploid sperm nucleus fertilizes the egg cell, the scutellum will exhibit phenotype *abb* and the endosperm will exhibit phenotype *a*.

RESULTS

Trisomic analysis of the m-MDH *genes* Mdh1 *and* Mdh2: Trisomics *2*, *4*, *6*, *7* and *10* were heterozygous for *Mdh1* and *Mdh2*. All were segregating for the isozymes MDH1, MDH3 and MDH2, MDH5, or were segregating for MDH2, MDH5 and repressed MDH1, MDH3. Heterozygous trisomic plants were back-

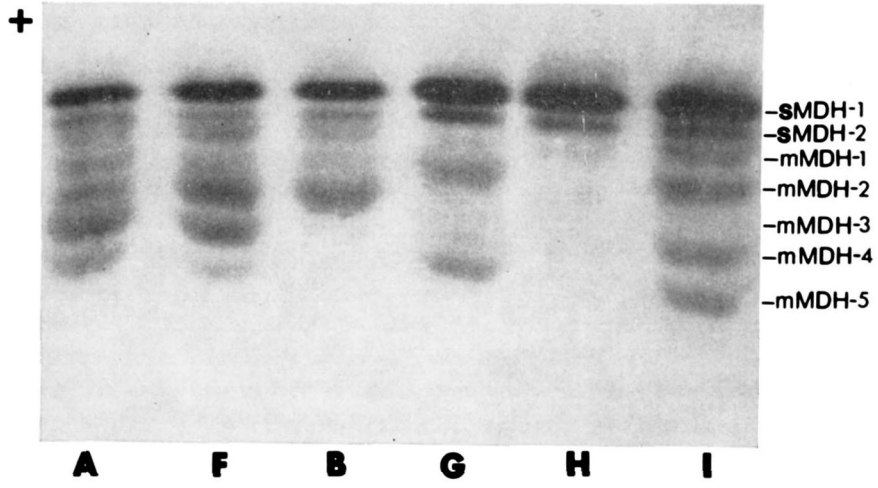

FIGURE 3.—Phenotypes observed among progeny of trisomic crosses shown in Table 2.

TABLE 2

Trisomic analysis of Mdh1 *and* Mdh2

Cross	Phenotypes A	F	B	G	G*	H	I
Tr2–A8–3 × W59	..	27	23	..	..	..	..
Tr4–A19–2 × D10			41				46
Tr7–A37–10 × W59		46	52				
Tr10–A54–2 × D10			69				74
Tr6–A32b–4 Selfed	84		5	16	14	1	

$$A = \frac{Mdh1\text{–}m2}{Mdh1\text{–}m2} \quad \frac{Mdh2\text{–}m5}{Mdh2\text{–}m5} \quad \frac{Mdh3\text{–}m1}{Mdh3\text{–}m1} \quad \frac{Mdh4\text{–}m3}{Mdh4\text{–}m3}$$

$$F = \frac{Mdh1\text{–}m2}{Mdh1\text{–}m1} \quad \frac{Mdh2\text{–}m5}{Mdh2\text{–}m3} \quad \frac{Mdh3\text{–}m1}{Mdh3\text{–}m1} \quad \frac{Mdh4\text{–}m3}{Mdh4\text{–}m3}$$

*G = Expresses only the isozymes MDH2 and MDH5.

crossed to either W59, which has the genotype *Mdh1-m1*, *Mdh2-m3*, *Mdh3-m1*, *Mdh4-m3*, or crossed to D10, which has the same genotype as W59. However, D10 exhibits a sharp reduction in MDH1 and MDH3 activity, so that the phenotype is almost a null for all m-MDH isozymes (Figure 3, phenotype H).

Trisomic analysis of the isozymes coded by the *Mdh1* and *Mdh2* loci is shown

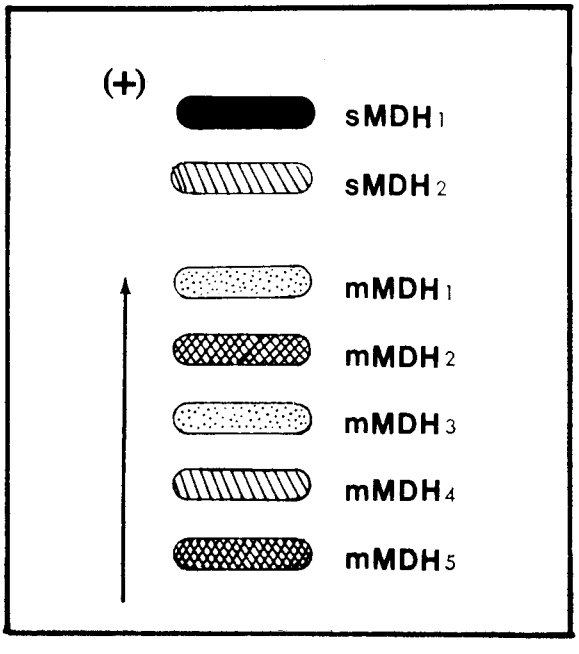

FIGURE 4.—Zymogram phenotype of Tr6-A32b-4. MDH2 and MDH5 are darker than phenotype A of Figure 1. Staining intensity relations from most intense to least intense are: ▬ > ▩ > ▨ > ▧

in Table 2 and Figure 3. All crosses except trisomic *6* exhibited a segregation ratio consistent with the premise that the isozyme genes are not encoded on the particular trisomic. The zymogram phenotype of Tr6-A32b-4 had reduced intensities of isozymes MDH1 and MDH3 and higher intensities of MDH2 and MDH5 (Figure 4). Phenotypes A and F (refer to Figures 1 and 3) were grouped together in the trisomic-*6* progeny because there were phenotypes that could not be classified, unequivocally, as A or F. The recovery of additional phenotypes between A and F is consistent with the segregation of an extra dose of *Mdh1*, *Mdh2* on chromosome *6*.

The ratio expected (A + F:B) for F_2 progeny of noncritical trisomics is 3:1, while a 17:1 ratio is expected for the critical trisomic. Indeed, Table 2 shows that a 17:1 ratio of A + F:B is observed for the trisomic *6* progeny. The phenotypes and frequencies expected if *Mdh1* and *Mdh2* are carried on chromosome *6* are shown in Table 3 and Table 4. The data for trisomic *6* demonstrate conclusively that *Mdh1* and *Mdh2* are located on chromosome *6*. Figure 5 shows a 21-chromosome plant resulting from selfing Tr6-A32b-4.

B-A *translocation mapping of* Mdh1 *and* Mdh2: Trisomic analysis has located *Mdh1* and *Mdh2* on chromosome *6*. In order to determine the chromosome arm location of *Mdh1* and *Mdh2*, Tb6Lc (obtained from D. S. ROBERTSON) was

TABLE 3

Phenotypes (refer to Figure 3) and frequencies expected if the structural genes Mdh1 *and* Mdh2 *are carried on Tr6–A32b–4*

Tr6–A32b–4 selfed

Genotype:

$$\frac{Mdh1\text{–}m2,\ Mdh2\text{–}m5}{Mdh1\text{–}m2,\ Mdh2\text{–}m5} \quad \frac{Mdh3\text{–}m1\ \ Mdh4\text{–}m3}{Mdh3\text{–}m0\ \ Mdh4\text{–}m0} \quad = \quad \begin{matrix} 2,5 & 1,3 \\ 2,5 & — \\ — & \end{matrix}$$

$$Mdh1\text{–}m0\ \ Mdh2\text{–}m0 \ \text{(null)}$$

♂ \ ♀	$\frac{2}{12}$ 2,5 1,3	$\frac{2}{12}$ 2,5 —	$\frac{1}{12}$ — —	$\frac{1}{12}$ — 1,3	$\frac{1\ 2,5\ 1,3}{12\ 2,5}$	$\frac{2\ 2,5\ 1,3}{12\ —}$	$\frac{1\ 2,5\ —}{12\ 2,5}$	$\frac{2\ 2,5\ —}{12\ —}$
$\frac{2}{6}$ 2,5 1,3	$\frac{4}{72}$ A	$\frac{4}{72}$ A,F†	$\frac{2}{72}$ A	$\frac{2}{72}$ F	$\frac{2}{72}$ A,F	$\frac{4}{72}$ A	$\frac{2}{72}$ A,F	$\frac{4}{72}$ A,F
$\frac{2}{6}$ 2,5 —	$\frac{4}{72}$ A,F	$\frac{4}{72}$ G*	$\frac{2}{72}$ G	$\frac{2}{72}$ A	$\frac{2}{72}$ A,F	$\frac{4}{72}$ A,F	$\frac{2}{72}$ G*	$\frac{4}{72}$ G
$\frac{1}{6}$ — —	$\frac{2}{72}$ A	$\frac{2}{72}$ G	$\frac{1}{72}$ H	$\frac{1}{72}$ B	$\frac{1}{72}$ A,F	$\frac{2}{72}$ A	$\frac{1}{72}$ G	$\frac{2}{72}$ G
$\frac{1}{6}$ — 1,3	$\frac{2}{72}$ F	$\frac{2}{72}$ A	$\frac{1}{72}$ B	$\frac{1}{72}$ B	$\frac{1}{72}$ A,F	$\frac{2}{72}$ F	$\frac{1}{72}$ A,F	$\frac{2}{72}$ A

† A,F cannot be unequivocally classified as A or F.

Phenotypes	A+F	G	G*	B	H
Expected frequencies	0.708	0.153	0.083	0.042	0.014

TABLE 4

Phenotypes and expected frequencies in Tr6–A32b–4 F_2 progeny

Phenotype	A + F	:	B	:	G	:	G*	:	H
Expected	85		5		18		10		2
Observed	84		5		16		14		1

$\chi^2 = 0.3865$

df_4

$0.95 < P < 0.99$

crossed to inbred line SD10. SD10 has the genotype *Mdh-1-m1*, *Mdh2-m3*, *Mdh3-m1* and *Mdh4-m3*, while Tb6Lc has the genotype *Mdh1-m2*, *Mdh2-m5*, *Mdh3-m1* and *Mdh4-m3*. If *Mdh1* and *Mdh2* are on the long arm of chromosome *6*, then nondisjunction gametes should show different zymogram phenotypes in the scutellum and the endosperm. Figure 6 shows phenotype A in the scutellum and phenotype B in the endosperm of plant number 4. Plants 5, 6 and 7 show the same phenotype in the scutella and endosperm indicating that they were produced by normal gametes (Figures 6, 7). Plants 8 and 9 show phenotype B in the scutellum and phenotype F in the endosperm (Figure 7). Phenotype F in the endosperm of plants 8 and 9 is expected since the endosperm is a triploid tissue and therefore contributes extra doses of the isozymes from SD10 (mater-

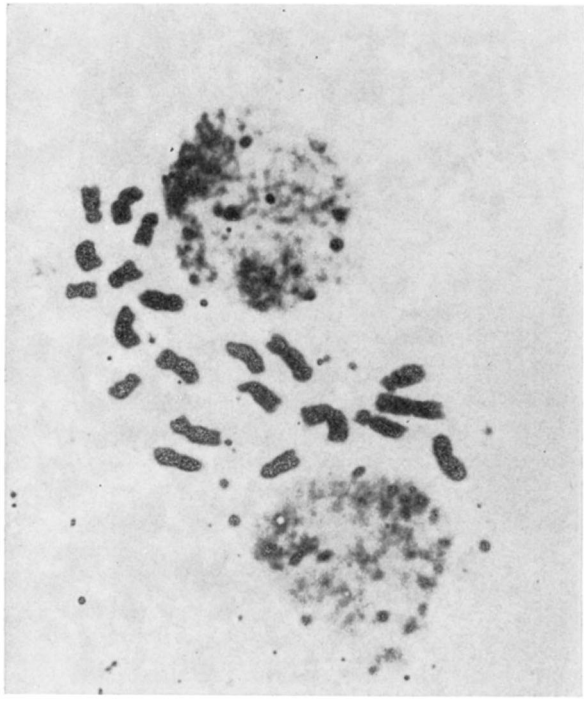

FIGURE 5.—Root tip spread of trisomic progeny from Tr6-A32b-4 selfed.

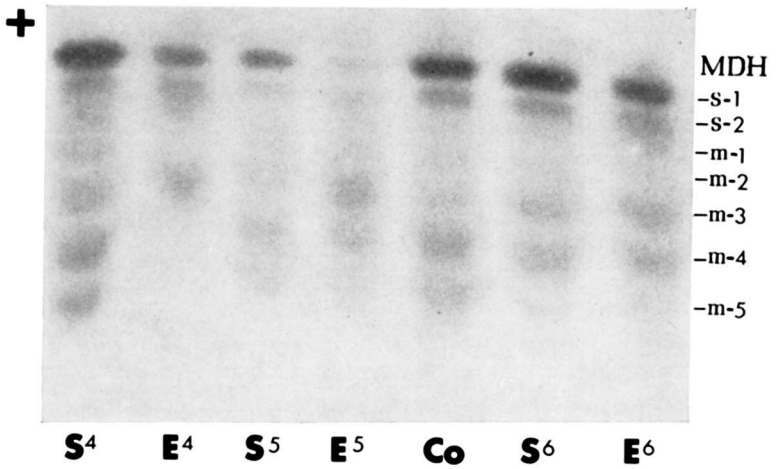

FIGURE 6.—Zymogram phenotypes of plants 4, 5, and 6 from the cross SD10 × Tb6Lc. S = scutella + embryo; E = endosperm; Co = W64A control.

nal dosing). The data presented localize *Mdh1* and *Mdh2* to the long arm of chromosome *6*. Further support is provided by the observation that the F_1 progeny of all the other *B-A* translocations studied always exhibit the same phenotype in both the scutella and endosperm. F_1 progeny of the following *B-A* translocation stocks were analyzed; Tb1Sb, Tb1La, Tb2S-3L6270, Tb1Sb-2L4464, Tb3Sb, Tb3La, Tb4Sa, Tb4L-1L4692, Tb1La-5S8041, Tb5La, Tb6Sa, Tb7Lb, Tb8La, Tb9Sb-4L6504, Tb9Lc, Tb10Sc and Tb10L19.

DISCUSSION

Trisomic analysis shown in Table 2 supports the conclusion that *Mdh1* and

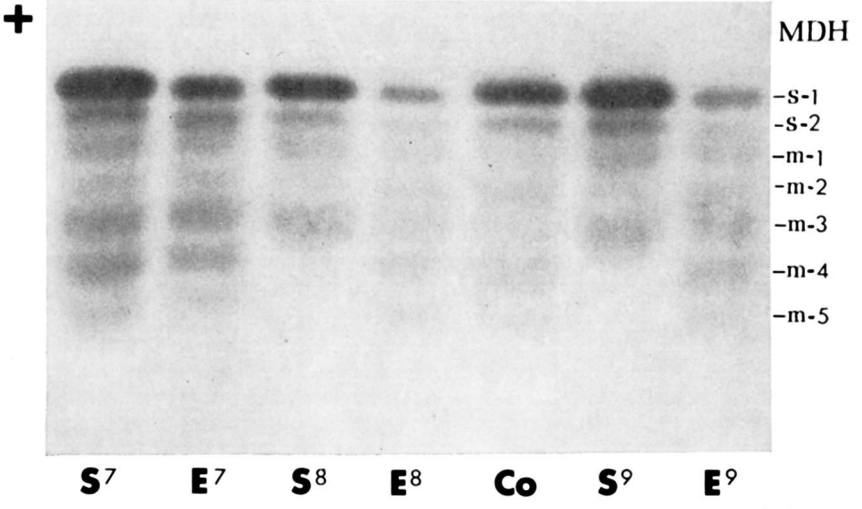

FIGURE 7.—Zymogram phenotypes of plants 7, 8 and 9 of the cross SD10 × Tb6Lc.

Mdh2 are located on chromosome *6*. Further studies utilizing *B-A* translocations confirmed this localization of the MDH structural genes on the long arm of chromosome *6* (Figures 6 and 7) .

The presence of the repressed (or null) phenotype observed in the progeny of the Tr6-A32b-4 cross in Table 2, together with the fact that D10 (null or repressed phenotype) originated from disomic progeny of trisomic *6* (supplied by the Maize Genetic Cooperation Stock Center), deserves careful consideration. In D10, the detection of the mitochondrial isozymes MDH1 and MDH3 is possible only if the whole scutellum is used and then only if a highly concentrated extract is made (Figure 3).

Zymogram analysis of Tr6-A32b-4 exhibited the phenotype shown in Figure 4. According to the genetic model for mMDH proposed by YANG, SORENSON and SCANDALIOS (1977), this phenotype can give only null or repressed progeny if Tr6-A32b-4 were segregating for null or repressed genes at *Mdh3*, *Mdh4*, as well as at *Mdh1* and *Mdh2* (Table 3). The probability of four null structural genes occurring simultaneously is quite remote. The fact that D10 shows m-MDH1 and m-MDH3 (although represssed) indicates that null genes (i.e., nontranscribing) do not exist in this inbred line. In addition, the observation of the phenotype mMDH1, m-MDH2 and m-MDH5 is not consistent with four null genes.

A possible explanation is that there is at least one regulatory or controlling locus involved in regulating the activity of m-MDH. This regulatory locus would activate the MDH1 and MDH3 isozymes when the normal form of the regulatory gene was present. The activation of MDH1 and MDH3 isozymes could occur at transcriptional, post-transcriptional, translational or post-translational levels. The regulatory gene could not be linked to *Mdh1* and *Mdh2*, since no MDH2, MDH5 phenotypes could be produced. However if the regulator is linked to the structural genes *Mdh3* and *Mdh4*, the resulting progeny are as expected in Table 3. Crosses to test this hypothesis are currently being constructed.

The developmental expression and tissue specificity of isozymes must be under intricate regulatory control. The chromosomal locations of the isozyme structural genes and the loci that regulate their expression are essential for understanding these developmental mechanisms. In this paper, we have demonstrated unequivocally that the structural genes *Mdh1* and *Mdh2* are located on the long arm of chromosome *6*.

Further experiments will be done to determine the level at which the postulated regulator exerts its effect on m-MDH. In addition, trisomic and *B-A* translocation crosses are being constructed in order to locate *Mdh3* and *Mdh4*.

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Note added in proof: We have recently used three markers to determine the linkage map of *Mdh1*, *Mdh2* on the long arm of chromosome *6*. Endopeptidase (*Enp-1*) located very close to yellow endosperm (y_1) at position 17, sugary endosperm (su_2) at position 57, and pigmy (*py*) at position 68 were used. *Mdh1*, *Mdh2* were not linked to *Enp-1*; however, *Mdh1*, *Mdh2* were linked to su_2 (24.4 map units) and *py* (12 map units). Therefore *Mdh1*, *Mdh2* are located at the very distal end of the long arm of chromosome *6*.