CHARACTERIZATION OF AN *SPM*-CONTROLLED BRONZE-MUTABLE ALLELE IN MAIZE

OLIVER E. NELSON AND ANITA S. KLEIN

Department of Genetics, University of Wisconsin, Madison, Wisconsin 53706

Manuscript received June 29, 1983
Revised copy accepted December 20, 1983

ABSTRACT

The association of a receptor (*Rs*) of the *Spm* system with a *Bz-1* allele has created a two-element *Spm*-controlled *bz*-mutable allele (*bz-m13*) of maize (*Zea mays* L.). In the absence of *Spm*, one copy of *bz-m13* (*bz/bz/bz-m13*) conditions full anthocyanin production in the aleurone layer of the seed. In the presence of this *Spm*, *bz-m13* produces a unique, coarsely variegated seed phenotype and has a high rate (50–83%) of gametic change to stable *bz'* or *Bz'* derivatives. Even one copy of a *Bz'* derivative allele conditions full anthocyanin production in the aleurone, but the enzyme (UFGT) level of the progenitor *Bz-1* allele is not restored in most *Bz'* derivatives.

MOBILE genetic elements were first identified and investigated in maize (MCCLINTOCK 1950, 1951), and although such elements have been found in a number of prokaryotic and eukaryotic organisms (SHAPIRO 1983), a number of laboratories are still investigating maize controlling element systems because of the number of different systems identified and the extensive genetic information concerning them. Interest has been heightened still further by the demonstrations that the controlling elements can be investigated at the molecular level (BURR and BURR 1981; DÖRING, GEISER and STARLINGER 1981) and the definite possibility that clones of controlling elements can be used to identify genomic clones of any gene in which a particular controlling element has been inserted.

To interpret fully nucleotide sequence data in relation to the manifold effects of a controlling element insertion on extent, timing and tissue specificity of gene function, it is necessary also to be able to specify the biochemical effects of the insertion. With the objective of elucidating the possible array of effects on gene function, this laboratory started several years ago an investigation of the *Ds*-controlled bronze-1 (*bz1*) mutable alleles of maize. The nonmutant allele(s) at this locus is the structural gene for uridine diphosphate glucose:flavonoid glucosyl transferase (UFGT; EC 2.4.1.91) which catalyzes the last step in anthocyanin biosynthesis (LARSON and COE 1977; DOONER and NELSON 1977a).

In this paper we review the characteristics of *Ds*-controlled bronze-1 mutable alleles since this will aid in distinguishing more fully the novel features of the newly identified bronze-1 mutable allele that is under the control of a different

system (*Spm*). The results of earlier investigations (DOONER and NELSON 1977b; DOONER 1980) have shown that different independent insertions of Dissociation (*Ds*) at the bronze locus may affect gene function in different ways as revealed in assays of enzyme activity of mature seeds of these stocks not carrying Activator (*Ac*). Enzyme activity in mature, dried *Bz/Bz/Bz* seeds is as high at maturity as at any time during development.

The bronze mutables, *bz-m1*, *bz-m2* (*DI*) and *bz-m2* (*DII*) alleles have no detectable UFGT activity and produce no protein that reacts with anti-UFGT antibodies (DOONER and NELSON 1977a). Bronze-weak mutable (*Bz-wm*) endosperms produce about 60% of the anthocyanin content present in *Bz/Bz/Bz* aleurones but have little or no UFGT activity in mature seeds. Low enzymatic activity can be detected in developing *Bz-wm/Bz-wm/Bz-wm* seeds 22 through 36 days postpollination if the activity is assayed immediately after harvest. Freezing, which does not affect the activity of the enzyme coded by *Bz*, destroys the activity of the *Bz-wm* enzyme. This enzyme is considerably more heat labile (half-life at 55°, 0.7 min) than the enzyme encoded by its progenitor *Bz* allele (half-life at 55°, 4.5 min). The processes accompanying seed maturation, which do not affect enzymatic activity in *Bz/Bz/Bz* endosperms, apparently also destroy the *Bz-wm* enzyme. It is clear that the *Bz-wm*-encoded enzyme differs qualitatively from that encoded by the *Bz* allele, although the exact nature of the difference cannot yet be specified.

The change induced by the *Ds* insertion producing the *bz-m4* allele is the most interesting since the enzyme produced cannot be shown to be different from that produced by its progenitor *Bz* allele. The early stage at which maximum enzyme activity is observed and the fact that the greatest enzyme activity is found in the endosperm tissue where no anthocyanins are synthesized rather than in the aleurone layer where virtually all UFGT activity in *Bz/Bz/Bz* seeds is found indicates that it is the regulation of gene activity that is perturbed in this mutable rather than the sequence coding for a protein product. Gerats *et al.* (1983) have suggested as an alternative explanation that deletion of the structural gene at the shrunken-1 locus has placed *bz-m4* under the control of a regulatory sequence for shrunken-1.

Since the phenotypes conditioned by the *bz* alleles are easy to score and since the enzymatic activity of any *bz*-mutable allele can be monitored and ultimately related to the type and position of the insertion sequence, the bronze-1 locus is a useful model with which to investigate controlling element effects. We were desirous of extending our investigations of the locus to include *Spm*-controlled *bz* mutables. The *Spm* system together with the *Ac*, *Ds* system has been intensively investigated from a genetic viewpoint. MCCLINTOCK (1965), FINCHAM and SASTRY (1974), PETERSON (1980) and FEDOROFF (1983) have reviewed both *Ac*, *Ds* and *Spm* as regulators of gene action. As is the case with *Ac*, *Ds*, *Spm*-controlled mutable genes may be either autonomous, in which case *Spm* is inserted in or close to the gene in question, or a two-element system in which the receptor of the system is present at the affected locus and responds to the presence of *Spm* anywhere on the genome. Since the receptors of the *Spm* system have not previously been given a symbol, we suggest that *Rs* (Re-

ceptor of *Spm*) would be appropriate and will so refer to it in this paper. Insertions of an *Spm* receptor (*Rs*) at a locus may produce mutable alleles conditioning a range of stable phenotypes from fully mutant through those approaching a nonmutant phenotype to others with a wholly nonmutant phenotype in the absence of *Spm* (McCLINTOCK 1965). Although we use the McCLINTOCK terminology for the *Spm* system, it should be noted that PETERSON (1953) independently isolated a homologous system in which the components were referred to as *I* and *En* (PETERSON 1965).

When *Spm* is present in the genome, the Suppressor (*Sp*) component inhibits completely gene function in those mutable alleles producing nonmutant or partially nonmutant phenotypes. The mutator (*m*) component of *Spm* then conditions a restoration of gene function in some cells (possibly by an excision of *Rs*), thus giving rise to clones of apparently fully functional cells distributed across a background of mutant cells. In sporogenous tissue, a certain proportion of the gametes produced can be shown to have changed either to stable nonmutant or to stable fully recessive alleles. It should be noted that BURR and BURR (1982) have reported that a nonmutant derivative of a *Ds*-controlled shrunken mutable has retained a 21- to 22-kb insert in the same position as in the shrunken mutable but that there has been extensive rearrangement within the insert.

The *Spm* system of controlling elements is clearly different from and more complex than the *Ac, Ds* system about which we have considerable information concerning its array of effects on gene function (DOONER and NELSON 1977b, 1979; DOONER 1980). We report here the isolation of a two-element *Spm*-controlled mutable bronze allele (*Rs* in a Bronze allele) with an interesting phenotype (coarse variegation rather than the more usual dotting pattern) and a very high rate of gametic change (*bz-m13* to *bz′* and *Bz′* derivatives) in the presence of *Spm*. The biochemical effects of this *Rs* insertion are reported separately (KLEIN and NELSON 1983).

MATERIALS AND METHODS

The starting point for our endeavor to isolate an *Spm* or *Rs*-controlled bronze-1 mutable was a stock originally furnished by BARBARA McCLINTOCK. This stock was *A1, A2, C2, R,* $\overline{\textit{Spm c1-m5}}$ *Sh Bz wx-m8* and had been crossed and backcrossed once to W22 (*A1, A2, Bz1, C1, C2, R*). The *A1, A2, C2* and *R* alleles are the functional alleles at complementary loci required for anthocyanin production in the aleurone layer of the seed. A functional allele at *C1* is also required. The mutable *c1-m5* was derived from the insertion of *Spm* in or adjacent to a functional allele at the *c* locus (McCLINTOCK 1965), and we indicate it here as $\overline{\textit{Spm c1-m5}}$. It is thus an autonomous mutable (carrying the information required for its transposition away from the locus). Its phenotype is a colorless seed on which numerous clones of colored cells can be observed. The *Sh* allele is the structural gene for sucrose synthetase (CHOUREY and NELSON 1976), and seeds with *sh/sh/sh* endosperms have a characteristic shrunken appearance. The *Bz* allele is the structural gene for UFGT, and *bz/bz/bz* seeds (in the presence of functional alleles at all other loci required for aleurone anthocyanin production) have a distinctive bronze color. The waxy alleles result in production by the endosperm of starch that is entirely amylopectin instead of a mixture of amylose and amylopectin. The mutable allele *wx-m8* resulted from the insertion of an *Rs* in a *Wx* allele (McCLINTOCK 1965). Thus, the *Bz* target was flanked proximally and distally by mutable genes of the *Spm* system maximizing the probability that a controlling element sequence excised from one

of the flanking mutables, *Spm c1-m5* or *wx-m8*, would be inserted at *Bz*. The tester stock, which was used both as a male and female parent in crosses with the *Spm c1-m5 Sh Bz wx-m8* stock was W22 *A1, A2, C2, R, C sh bz Wx* or *wx*.

The assays of UFGT activity in endosperms of dry, mature seeds were made by the method of DOONER and NELSON (1977b) as modified by KLEIN and NELSON (1983).

RESULTS

In 1978, plants of the *Spm c1-m5 Sh Bz wx-m8/Spm c1-m5 Sh Bz wx-m8* stocks were used as both male and female parents in crosses with the *C sh bz Wx* tester stock. With the *Spm*-containing stock as a female parent, 466 crosses were made with an estimated 122,200 kernels. Although 11 kernels with color variegation suggesting that they were bronze mutables were identified, none of these kernels produced a plant carrying a *bz*-mutable allele. With several plants of the *Spm*-containing stock as male parents, 23 crosses were made with a total of 4582 kernels, and seven possible *bz*-mutable kernels were identified. In 1979, plants from two of these kernels proved to be carrying a *bz* mutable as demonstrated by the production of variegated kernels (clones of cells with full anthocyanin pigment scattered about on a bronze background) when crossed by a *C sh bz wx* tester stock and were ultimately shown to be under *Spm* control. These bronze-mutable alleles were designated as *bz-m11* and *bz-m13*. We will refer to the phenotype of seeds with *bz-m13* and *Spm* as bronze(bz) variegated. It is clear that there was a significant difference in the frequency of transposition of either *Rs* or *Spm* to *Bz* when the *Spm c1-m5 Sh Bz wx-m8* stock was used as a male parent in crosses to the *C sh bz Wx* tester as compared to its use as a female parent. This disparity has not been investigated further.

Crosses in 1979 onto a *I Ds* tester established that the *bz-m11/bz* and *bz-m13/bz* plants did not carry *Ac* since they were incapable of inducing chromosome breakage at *Ds* with a concomitant loss of *I* (a dominant inhibitor of aleurone pigmentation). In 1980, by crosses onto a *C sh bz wx-m8* tester, both the *bz-m11* and the *bz-m13* stocks were shown to be carrying *Spm* by virtue of their ability to induce *Wx* sectors in *wx-m8*. Additional test crosses to a *C sh bz Wx* tester also were in agreement with the tentative conclusion from the 1979 crosses of *Sh bz-m11 wx/sh bz Wx* and *Sh bz-m13 "wx"/sh bz Wx* by the *C sh bz wx* tester that *bz-m11* was an autonomous mutable and that *bz-m13* was a two-element mutable. In the *bz-m11* crosses, the great majority of the nonshrunken kernels were bronze with nonbronze spots, whereas in the *bz-m13* crosses the nonshrunken kernels might or might not be bronze variegated. The data for *bz-m11* do not exclude the possibility that the regulatory element might be closely linked to *bz* rather than at the locus. Since our objective has been to determine, on a biochemical level, the consequences of an *Rs* insertion within or close to a *Bz* allele, and this can be done only with a two-element system in the absence of *Spm*, our attention has been concentrated on the *bz-m13* allele. Investigations of this allele alone will be reported in this paper.

The chromosome derived from the *Spm c-m5 Sh Bz wx-m8* stock which carried *bz-m13* had the constitution *c Sh bz-m13 "wx"*. The *"wx"* symbol indicates an

intermediate *wx* allele distinguishable from *wx* by staining more deeply with a KI-I_2 solution. It is no longer responsive to *Spm*, but in the presence of *Spm*, there is an infrequent appearance of a *wx*-mutable allele which responds to *Spm* by producing sectors of *Wx* tissue in the endosperm. Although there had been changes at both *c-m5* (to *c*) and *wx-m8* (to "*wx*") on the *bz-m13* chromosome, one cannot conclude that these changes occurred simultaneously with the insertion of *Rs* in the *Bz* allele. Since the *Spm*-containing stock was *Spm c-m5 Sh Bz wx-m8/Spm c-m5 Sh Bz wx-m8* and the seeds for planting in 1978 were selected to ensure that they displayed both $c \rightarrow C$ and $wx \rightarrow Wx$ variegation, a change from *c-m5* to *c* or *wx-m8* to "*wx*" could have occurred in the previous generation or at any time in the development of the plant from which pollen was taken without being detectable.

The types of seeds produced when the original plant carrying *bz-m13* (*C sh bz Wx/c Sh bz-m13* "*wx*") was used as a female parent in a cross with a *C sh bz wx* or *C sh bz Wx* tester are given in Table 1. It is not possible to ascertain from these data what the phenotype of *bz-m13* in the absence of *Spm* is, although the data are compatible with the hypothesis that *bz-m13* has a bronze phenotype in the absence of *Spm*, that there is one copy of *Spm* present that is unlinked to the *bz* locus and that *bz-m13* in the presence of *Spm* mutates frequently to a stable *Bz'* allele but not to *bz*.

That this is not the case was shown by growing plants from *Sh bz* kernels and from *Sh Bz* kernels and crossing them onto a *C sh bz wx*; *Spm* tester stock. The crosses involving plants from *Sh bz* kernels gave only bronze kernels, whereas crosses of some of the plants from *Sh Bz* kernels produced bronze variegated (*bz-m13*) kernels and crosses from other plants gave nonvariegated *Bz* kernels. It was then apparent that the *bz-m13* allele in the absence of *Spm* conditions fully colored (nonmutant) kernels and that these colored, nonshrunken kernels in the cross of 21172A2 (*c Sh bz-m13*; *Spm/C sh bz*; no *Spm*) × *C sh bz* might be of two different sorts—those in which the color is due to *bz-m13* in the absence of *Spm* and those in which the color is conditioned by a derivative of *bz-m13* from which *Rs* has transposed away or an internal rearrangement permitting gene function has taken place. As has been shown by McCLINTOCK (1965), there are numerous instances in which the insertion of a receptor of the *Spm* system in or near a functional allele allows sufficient gene function in the absence of *Spm* that a nonmutant phenotype results.

TABLE 1

Seed phenotypes produced in 1979 when plant 21172A2 (c sh bz Wx/c Sh bz-m13 "wx") *was crossed as a female by a* C sh bz wx *tester*

Cross	Progeny phenotypes						Total
	sh bz	Sh bz	sh Bz	sh bz-var	Sh Bz	Sh bz-var	
21172A2 / C sh bz wx	229	130	2	2	87	60	510

These derivatives of *bz-m13* do not respond to the presence of *Spm*. We shall refer to such a derivative allele as a *Bz'* as has been done for colored derivatives of the *Ds*-controlled *bz* mutable, *bz-m2* [Derivative I (DI)], in a previous paper (DOONER and NELSON 1979). These derivative *Bz'* alleles are designated as *Bz1'-1*, *Bz1'-2*, *Bz1'-3*, etc.

The phenotype of kernels with *bz/bz/bz-m13*; *Spm* endosperms is a coarsely variegated pattern with large clones of colored cells distributed on a bronze background in contrast to the dotted pattern characteristic of most mutable genes affecting anthocyanin production. This pattern results from the interaction of *bz-m13* with an *Spm* that is producing early, but infrequent, events during endosperm development. When the genotype of the endosperm tissue is *bz-m13/bz-m13/bz-m13*; *Spm*, the aleurone is extensively pigmented. In some seeds, it may appear that the aleurone is self-colored (Figure 1).

Coarse variegation of the type shown by *bz-m13* in the presence of *Spm* is not unprecedented for two-element *Spm* system mutables. MCCLINTOCK (1971) has reported such a pattern for the original state of *a2-m1* in response to an *Spm* with an early-acting mutator component, and Peterson (1963) has reported examples of coarse variegation also.

Since *bz-m13* in the absence of *Spm* conditions a colored seed phenotype, it is necessary to account for the large excess of phenotypically *Sh bz* kernels in the crosses in Table 1 over those expected to result from recombination between the *sh* and *bz* loci which are 2 map units apart. The kernels of the complementary crossover class (*sh Bz'* and *sh bz*-variegated) are present in approximately the expected numbers. The *Sh bz/sh bz* plants from these seeds do not respond when crossed by a *sh bz*; *Spm* stock by producing variegated seeds; therefore, it is clear that the vast majority of the *Sh bz* seeds are also derivatives of *bz-m13*, but in these instances, the transposition or rearrangement of *Rs* has resulted in the production of nonfunctional alleles.

From the large number of *Sh bz* seeds in the cross reported in Table 1, one can conclude that there is a high rate of change in *bz-m13* in the presence of *Spm*. To estimate accurately the frequency of germinal changes at *bz-m13* for

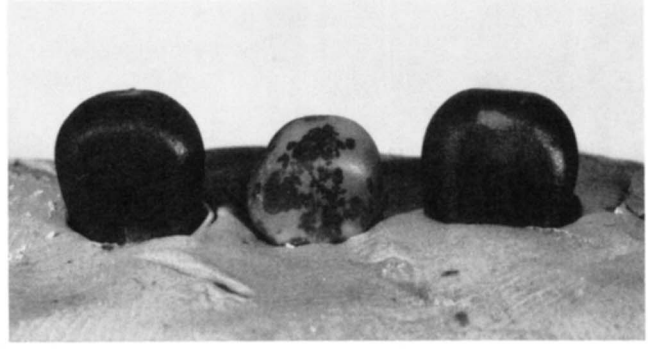

FIGURE 1.—The phenotypes of seeds which are (left to right) *bz/bz/bz-m13*, no *Spm*; *bz/bz/bz-m13*, *Spm* and *bz-m13/bz-m13/bz-m13*, *Spm*.

any plant, however, it is necessary to know what proportion of the *Sh Bz* kernels are stable *Bz'* derivatives. This datum for any plant can be obtained by crossing the plants grown from *Sh Bz* kernels with a tester stock which is *C sh bz wx; Spm*. The plants from *Sh Bz'* seeds produce self-colored kernels from this cross, whereas those from *Sh bz-m13*; no *Spm* seeds produce kernels that are variegated in color.

When colored, nonshrunken kernels resulting from crossing the original plant, 21172A2, carrying *bz-m13* (*C sh bz Wx/c Sh bz-m13* "*wx*") by a *C sh bz wx/C sh bz wx* tester were planted and the plants crossed by a *C sh bz wx; Spm* tester stock, 18 plants carried stable *Bz'* derivatives of *bz-m13*, whereas seven carried *bz-m13*. Assuming this to be a valid estimate of the proportions of *Bz'* and *bz-m13* (no *Spm*) alleles among the fully colored seeds, we can apportion 28% of the *Sh Bz* kernels into the *Sh bz-m13* (no *Spm*) category. Furthermore, since the number of *Sh bz* kernels far exceeds the number expected on the basis of two% recombination between *sh* and *bz* while the reciprocal crossover class (*sh Bz* and *sh bz-m13*) is close to an expected frequency, it is reasonable to assume that the excess of *Sh bz* kernels over expectation is attributable to germinal changes producing stable *bz'* derivatives from *bz-m13*. On this assumption, and using the datum as to the proportion of nonshrunken, colored kernels that are colored derivatives (*Bz'*) of *bz-m13*, it is possible to adjust the data in Table 1 to reflect the germinal changes (*bz-m13* to *Bz'* or *bz'*) that occurred in plant 21172A2.

These adjusted data are given in Table 2 for plant 21172A2 and for its progeny plants that were similarly tested. Since all of these data were derived from crosses of *bz-m13/bz(+Spm)* plants as females by a *C sh bz* tester, premeiotic changes giving rise to large sectors of *Bz'* or *bz'* kernels would have been noted. Such sectoring was not observed. We assume, therefore, that the changes of *bz-m13* to *bz'* and *Bz'* derivatives on the female side are postmeiotic. It can be noted that the rate of gametic change from *bz-m13* to stable *bz'* or stable *Bz'* is very high for the original plant carrying *bz-m13* (21172A2) and the majority of its tested progeny and that changes to *bz'* derivatives are more common than changes to *Bz'* derivatives. The high germinal change rate is not characteristic of all of the progeny of 21172A2, however, as evidenced by the value for 23367-11. Although the gametic change rate has diminished dramatically for this plant, as compared with sib plants, the pattern of variegation on the aleurone layer has not been altered. It appears that the rates of germinal and somatic change may not be closely coupled.

It should be noted that our data concerning the diminution in rates of gametic change in one progeny plant of 21172A2 do not distinguish between the possibilities of alteration in the structure of *Rs* or its relation to the *Bz* allele and that of a change in *Spm* that lessens its mutator activity. A decision as to which component of this two-element system has been affected can be made only after replacing the *Spm* present with an *Spm* from the original stock to ascertain whether the gametic change rate then returns to the high rate characteristic of most descendents of 21172A2.

TABLE 2

Kernel types produced when 21172A2 and a sample of its progeny grown from nonshrunken bronze-variegated seeds (c Sh bz-m13 "wx"/C sh bz Wx/wx; + Spm) *were pollinated by* C sh bz/C sh bz *tester stocks*

	Genotypes produced[a]									
						Sh bz-m13				
Plant	*sh bz*	*Sh bz'* and *Sh bz*[b]	*sh Bz'*	*sh bz -m13*	*Sh Bz'*	*+Spm*	*−Spm*	Total	% Germinal change[c]	% Germinal changes to *bz'*
21172A2	229	130 (5)	2	2	63	60	24	510	76	66
23367-1	126	40 (2)	1	0	17	38	21	243	59	69
23367-4	74	54 (2)	0	1	9	30	17	185	68	85
23367-5	275	39 (6)	0	7	12	89	154	576	34	73
23367-7	245	113 (5)	1	2	25	49	76	511	73	81
23367-8	310	143 (6)	1	4	72	42	57	629	83	66
23367-9	179	73 (4)	1	1	31	31	66	382	76	69
23367-10	194	79 (4)	1	2	20	83	32	411	53	79
23367-11	156	7 (3)	0	4	0	69	76	312	5	100
23367-12	235	106 (5)	0	0	33	96	29	499	58	75
23367-13	125	44 (3)	1	1	18	27	43	259	69	69

[a] The phenotypes of kernels produced by these crosses are as given except that the number of *Sh Bz* (nonshrunken, fully colored) kernels is found by adding the *Sh Bz'* and *Sh bz-m13*, no *Spm* classes. The proportion of nonshrunken, fully colored kernels that were *Sh bz-m13*, no *Spm* was ascertained for each progeny as described in the RESULTS.

[b] The numbers in the parentheses are estimates of the numbers of *Sh bz* kernels expected on the assumption of 2% recombination between *sh* and *bz*. The estimate for most plants agrees well with numbers observed for the complementary crossover class (*sh Bz'* plus *sh bz-m13*).

[c] The percentage of germinal change for *bz-m13* in the presence of *Spm* is calculated as 100 × the sum of (1) excess of *Sh bz* kernels over the expectation from recombination between *sh* and *bz* and (2) the number of *Sh Bz'* kernels divided by the sum of (1) and (2) plus the number of *Sh bz-m13* + *Spm* kernels. The *sh* kernels are not considered in this calculation.

As might be expected of *Sh bz-m13/sh bz*; + *Spm* plants that show a high rate of change to *bz'* and *Bz'* derivatives, there were frequent apparent changes of state altering the phenotype produced when *bz-m13* responds to the presence of *Spm* in the genome. MCCLINTOCK (1965) has discussed the changes of state derived from mutable alleles. Although it remains to be rigorously demonstrated that these alterations are not due to changes in *Spm*, we suspect that the majority stem from changes in *Rs* or its relation to *Bz*. If so, an analysis of nucleotide sequences in these mutable alleles will be illuminating when considered in relation to the expression of pigment by these mutables with apparent changes of state.

Seven stable *Bz'* derivatives of *bz-m13* have been investigated with regard to enzyme activity in mature seeds. The results of these assays are presented in Table 3 with the activities of the derivatives compared to *bz-m13-R5*, the mutable allele from which they were derived, and to *Bz-Mc*, the *Bz* allele which was the progenitor of *bz-m13*. Reference to Table 3 shows that, with the exception of *Bz'-3*, the enzyme activity of the derivatives is markedly lower than *Bz-Mc*, but all are considerably higher than *bz-m13-R5*. It appears then that whatever event(s)—transposition or rearrangement—converted *bz-m13* into stable *Bz'* derivatives, the original organization of the progenitor *Bz-Mc*

TABLE 3

UFGT activity in mature endosperms of stable Bz′ *derivatives of* bz-m13

Genotype	Activity[a]
Bz-Mc[b]	846 ± 53; 692 ± 37
bz-m13R5[b]	22 ± 0.8; 13 ± 0.3
Bz′-1	213 ± 9.8
Bz′-2	317 ± 12; 394 ± 22
Bz′-3	585 ± 32; 743 ± 29
Bz′-4	374 ± 14
Bz′-6	301 ± 13
Bz′-7	134 ± 47

Endosperms were homozygous for the specified allele.

[a] Activity in milliunits per endosperm, where 1 mu equals 1 nmol isoquercetrin formed per hour. The amount of UFGT activity was calculated from the initial rate of incorporation of ^{14}C-glucose into product. This rate was determined from the linear regression of product formed on time of incubation. There were duplicate assay tubes at each of three time points. The error terms are the sample standard deviations of the regression coefficients (SNEDECOR and COCHRAN 1967). Where two values are given for a genotype, different ears were sampled.

[b] *Bz-Mc* is the progenitor *Bz* allele from which *bz-m13* was derived. All *Bz′* derivatives (1 through 7) arose as independent events from *bz-m13-R5* which was one of *bz-m13/bz*; + *Spm* plants from 21172A2 × a *C sh bz* tester.

allele was rarely restored. Although the number of derivatives is small, the results appear to contrast with the stable *Bz′* derivatives of *bz-m2* (*DI*), a *Ds*-controlled bronze-mutable allele, where DOONER and NELSON (1979) found that five of 15 *Bz′* derivatives were indistinguishable from their *Bz-Mc* progenitor with regard to enzyme activity, electrophoretic mobility and thermal stability. Nine of the other ten *Bz′* derivatives had less than 4% of the enzyme activity of the *Bz-Mc* allele. That also contrasts with our observations on *Bz′* derivatives of *bz-m13* in which enzyme activity is considerably higher. It is not clear, however, whether the differences in types of *Bz′* derivatives from *bz-m2* (*DI*) and *bz-m13* are typical of *Ds*-controlled and *Rs*-controlled mutable alleles or whether each type of mutable system could generate a spectrum of *Bz′* derivatives depending on the location of the insertion within the *Bz* allele.

DISCUSSION

The outstanding attribute of *bz-m13* is the high rate of germinal change in the presence of *Spm* compared to most mutable genes. The observed gametic change rate of greater than 70% for the original *bz/bz-m13* plant (21172A2) and 50 to 83% in most of its progeny is very high. The only comparably high rates of gametic change for a mutable allele in maize are those reported by PETERSON (1970) for *a1-m(pa-pu)*, an autonomous mutable with *En* (*Spm*) inserted in an *A1* allele. We note also in Drosophila the high rate of transpositions of *P* elements into *M*-derived *X* chromosomes from *P*-derived autosomes in dysgenic males (BINGHAM, KIDWELL and RUBIN 1982; ENGELS 1983). The figure of 0.82 transpositions/chromosome arm/generation is not directly comparable to our estimates of gametic change per *bz-m13* allele since it measures

transpositions into a chromosome arm from an unknown but large number of *P* elements. We are measuring transposition from and rearrangements in a single site.

If the gametic changes from *bz-m13* to *bz'* and *Bz'* derivatives usually involve a transposition of *Rs* away from the *bz* locus, then *bz-m13*; + *Spm* stocks should be favorable genotypes in which to attempt placing a target locus under control of the *Spm* system as a preliminary step to identifying genomic clones of the locus using *Rs* clones as a probe.

The frequent changes of state that result in phenotypic changes at the *bz* locus could also produce useful experimental material. If most of these changes involve alterations in *Rs* or its relation to the locus as we believe to be the case, these state changes, together with their stable functional or nonfunctional derivatives in conjunction with nucleotide sequences, could be an informative method of dissecting a structural gene and contiguous regulatory sequences. Furthermore, TUSCHALL and HANNAH (1982), who examined five derivative alleles from *sh2-m1* (a *Ds*-controlled mutable allele of a structural gene for ADPglucose pyrophosphorylase) that condition nonmutant phenotypes, found one derivative allele producing 140% of the enzymatic activity of the progenitor *Sh2* allele. Another derivative coded for an enzyme that was markedly less inhibited by inorganic phosphate. As they note, a transposable element insertion in a gene constitutes an efficient locus-specific mutagen capable of generating not only null mutants but interesting variations on a preexisting enzyme.

We are indebted to BARBARA MCCLINTOCK for the *Spm c-m5 Sh Bz wx-m8* stock as well as the *C sh bz wx*; *Spm* and *C sh bz wx-m8* tester stocks, to RUSSELL HUSETH for assistance with our field work and to AMY SMITH for assays of enzyme activity in *Bz'* derivatives of *bz-m13*.

Research supported by the College of Agriculture and Life Sciences, University of Wisconsin-Madison, National Science Foundation grant PCM-8207987 and by National Institutes of Health grant 5 T32 GMO 7131-06A1. This is paper 2663 from the Laboratory of Genetics, University of Wisconsin-Madison.

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Corresponding editor: W. F. SHERIDAN