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NOTES AND COMMENTS

ORIGIN OF THE INACTIVE REGULATORY ELEMENT *Bg-in* OF THE MAIZE *o2m(r)-Bg* SYSTEM OF CONTROLLING ELEMENTS

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1. Introduction

Salamini (1980, 1981) discovered a case of genetic variegation at the opaque-2 locus of maize, controlled by a two element system. A regulatory element, *Bg*, located at an unknown position in the genome, is responsible for the mutability of an opaque-2 responsive allele, *o2m(r)*, which, in the presence of *Bg*, mutates giving rise to sectors of flint-like endosperm in an opaque background. The genetic properties of the system are apparently similar to those previously described for other systems of maize controlling elements (Rhoades, 1938; McClintock, 1950, 1956; Peterson, 1953).

In a recent study (Salamini, 1981) it was found that a significant fraction of the recessive *o2* alleles of spontaneous origin responded to the regulatory element *Bg*. The ability of these receptive alleles to give sectors of normal tissue in the presence of the regulator was, moreover, related to the loss of the receptive function from the controlled locus. This event may take place as early as in one of the two sperm of the male gametophyte or in one of the mitotic divisions of the female gametophyte. In such cases, starting from *o2m(r) Bg* genotypes, normal (wildtype) derivatives were recovered when the gametophyte which had lost the inhibiting element generated the embryo. Such events were found with a frequency of 0·86 per cent (Salamini, 1981); however it was demonstrated that the stable *O2* derivatives still bore two active *Bg* elements (Salamini, 1981).

This paper describes an event which has been observed more rarely, *i.e.*, the origin and behaviour of a mutated state of the regulatory element *Bg*.

2. Materials and methods

Genetic stocks and alleles used in this study were previously described (Salamini, 1981). Seeds of ears from plants grown on the farm of the Experimental Institute for Cereal Research (Bergamo section) during the summer generations of 1977, 1979, 1980 and 1981 and during the greenhouse generations of 1978 and 1981 were classified according to their phenotype. Classification of variegated kernels followed the patterns of variegation reproduced in fig. 1.

3. Results and discussion

(i) *Origin of an ear segregating variegated and opaque kernels in a progeny derived from a* o2m(r) o2m(r) Bg Bg *strain*

The phenotypes of seeds from *o2m(r) o2m(r) Bg Bg* ears were of two types: variegated seeds (about 99%) and wildtype seeds (about 1%; Salamini, 1981). The appearance of the latter was interpreted as the result of events leading from *o2m(r)* to *O2* in one of the two sperm of the male gametophyte or in the female gametophyte. The embryo associated with such wildtype endosperm always had the genotype *o2m(r) o2m(r) Bg Bg*, as revealed by progeny testing.

Such behaviour was seen in a large number of instances. In one such case a selfed plant of genotype *o2m(r) o2m(r) Bg Bg* produced a phenotypically wildtype seed on a fully variegated ear. This seed was grown, and gave, as expected, a fully variegated ear. To check the stability of this culture, a plot of ten plants was grown in the following year. Six plants were selfed and two crossed with *o2R* pollen. Seven out of the eight resulting ears were fully variegated; one selfed ear, however, was exceptional in segregating about $\frac{1}{4}$ opaque seeds (197 variegated and 64 opaque; ear 1979-4069-6 ⊗). This unexpected result led to studies on the nature of the genetic alteration which gave rise to fully opaque kernels.

In 1980, both variegated and opaque seeds from the exceptional ear 1979-4069-6 ⊗ were grown and the resulting plants selfed. Table 1 shows the classification of ears derived from variegated kernels. About one third were fully variegated. Two thirds segregated variegated and opaque seeds with ratios close to 3:1. Such a distribution between the two classes of ears (one third homozygous variegated, two thirds segregating opaques) and the ratios found in the segregating progenies were suggestive of a simple type of genetic alteration associated with the origin of the abnormal ear 1979-4069-6 ⊗. Two findings, however, opposed such an interpretation. Firstly, ears 1980-10116-4 ⊗, 1980-10117-5 ⊗ and 1980-10118-1 ⊗ segregated variegated and opaque kernels with ratios definitely different from 3:1; two of them had a pronounced excess of opaque kernels. Secondly, in the ears 1980-10119-2 ⊗, 4 ⊗, 7 ⊗, 8 ⊗, a consistent proportion of *fine* and *very fine* types of variegated kernels were present.

Even more surprising were the results from progenies derived from opaque seeds of ear 1979-4069-6 ⊗. Two out of four ears were, as expected, opaque (ears 1980-10120-2 ⊗ and 4 ⊗), but the other two did not breed true; they gave respectively 182 variegated and 71 opaques (ear 1980-10120-1 ⊗) and 148 variegated and 44 opaques (ear 1980-10120-3 ⊗).

(ii) *An abnormal* Bg *is present in the progenies of ear 1979-4069-6* ⊗

At the end of the 1980 season, fully homozygous progenies deriving from opaque seeds of plant 1979-4069-6 ⊗ were available. The stability of these opaque derivatives (ears 10120-2 ⊗ and 4 ⊗) was confirmed in 1981. Tests were also done to check which of the two elements of the *o2m(r) Bg* system had mutated. Stable opaque plants were crossed with strains containing *Bg* and an *o2* allele not responsive to it, such as A69Y *o2R o2R Bg Bg*, and strains lacking *Bg* but carrying *o2* alleles responsive to it, such as W64A *o2-ch o2-ch* $+^{Bg}+^{Bg}$, W64A *o2-col o2-col* $+^{Bg}+^{Bg}$,

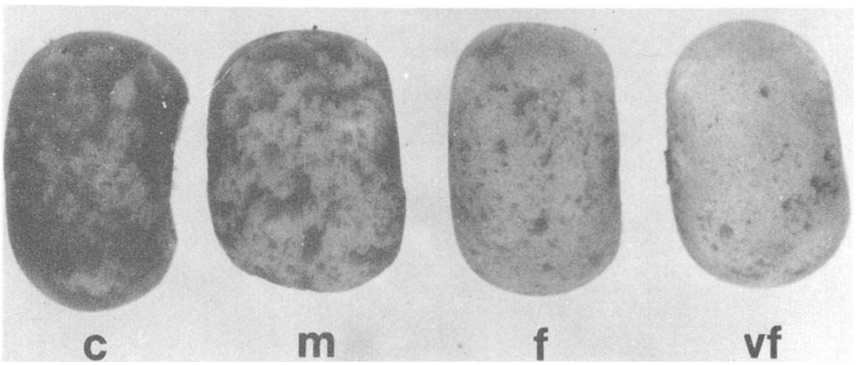

FIG. 1.—Patterns of variegation observed in the materials studied; the four phenotypes were defined as *coarse* (c), *medium* (m), *fine* (f), *very fine* (vf). When classifying variegated kernels, similar types were considered.

TABLE 1

Endosperm phenotypes on ears derived from variegated seeds of plant 1979-4069-6 ⊗

Plot and ear number	Endosperm phenotype		$\chi^2_{3:1}$	Type of variegation[2]
	Variegated[1]	Opaque		
1980-10116-1 ⊗	192	–	–	c
-2 ⊗	230	–	–	c
-3 ⊗	101	24	2·24	c
-4 ⊗	139	68	6·80**	c
-5 ⊗	139	49	0·11	m
1980-10117-1 ⊗	260	–	–	c
-2 ⊗	184	–	–	c
-3 ⊗	191	–	–	m
-4 ⊗	210	48	5·63*	c
-5 ⊗	54	25	1·86	c
-6 ⊗	159	64	1·63	c
1980-10118-1 ⊗	151	124	59·20**	c and f
-2 ⊗	166	50	0·39	c
-3 ⊗	183	63	0·05	c
1980-10119-1 ⊗	351	–	–	c
-2 ⊗	310	–	–	293c, 17vf
-3 ⊗	182	63	0·07	c
-4 ⊗	235	–	–	172c, 46f, 17vf
-5 ⊗	272	76	1·85	c
-6 ⊗	181	59	0·02	c
-7 ⊗	151	65	2·99	f
-8 ⊗	143	46	0·04	f and vf

[1] In this and in the following tables normal seeds always present among variegated seeds (1·1 per cent and 0·78 per cent according to data of Salamini, 1980 and 1981, respectively) deriving from early reversions ($o2m(r) \rightarrow O2$) affecting whole endosperm, were counted under variegated or under coarse variegated.

[2] See fig. 1 for symbols.

* Significant; $P = 0{\cdot}05$.

** Significant; $P = 0{\cdot}01$.

A69Y $o2m(r)\, o2m(r) + {}^{Bg} + {}^{Bg}$. The results of such crosses were unequivocal. When an active regulator was introduced into the cross, fully variegated ears of the *coarse* type were obtained; when the tester strains were devoid of *Bg*, their F_1s with the stable opaque derivatives gave fully opaque ears. The conclusion was that the stable opaque seeds still carried the original responsive $o2m(r)$ allele but their *Bg* activity disappeared. This genetic condition of *Bg* was indicated by the symbol *Bg-in.*

(iii) *Patterns of endosperm variegation in progenies from ears with abnormal segregation*

Ear 1980-10118-1 ⊗ segregated 151 variegated and 124 opaque seeds. Such an abnormal segregation ratio suggested the possibility that in this plant high losses or inactivation of *Bg* had taken place during the last divisions of micro- or megagametophytes. This was verified by studying sample progenies from variegated (table 2) and opaque (table 3) seeds.

Of seven ears from variegated seeds, one was fully variegated (1981-525-1 ⊗; table 2), and three segregated variegated and opaque kernels with ratios near to 3:1 (ears 1981-525-2, 5, 6, 7 ⊗); these ears showed

TABLE 2

Endosperm phenotype on ears derived from variegated seeds of plant 1980-10118 ⊗ which segregated 151 variegated and 124 opaque seeds

Plot and ear number	Endosperm phenotype Variegated[1] c	m	f	vf	total	Opaque	$\chi^2_{3:1(v:o)}$
1981-525-1 ⊗	208	–	–	–	208	–	–
-2 ⊗	29	17	16	43	105	48	3·3
-3 ⊗	3	10	9	17	39	241	557·0**
-4 ⊗	73	11	4	2	90	13	8.4**
-5 ⊗	189	12	–	14	215	79	0·5
-6 ⊗	107	17	–	1	125	45	0·2
-7 ⊗	112	15	5	10	142	62	3·1

[1] See fig. 1 for symbols
** Significant; $P = 0{\cdot}01$.

coarse variegation. One ear had a clearly abnormal segregation ratio (*i.e.*, 39 variegated and 241 opaque seeds (ear 1981-525-3 ⊗); the variegated kernels showed *medium, fine* or *very fine* variegation. The seventh ear of the plot (1981-525-4 ⊗) had a segregation ratio showing an excess of variegated kernels (90 variegated, 13 opaque).

Results obtained with plants grown from opaque seeds were even more surprising (table 3). It was found that some of the phenotypically opaque seeds gave rise to uniformly opaque ears (the case of 1981-524-1, 3, 8 ⊗) but a majority of these seeds yielded ears segregating variegated seeds. These latter progenies, moreover, were all characterized by abnormal segregation ratios (deficiency of variegated seeds) and again, as for ear 1981-525-3 ⊗ of table 2, the majority of the variegated kernels were of the *medium, fine* and *very fine* patterns. Tables 2 and 3 show that when loss or inactivation of *Bg* takes place a concomitant appearance of new patterns of mutability is observed; in the origin or these new patterns the trend is from *coarse* to *medium, fine* and *very fine* types of variegation.

The results presented here suggest that the *Bg* element, the regulator of our system of somatic mutability, could either mutate to inactivity or be lost. It is however noteworthy that the process of loss or inactivation

TABLE 3

Endosperm phenotypes on ears derived from opaque seeds of plant 1980-10118-1 ⊗ which segregated 151 variegated and 124 opaque seeds

Plot and ear number	Endosperm phenotype Variegated[1] c	m	f	vf	total	Opaque	$\chi^2_{3:1(v:o)}$
1981-524-1 ⊗	–	–	–	–	–	250	–
-2 ⊗	78	21	–	30	129	91	31·4**
-3 ⊗	–	–	–	–	–	256	–
-4 ⊗	27	17	–	17	61	218	420·0**
-5 ⊗	102	–	47	–	149	107	38·5**
-6 ⊗	34	–	48	51	133	138	97·1**
-7 ⊗	–	85	–	–	85	106	94·7**
-8 ⊗	–	–	–	–	–	212	–

[1] See fig. 1 for symbols.
** Significant; $P = 0{\cdot}01$.

of *Bg* seems to be preceded by a state of instability as revealed by abnormal segregations and the appearance, starting from a *coarse* type, of new states of mutability, *i.e.*, *medium*, *fine* and *very fine* patterns of seed variegation. The new types of variegation reflect the behaviour of the unstable *Bg* because the stable *o2* derivatives crossed to *o2R Bg* testers not only always gave variegated seeds but they showed the *coarse* phenotype as expected in the absence of mutation at the receptor component of *o2m(r)*.

Ears where opaque phenotypes exceed significantly their predicted 25% may bear at least one unstable regulatory element which generates a fraction of inactive *Bg*'s (*Bg-in* state).

In other systems of maize controlling elements, mutation of the regulatory component has also been reported (reviewed and discussed in McClintock, 1965; Nevers and Saedler, 1977; Peterson, 1981). Mutations affecting the regulator frequently modify the time of occurrence of responses. McClintock (1958) described in detail an unstable behaviour of *Spm* strikingly similar to the unstable *Bg* which anticipates the appearance of *Bg-in*. The study of this particular *Spm* led to the discovery of alternating phases of activity and inactivity of that element. From that time changes in phase of activity of regulators such as *Ac*, *Spm* and *En* have been reported (McClintock, 1964; Peterson, 1966). The *Bg-in* element described here seems stable, at least in the case of those opaque derivatives which breed true for two consecutive generations. At present however, we cannot attribute the mutation to *Bg-in* to inactivation of the element or to a physical loss of *Bg*.

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