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Chromosomal Location of the Catalase Structural Genes in *Zea mays*, Using B-A Translocations*

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Summary. B-A translocations have been used to map the catalase genes, *Cat1*, *Cat2*, and *Cat3* of *Zea mays*. *Cat1* was found to be on the short arm of chromosome 5, 9.1 map units from brittle endosperm (bt_1). *Cat2* was located on chromosome 1S, while *Cat3* was located on the distal half of chromosome 1L. There was no linkage between *Cat2* and *Cat3*. The significance of mapping the catalase structural genes is discussed.

Key words: B-A translocations – Catalase, Mapping – Structural genes – *Zea mays*

Introduction

Maize catalase (H_2O_2:H_2O_2 oxidoreductase, E.C. 1.11.1.6) is a tetrameric enzyme (Scandalios 1965) encoded in at least three distinct genetic loci; *Cat1*, *Cat2* and *Cat3* which code for the isozymes CAT-1, CAT-2 and CAT-3, respectively (Scandalios 1970; Scandalios et al. 1980a). *Cat1* is primarily expressed in the endosperm and scutellum of developing kernels; *Cat2* is primarily expressed in the scutellum, and *Cat3* in the shoot of seedlings during early sporophytic development (Scandalios 1979). At times when both *Cat1* and *Cat2* are simultaneously expressed (i.e., in scutella after seed imbibition), their products interact to generate hybrid catalase molecules (Scandalios et al. 1972). The general developmental pattern of CAT-1 and CAT-2 isozymes following germination is one in which the CAT-2 isozyme increases in activity as CAT-1 gradually disappears (Quail and Scandalios 1971; Scandalios 1974). Furthermore, Quail and Scandalios (1971) have shown that *Cat1* and *Cat2* differ both in rates of synthesis and degradation and they suggested that this post-translational control may account, at least in part, for the differential expression of catalase. Thus, the maize catalase system presents an opportunity for the study of differential gene expression in higher plants. Further study, however, into the mechanisms of catalase regulation in the developing plant requires knowledge of the precise location of the catalase loci on the maize genome.

To date few biochemical markers have been located on individual chromosomes of the maize genome (Nielsen and Scandalios 1974; Neuffer and Coe 1975; Ott and Scandalios 1978; McMillin et al. 1979). In this paper we report the chromosomal localization of these three catalase structural genes on the maize genome through the use of B-A translocations.

Materials and Methods

The B-A translocation strains used and the catalase variants found in each of the translocation stocks are given in Table 1. The translocation strains were kindly supplied by J.B. Beckett (University of Missouri), R.J. Lambert (Maize Genetics Cooperation Stock Center) and D.S. Robertson (Iowa State University).

The inbreds Oh51A and SD10 were obtained from W.R. Findley, Ohio Agricultural Research and Development Center and from D.B. Shank, South Dakota State University, respectively. The inbreds R6-45, R6-48 and R6-49 were provided by R.H. Moll, North Carolina State University, while W10, W59, and W64A have been maintained by our laboratory.

The use of B-A translocations to locate genes on chromosome arms had been previously described (Roman and Ullstrup 1951; Beckett 1978). The B^A chromosome may undergo nondisjunction during the second pollen mitosis (Fig. 1) (Roman 1947). Nondisjunction produces two sperm nuclei, one deficient for a chromosome arm (Fig. 1,1) and one having two doses of the same arm (Fig. 1,2). When a B-A translocation line is crossed as a male to an inbred line carrying a different isozyme variant, seed resulting from nondisjunction of the critical B-A translocation chromosome (i.e., chromosome on which the gene in question resides) will ex-

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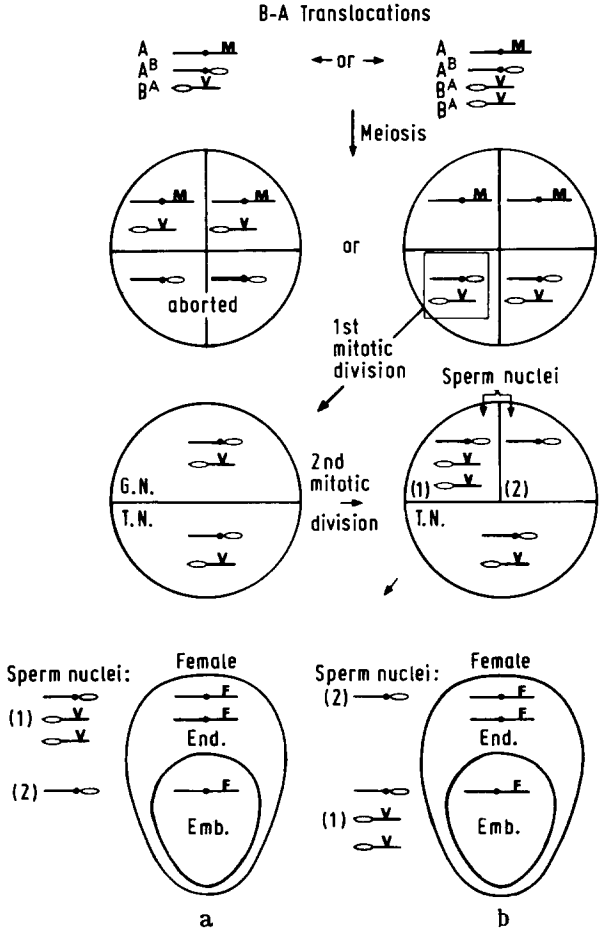
Table 1. The CAT-1, CAT-2 and CAT-3 isozymes carried by each of the B-A translocation strains used[a]

B-A translocations	1*			2*			3*		
	CAT-1	CAT-2	CAT-3	CAT-1	CAT-2	CAT-3	CAT-1	CAT-2	CAT-3
Tb-1Sb	M	Z	A	V	Z,PZ,P		–		
Tb-1La	M	Z,PZ	A	V	Z	A	–		
Tb-3La-2S6270	M	Z	A	V	Z		–		
Tb-1Sb-2L4464	M,FM,MV	Z	A	–	–		V		
Tb-3Sb	M	Z	A	V	Z		–		
Tb-3La	M,MV	Z	A	V,FV	Z		–		
Tb-4Sa	M	Z	A	V	Z	A	–		
Tb-9Sb-4L6504	M,FM	Z,PZ	A	–	–		–		
Tb-4L-1La4692	–	–	–	V	Z,PZ,P		–		
Tb-1La-5S8041	M,MV	Z	A	–	–		V		
Tb-5La	M	Z	A	V,FV	Z		–		
Tb-6Sa	M	Z	–	–	–		–		
Tb-6Lc	M,MV	Z	–	–	–		V,MV	PZ	A
Tb-7Lb	M	Z	A	V	Z	A	–		
Tb-8Lb	M	Z	A	V	Z		–		
Tb-9Sb	M,MV	Z	–	V,FV	Z		–		
Tb-9La	–	–	–	V	Z		–		
Tb-10Sc	M	Z,PZ	A	V,FV	Z,PZ	AB,A	–		
Tb-10La	–	–	–	F	Z,PZ	AB,A			
Tb-10L19	M,MV	Z	A	–	–	–	–		

[a] The B-A translocation strains were obtained from three different sources: 1* J.B. Becket, 2* R.J. Lambert, 3* D.S. Robertson



hibit different zymogram phenotypes in the scutellum and in the endosperm (Fig. 1) (McMillin et al. 1979). The F_1 progeny of all the other B-A translocations (non-critical B-A translocations) will exhibit the F_1 hybrid form. For enzymes that are not expressed in the endosperm, such as CAT-2 and CAT-3, a different strategy is used for mapping. In the tissue screened, three phenotypes will be observed for the critical B-A translocation chromosome; a maternal, an F_1 and an F_1 phenotype dosed to the paternal parent. Furthermore, the chromosome number can be used as an additional control since the maternal phenotype will have 20 chromosomes, the F_1 phenotype will have 20 or 21 chromosomes, and the F_1 phenotype dosed to the paternal parent will have 22 chromosomes.

Root-tips were collected and chromosomes counted using the following Feulgen procedures. Roots were pretreated in 3 mM 8-Hydroxyquinoline for 4 hr, fixed in 3:1 ethanol:glacial acetic acid overnight, hydrolized for 9 min in 1 N HCl, and then stained

Fig. 1a and b. Behavior of B^A chromosomes during meiosis and pollen development (modified from Roman 1947). B^A chromosomes may undergo nondisjunction at the second microspore mitotic division resulting in a hyperploid sperm nucleus (1) and a hypoploid sperm nucleus (2). If a diploid female homozygous for CAT-1F is crossed to the critical B-A translocation (the translocation where *Cat1* is located) carrying CAT-1VM, different phenotypes will be observed in the scutellum and in the endosperm. In case (a) the endosperm will be FFVV while the scutellum and embryo will be F. In case (b) the endosperm will be FF, while the scutellum and embryo will be FVV. G.N., generative nucleus; T.N., Tube nucleus, END., endosperm; EMB., embryo; A, normal chromosome; A^B, A chromosome-B chromosome translocation with A centromere; B^A, B chromosome-A chromosome translocation with B centromere; M, V, F are the respective catalase isozymes specified by the allelic genes *Cat1M, Cat1V* and *Cat1F*

Table 2. The CAT-1 phenotypes observed in F_1 progeny of crosses made between B-A translocations carrying the M,V or MV CAT-1 isozymes, as males, and inbred strains homozygous for the fast CAT-1 variant (FF), as females

Cross			CAT-1 variants of the male plants used	The CAT-1 phenotypes observed in scutellum			
♀		♂		FM	FV	FF	Total
W59	X	Tb-1Sb	M	20			20
W10	X	Tb-1Sb	M	14			14
W59	X	Tb-1La	V		24		24
W10	X	Tb-1La	M	10			10
W59	X	Tb-3La-2S6270	M	21			21
W59	X	Tb-1Sb-2L4464	M	28			28
R6-45	X	Tb-3Sb	M	21			21
W59	X	Tb-3La	MV	9	12		21
W59	X	Tb-3La	V		22		22
W10	X	Tb-4Sa	V		21		21
W59	X	Tb-9Sb-4L6504	M	21			21
W59	X	Tb-1La-5S8041	MV	11	17	6	34
W10	X	Tb-1La-5S8041	MV	2	10	2	14
R6-48	X	Tb-1La-5S8041	V		4	2	6
W59	X	Tb-5La	M	21			21
W10	X	Tb-6Lc	MV	12	9		21
W59	X	Tb-7Lb	M	21			21
W59	X	Tb-8Lb	M	20			20
W59	X	Tb-9La	V		20		20
W59	X	Tb-10Sc	V		20		20
Oh51A[a]	X	Tb-10La	F		14		14

[a] Oh51A is homozygous for the V variant of *Cat1* isozymes. F, M and V are allelic forms of CAT-1 (Scandalios 1969)

in Feulgen stain. Seedlings with 21 or 22 chromosomes were planted in the greenhouse or in the field. Pollen abortion was determined for all B-A translocation plants using a pocket microscope. Heterozygous translocation plants with 20% pollen abortion or greater and hyperploid heterozygous plants were crossed, as males, to inbred strains homozygous for different catalase variants (Fig. 1). All seeds, both inbreds and B-A translocations, were screened for CAT-1, CAT-2 and CAT-3 electrophoretic variants. In addition, the seeds obtained from the crosses were screened for CAT-1, CAT-2 and CAT-3 isozymes. The tissues screened for each of the three catalase loci were as follows: 1) CAT-1 isozymes: a) a piece of mature scutellum from seeds soaked in tap water for 24 hours was ground in 25 mM glycylglycine buffer (pH 7.4). The crude extract was used for electrophoresis. b) Liquid endosperm and scutellum from 23-25 day old post-pollinated seeds were ground separately in 25 mM glycylglycine buffer, pH 7.4. The extract was used for electrophoresis. 2) CAT-2 isozymes: single scutella 8-10 days after germination were ground in 0.8 ml 25 mM glycylglycine (pH 7.4) and 2-3 mg of polyvinylpyrrolidone (PVP) was added to remove phenolic compounds. The extract was centrifuged in a Beckman microfuge for 3.5 minutes. The crude supernatant was used for electrophoresis. 3) CAT-3 isozymes: five day old post-germination coleoptile tissue was ground with a drop of 25 mM glycylglycine buffer and 1-2 mg of PVP. The crude extract was used for electrophoresis. The CAT-3 phenotypes are not easily distinguished unless the gel preparation and grinding procedures are carefully controlled.

Catalase isozymes were separated by electrophoresis on 12% starch gels utilizing the electrophoretic and histochemical procedures described previously (Scandalios 1969).

Results

Chromosomal Location of the Cat-1 Gene

Plants carrying specific B-A translocations were crossed as males to inbred lines with the genotype *Cat1F* (Table 2). Every B-A translocation except Tb-1La-5S8041 gave results expected for a non-critical translocation. The CAT-1F phenotype observed in progeny from the crosses W59 x Tb-1La-5S8041, W10 x Tb-1La-5S8041, and R6-48 x Tb-1La-5S8041 can only be produced if the eggs were fertilized with hypoploid sperm nuclei lacking the *Cat1* structural gene (critical chromosome) (Fig. 1). The possibility of contamination was ruled out by using other isozymes as markers (data not shown). In addition to the CAT-1F phenotype, plants were observed exhibiting dosage toward the paternal phenotype. This is a result of the hyperploid sperm nucleus fertilizing the egg. All plants which exhibited the F_1 hybrid pattern, but dosed to the paternal phenotype, had 22 chromosomes (Fig. 2a). Therefore, the zymogram phenotypes and the chromosome counts always gave results consistent with *Cat1* being located on either chromosome 1L or chromosome 5S.

Since nondisjunction occurs in the second pollen mitotic division, whenever a hypoploid sperm nucleus

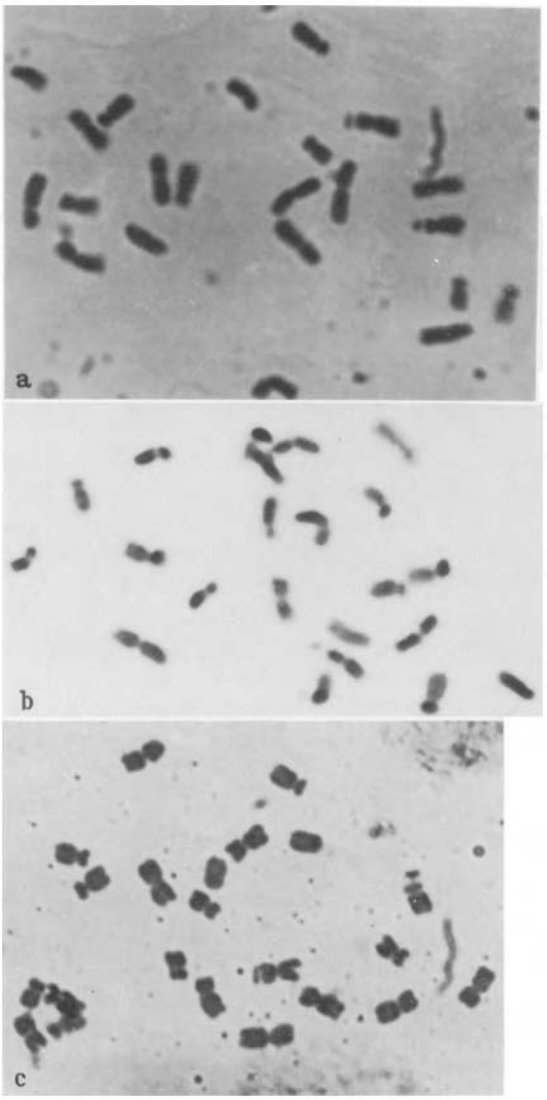


Fig. 2a-c. **a** Root tip spread of a 22 chromosome seedling which exhibited the FVV CAT1 phenotype in the scutellum; **b** root tip spread of a 22 chromosome plant exhibiting CAT-2PZZ; from the cross R6-49 × Tb-1Sb. **c** root tip spread of a 22 chromosome plant exhibiting CAT-3AAB from the cross W59 × Tb-1La

fertilizes the egg a hyperploid sperm nucleus will fertilize the endosperm. When a hyperploid sperm nucleus fertilizes the egg a hyperploid sperm nucleus will fertilize the endosperm. Therefore, if an isozyme is expressed in both the endosperm and scutellum, seed resulting from nondisjunction of the critical B-A translocation chromosome will exhibit different zymogram phenotypes in the scutellum and the endosperm (Fig. 1) (McMilin et al. 1979). The cross W10 × Tb-1La-5S8041 was harvested 25 days after pollination, while the endosperm was still in liquid form. Plant numbers 2 and 8 show phenotype CAT-1F in the scutellum and the F_1 hybrid form CAT-1FVV in the endosperm (Fig. 3a, b). In addition, plant number 9 shows the phenotype CAT-1FVV in the scutellum and the phenotype

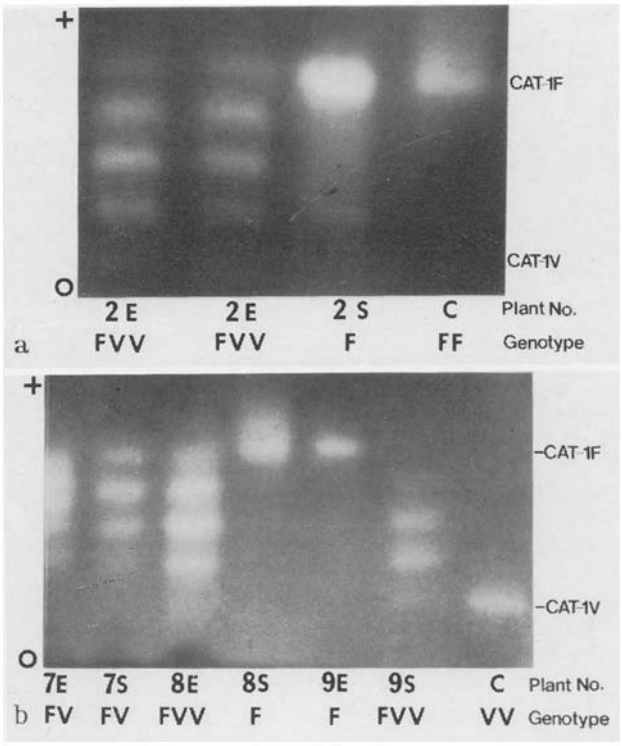


Fig. 3a and b. Zymograms of catalase phenotypes observed in plants derived from the cross W10 × Tb-1La-5S8041. **a** plant no. 2; **b** plant no. 7, 8 and 9. *Cat1* genotype of each plant sample is indicated. S = scutellum and embryo; E = endosperm; C = control; O = origin; migration is anodal

CAT-1F in the endosperm (Fig. 3b). This is conclusive evidence that *Cat1* is located either on chromosome 1L or chromosome 5S. Since W59 × Tb-1La and W10 × Tb-1La gave no nondisjunction progeny, the *Cat1* gene is most likely on chromosome 5S (Table 2). Unfortunately no lines involving a translocation of chromosome 5S exist to confirm this, But, linkage data with the marker brittle endosperm (bt_1) located near the centromere on chromosome 5L (Neuffer et al. 1968) confirm that *Cat1* is located on chromosome 5S. F_2 linkage analysis gave a linkage value of 9.1 map units (Table 3).

Table 3. Analysis of progeny of self-pollinated plants heterozygous for the marker gene, brittle endosperm (bt_1) and for two CAT-1 isozymes $\frac{+F}{bt_1\ V}$

Type of endosperm	Genotype of Scutellar tissue	The CAT-1 phenotypes observed in scutella tissue			
		FF	FV	VV	Total
Normal	(+ –)	118	253	24	395
Brittle	$bt_1 bt_1$	1	13	73	87
Total		119	266	97	482

Chromosomal Location of the Cat2 Gene

There are two common allelic variants for the *Cat2* gene; CAT-2P and CAT-2Z. Since *Cat2* is not expressed in the endosperm, two criteria were used to map *Cat2*. First the scutella should give three phenotypes; maternal, F_1 and an F_1 phenotype dosed to the variant carried by the paternal parent. Secondly, the chromosome constitution of each plant should correspond to the phenotype.

Plants carrying a specific B-A translocation were crossed as males to inbred lines exhibiting either the CAT-2Z or CAT-2P phenotype (Table 4).

The progeny of the crosses R6-45 × Tb-1Sb and R6-49 × Tb-1SB exhibit all the phenotypes expected from non-

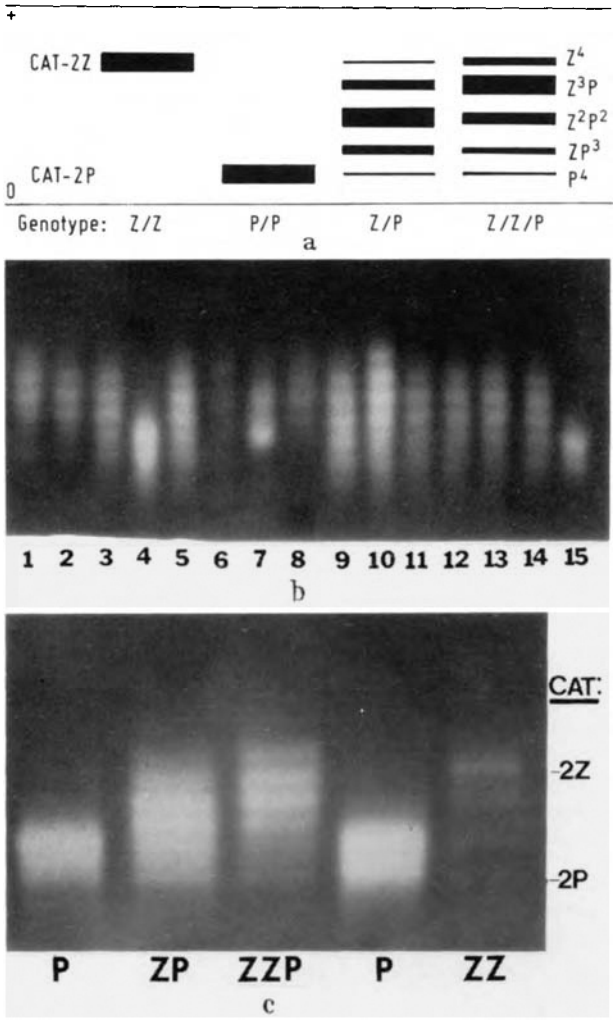


Fig. 4a-c. a Schematic drawing of 1) CAT-2Z, 2) CAT-2P, 3) CAT-2PZ and 4) CAT-2ZZP. b Progeny from the cross R6-49 × Tb-1Sb (CAT-2PP × CAT-2ZZ) showing the nondisjunction phenotypes expected. No. 6 CAT-2Z control, 7 CAT-2P control, 1 and 10 are CAT-2ZZP, 4 and 15 are CAT-2P, 2, 3, 5, 8, 9, 11, 12, 13 and 14 are CAT-2ZP. c Nondisjunction phenotypes CAT-2P and CAT-2ZZP observed in the cross R6-49 × Tb1Sb. CAT-2P control, CAT-2ZP, CAT-2ZZP, CAT-2P, CAT-2Z control

Table 4. CAT-2 variants of the inbred strains and B-A translocations used

Cross			CAT-2 variants of the plants used	
♀		♂	♀	♂
R6-45	×	Tb-1Sb	PP	ZZ
R6-49	×	Tb-1Sb	PP	ZZ
W59	×	Tb-1La	PP	ZZ
W59	×	Tb-2S-3L6270	PP	ZZ
W10	×	Tb-1Sb-2L4464	ZZ	PP
W59	×	Tb-1Sb-2L4464	PP	ZZ
R6-45	×	Tb-3Sb	PP	ZZ
W59	×	Tb-3La	PP	ZZ
W59	×	Tb-9Sb-4L6504	PP	ZZ
SD10	×	Tb-4Sa	PP	ZZ
W59	×	Tb-1La-5S8041	PP	ZZ
W59	×	Tb-5La	PP	ZZ
SD10	×	Tb-6Sa	PP	ZZ
W10	×	Tb-6Lc	ZZ	PZ
SD10	×	Tb-6Lc	PP	PZ
W59	×	Tb-7Lb	PP	ZZ
W59	×	Tb-8Lb	PP	ZZ
W59	×	Tb-9La	PP	ZZ
W59	×	Tb-10Sc	PP	PZ
Oh51A	×	Tb-10Sc	ZZ	PZ
W59	×	Tb-10La	PP	PZ
Oh51A	×	Tb-10La	ZZ	PZ
W64A	×	Tb-10La	ZZ	PP

Table 5. The CAT-2 phenotypes observed in F_1 progeny of crosses made between B-A translocations, as males, and inbred strains as females

Cross			CAT-2 variants observed in scutellum of F_1 and backcross progeny				
♀		♂	ZZ	PZ	PZZ	PP	Total
R6-45	×	Tb-1Sb		36	7	9	52
R6-49	×	Tb-1Sb		46	8	17	71
W59	×	Tb-1La		21			21
W59	×	Tb-2S-3L6270		24			24
W10	×	Tb-1Sb-2L4464		50			50
W59	×	Tb-1Sb-2L4464		21			21
R6-45	×	Tb-3Sb		21			21
W59	×	Tb-3La		50			50
W59	×	Tb-9Sb-4L6504		21			21
SD10	×	Tb-4Sa		16			16
W59	×	Tb-1La-5S8041		21			21
W59	×	Tb-5La		28			28
SD10	×	Tb-6Sa		21			21
W10	×	Tb-6Lc	9	12			21
SD10	×	Tb-6Lc		12		10	22
W59	×	Tb-7Lb		21			21
W59	×	Tb-8Lb		20			20
W59	×	Tb-9La		20			20
W59	×	Tb-10Sc		18		22	40
Oh51A	×	Tb-10Sc	38	36			74
W59	×	Tb-10La		50		45	95
Oh51A	×	Tb-10La	71	108			179
W64A	×	Tb-10La		17			17

disjunction progeny involving the critical B-A translocation for *Cat2* (Table 5). The recovery of the CAT-2P phenotype is expected only if the hypoploid sperm nucleus fertilized the egg. The possibility of contamination was ruled out using other isozymes as markers (data not shown). In addition, the appearance of the phenotype CAT-2PZZ is expected if the hyperploid sperm nuclei fertilized the egg. Figure 4a shows a schematic drawing of CAT-2Z, CAT-2P, CAT-2PZ and CAT-2PZZ. The recovery of the phenotypes CAT-2P, CAT-2PZ and CAT-2PZZ (Fig. 4b, c) is expected if *Cat2* is on chromosome 1S. In addition, cytological examination of four seed with CAT-2PZZ demonstrated that all had 22 chromosomes (Fig. 2b), indicating that *Cat2* is located on the short arm of chromosome 1.

The translocation line Tb-1Sb-2L4464 carries on the B^A chromosome the first half (105-53) of the short arm of chromosome 1 (Beckett 1978). The compound translocation Tb-1Sb-2L4464 when crossed to inbred strains produced F_1 seeds which were heterozygous for CAT-2 (Table 5). This may indicate that the *Cat2* gene is not located on the first half of the short arm of chromosome 1. Therefore, the *Cat2* gene is located on the short arm of chromosome 1 and most likely on the distal part of this arm.

Chromosomal Location of the Cat3 Gene

CAT-3 is expressed as the major catalase isozyme in 5 day old coleoptile. CAT-1 is also expressed in this tissue in some maize lines, although the CAT-1 activity level is lower (Scandalios et al. 1980a).

Table 6. CAT-3 variants of the inbred strains and B-A translocations used

Cross			CAT-3 variants of the plants used	
♀		♂	♀	♂
W59	×	Tb-1La	BB	AA
W59	×	Tb-1Sb-2L4464	BB	AA
W59	×	Tb-3La-2S6270	BB	AA
W59	×	Tb-3La-2S6270	BB	AB
W59	×	Tb-3La	BB	AA
W59	×	Tb-3La	BB	AB
W59	×	Tb-9Sb-4L6504	BB	AA
W59	×	Tb-1La-5S8041	BB	AA
W59	×	Tb-5La	BB	AA
W59	×	Tb-5La	BB	AA
W59	×	Tb-7Lb	BB	AA
W59	×	Tb-8Lb	BB	AA
W59	×	Tb-9La	BB	AB
Oh51A	×	Tb-10Sc	AA	AB
W59	×	Tb-10La	BB	AB
Oh51A	×	Tb-10La	AA	AB

Table 7. The CAT-3 phenotypes observed in F_1 progeny of crosses made between B-A translocations, as males, and inbred strains homozygous for Cat3, as females

Cross			CAT-3 variants observed in coleoptile tissue of F_1 and backcross progeny				
♀		♂	AAB	AA	AB	BB	Total
W59	×	Tb-1La	17		15	5	37
W59	×	Tb-1Sb-2L4464			21		21
W59	×	Tb-3La-2S6270			21		21
W59	×	Tb-3La-2S6270			10	13	23
W59	×	Tb-3La			21		21
W59	×	Tb-3La			39	34	73
W59	×	Tb-9Sb-4L6504			21		21
W59	×	Tb-1La-5S8041			21		21
W59	×	Tb-5La			21		21
W59	×	Tb-5La			36		36
W59	×	Tb-7Lb			21		21
W59	×	Tb-8Lb			20		20
W59	×	Tb-9La			9	11	20
Oh51A	×	Tb-10Sc		38	36		74
W59	×	Tb-10La			51	44	95
Oh51A	×	Tb-10La		96	77		173

Table 8. Linkage Analysis of *Cat3* and *Amp1*

Cross: $\frac{\text{W64A}}{\text{K41}} \times \text{W64A}$

Genotype: *Cat-3A, Amp-1S* × *Cat-3A, Amp-1S*
Cat-3B, Amp-1F × *Cat-3A, Amp-1S*

Phenotypes observed		Number of progeny
CAT3A	AMP1S	56
CAT3A	AMP1FS	46
CAT3AB	AMP1S	43
CAT3AB	AMP1FS	56

1. Chi square testing 1:1 segregation of CAT3A:CAT3AB
   $\chi^2 = 0.04$ P = 0.83
2. Chi square testing 1:1 segregation of AMP1S:AMP1FS
   $\chi^2 = 0.04$ P = 0.83
3. Chi square testing independent of *Cat3* and *Amp1*
   $\chi^2 = 2.73$ P = 0.44

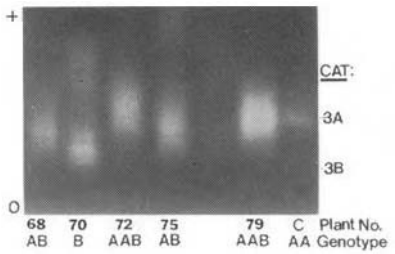


Fig. 5. Zymogram phenotypes observed in progeny of the cross W59 × Tb-1La. The phenotypes CAT-3B and CAT-3AAB are phenotypes expected from nondisjunction gametes

Plants carrying a specific B-A translocation were crossed as males to inbred lines exhibiting either CAT-3A or CAT-3B (Table 6). Only the progeny from the cross W59 × Tb1La exhibited the phenotypes expected from nondisjunction involving a critical B-A translocation (Fig. 5, Table 7). In Fig. 5, it can be seen that plant number 70 shows the maternal phenotype CAT-3B, plants 72 and 79 show the phenotype CAT-3AAB, while plants 68 and 75 show the phenotype CAT-3AB. Furthermore, all plants which exhibited the maternal phenotype CAT-3B had 20 chromosomes, while the plants that were definitely CAT-3AAB had 22 chromosomes (Fig. 2c). This can only occur if *Cat3* is located on chromosome 1L. In addition, isozyme markers were used to establish that the maternal phenotype was not due to contamination (data not shown). Backcrosses were made to test for linkage between *Cat3* and aminopeptidase 1 (*Amp1*), which is located on chromosome 1L near the centromere (Ott and Scandalios 1978). The results indicate that *Cat3* and *Amp1* are either very loosely linked or not linked at all (Table 8). Therefore *Cat3* must be on the distal half of chromosome 1L.

Discussion

We have used B-A translocations to determine the chromosomal location of *Cat1*, *Cat2* and *Cat3* in *Zea mays*. *Cat1* is located on chromosome 5S (Table 2, Fig. 3a, b). Furthermore, a linkage value of 9.1 map units was found between *Cat1* and brittle endosperm (bt_1) (Table 3).

Previous data have indicated that *Cat1* is linked to one of the genes coding for amylase, *Amy2*, by 5 map units (Chao and Scandalios 1969). In addition, *Cat1* has been determined to be linked by three map units to the aminopeptidase gene *Amp3* (Ott and Scandalios 1978). Therefore, *Amp3* and *Amy2* are also located on chromosome 5S. The linkage order for *Cat1*, *Amy2* and *Amp3* has not been established.

Cat2 has been located on chromosome 1S (Table 5, Figs. 4a, 4b, and 4c). The abnormal ratio observed in the cross Oh51A × Tb-10La is not due to *Cat2* being on chromosome 10. First, the crosses W59 × Tb-10La and W64A × Tb-10La do not give abnormal ratios. Rather the results are consistent with *Cat2* not being on chromosome 10 (Table 5). In addition, no phenotypes dosed to the paternal parent were ever observed. The skewed ratio observed could be the result of preferential segregation, since chromosome 10 has been implicated in this phenomenon (Rhoades and Dempsey 1968). The results from crosses involving CAT-3 are consistent with *Cat3* being located on chromosome 1L (Fig. 6, Table 7). Linkage analysis suggests that *Cat3* resides on the distal half of chromosome 1L. This supports linkage data which indicated no linkage between *Cat2* and *Cat3* (data not shown). The use of B-A translocations to map isozyme loci is reliable and conclusive. The determination of the chromosomal locations of the catalase structural genes, the catalase inhibitor gene(s) (Scandalios 1979), and any possible regulatory genes is necessary in order to elucidate the mechanisms underlying the temporal and spatial control of the catalase loci. We have recently identified a temporal regulatory gene (*Car1*) for catalase in *Zea mays*. This gene is 37 map units from *Cat2* (Scandalios et al. 1980b). *Car1* affects mainly the expression of *Cat2*. The determination of the chromosomal location of *Cat2* and *Car1* will enable us to further characterize the system by varying the dosage of *Cat2* and *Car1*, and studying the effects. Furthermore, a study using B-A translocations has demonstrated that *Cat1* and *Cat2* respond linearly to one, two or three doses of the catalase structural genes as determined by cytological analysis (Tsaftaris et al. 1980). This provides further proof that the *Cat1* and *Cat2* chromosome assignments are correct. In addition to the contribution that mapping of the catalase structural genes will have on elucidating the mechanisms of catalase regulation, mapping biochemical markers is a necessity for contributing to the genetic map of *Zea mays*.

Literature

Beckett, J.B. (1978): A-B translocations in maize. 1. Use in locating genes by chromosome arms. J. Hered. **69**, 27-36

Chao, S.E.; Scandalios, J.G. (1969): Identification and genetic control of starch-degrading enzymes in maize endosperm. Biochem. Genet. **3**, 537-547

McMillin, D.E.; Roupakias, D.G.; Scandalios, J.G. (1979): Chromosomal location of two mitochondrial malate dehydrogenase structural genes in *Zea mays* using trisomics and B-A translocations. Genetics **92**, 1241-1250

Nielsen, G.; Scandalios, J.G. (1974): Chromosomal location by use of trisomics and new alleles of endopeptidase in *Zea mays*. Genetics **77**, 679-686

Neuffer, M.G.; Coe, Jr., E.H. (1975): Corn (Maize). In: Handbook of Genetics, Vol. 2 (ed.: King, Robert C.), pp. 3-30. New York: Plenum Press

Neuffer, M.G.; Jones, L.; Zuber, M.S. (1968): The Mutants of Maize. Madison, Wisc. Crop Sci. Soc. America

Ott, L.; Scandalios, J.G. (1978): Genetic control and linkage relationships among aminopeptidases in maize. Genetics **89**, 137-146

Quail, P.H.; Scandalios, J.G. (1971): Turnover of genetically defined catalase isozymes in maize. Proc. Nat. Acad. Sci. (Wash.) **68**, 1402-1406

Rhoades, M.M.; Dempsey, E. (1966): The effect of abnormal chromosome 10 on preferential segregation and crossing over in maize. Genetics **53**, 989-1020

Roman, H. (1947): Mitotic nondisjunction in the case of interchanges involving the B-type chromosome in maize. Genetics **32**, 391-409

Roman, H.; Ullstrup, A.J. (1951): The use of A-B translocations to locate genes in maize. Agron. J. **43**, 450-454

Scandalios, J.G. (1965): Subunit dissociation and recombination

of catalase isozymes. Proc. Natl. Acad. Sci. (Wash.) **53**, 1035-1040

Scandalios, J.G. (1969): Genetic control of multiple molecular forms of enzymes in plants. A review. Biochem. Genet. **3**, 37-79

Scandalios, J.G. (1970): Developmental genetics of catalase in maize. Plant Res. **70**, 140-141.

Scandalios, J.G. (1974): Subcellular localization of catalase variants coded by two genetic loci during maize development. J. Hered. **65**, 28-32

Scandalios, J.G.; Tong, W.F.; Roupakias, D.G. (1980a): *Cat3*, a third gene locus coding for a tissue-specific catalase in maize: genetics, intracellular location, and some biochemical properties. Mol. Gen. Genet. **179**, 33-41.

Scandalios, J.G.; Liu, E.H.; Campeau, M.A. (1972): The effects of intragenic and intergenic complementation on catalase structure and function in maize: A molecular approach to heterosis. Arch. Biochem. Biophys. **153**, 695-705

Scandalios, J.G. (1979): Physiological Genetics. New York: Acad. Press

Scandalios, J.G.; Chang, D.Y.; McMillin, D.E.; Tsaftaris, A.S.; Moll, R.H. (1980b): Genetic regulation of the catalase developmental program in maize scutellum: identification of a temporal regulatory gene. Proc. Natl. Acad. Sci. (Wash.) (in press)

Tsaftaris, A.S.; Scandalios, J.G.; McMillin, D.E. (1980): Gene dosage effects on catalase expression in maize. J. Hered. (in press)

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