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Analysis of a case of somatic instability in a strain of maize from India

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Abstract. A case of somatic instability affecting aleurone colour in a strain of maize from India with flint background was analysed. The somatic instability is localized to the C^1 (Inhibitor) allele of *C* locus on the short arm of chromosome 9. Molecular tests indicated that *Ac* is not present in the Indian stock and the evidence is consistent with the involvement of the *En* (*Spm*) transposable element in the instability. The presence of the *En* (*Spm*)-like element in the stock would suggest that these elements have been present in the maize genome for a long time. A new allele of *shrunken* (*sh1*) gene with a somewhat unorthodox breeding behaviour is also described.

Keywords. Indian maize; aleurone colour instability; C^1 allele; chromosome 9; transposable element *En* (*Spm*); presence of DNA sequences; new *shrunken* 1 allele $sh1^B$.

1. Introduction

Barbara McClintock (1950) first reported the occurrence of transposable elements in maize. Such elements have since been found in several other organisms and in maize alone more than a dozen families have been discovered (see reviews by Nelson 1988; Wessler 1988; Fedoroff 1989). McClintock (1951) noted insertions of transposable elements *Ds* and/or *Ac* at several loci including *C1*. She also detected cases of instability due to another element *Spm*, which was termed *En* and discovered independently by Peterson (1960). Reddy and Peterson (1983) isolated *En*-induced mutability cases at the *C1* locus. Three cases of instability at C^1 have been reported by Peterson, two of which show colourless dots on a coloured background and one which shows coloured dots on a colourless background (Peterson 1986).

Maize cobs that showed some kernels with somatic instability for aleurone colour were obtained from the local market. In addition, stray *shrunken* kernels sometimes appeared in selfed progeny. Studies were carried out to determine the locus and other elements involved in the aleurone colour instability and the basis for the occurrence of *shrunken* kernels. We describe our results in this paper.

2. Materials and methods

2.1 *Maize stocks*

A stock of maize obtained from the local market showed kernels having coloured dots on a colourless aleurone background. This stock is named as PRAN I. This maize has a flint background. The other maize stocks used are listed in table 1.

Table 1. Maize stocks.

Genotype	Source	Phenotype
$a_1 : A_2CR$	Maize Genetics Co op. Stock Centre	Colourless aleurone
$a_2 : ACR$	Maize Genetics Co op. Stock Centre	Colourless aleurone
$c_1 : AA_2RC_2$	Maize Genetics Co op. Stock Centre	Colourless aleurone
$c_2 : AA_2RC$	Maize Genetics Co op. Stock Centre	Colourless aleurone
$r : RAA_2C$	Maize Genetics Co op. Stock Centre	Colourless aleurone
ACR	Local isolate	Coloured aleurone (background : flint)
Ds Sh1 (no *Ac*)	Nina Fedoroff, Carnegie Institution of Washington, Baltimore, USA	Colourless aleurone, shrunken endosperm, used as *Ac* tester stocks
$a^{m(r)}$-$a^{m(r)}$	P. A. Peterson Iowa State University, USA	Colourless aleurone *En* tester stock

2.2 *DNA clones*

Clones carrying transposable element DNA inserts are listed in table 2.

2.3 *Isolation of DNA*

Plant nuclear DNA was isolated as described by Shure *et al.* (1983). Plasmid DNA from *Escherichia coli* was isolated as described by Maniatis *et al.* (1982).

2.4 *Restriction endonuclease digestion*

Restriction enzymes were used in buffers as per the methods specified by the suppliers (BRL, USA).

2.5 *Southern hybridization*

DNA fragments separated on agarose gel were transferred to nitrocellulose paper as

Table 2. Description of DNA clones used.

DNA clones	Source	Characteristics
Ac clone	Nina Fedoroff, Carnegie Institution of Washington, USA	4·3 kb insertion from *wx-m7* allele.
En/Spm clone	A. Gierl, Max-Planck Institut fur Zuchtungs-forschung; Köln, Germany	*En* element is cloned as a Xho-I fragment into pBR322, at its *Sal I* site. It contains 300 bp of flanking *waxy* gene sequences

described by Maniatis *et al.* (1982). The gel was treated with 0·25 N HCl for 8 minutes before soaking in an NaCl–NaOH mixture. Nick translation was performed using a kit from Amersham International, UK, according to their instructions. α-^{32}P-dCTP with a specific activity of 3000 Ci/mmol was used. Hybridization of Southern filters was performed, as described by Maniatis *et al.* (1982), in air-tight, heat-resistant, flat-bottomed plastic boxes.

2.6 *Identification of sucrose synthetase (product of gene Sh1) by polyacrylamide gel electrophoresis*

The method described by Chourey (1981) was used for the identification of sucrose synthetase in non-denaturing polyacrylamide gels.

2.7 *Sucrose synthetase assay*

Developing kernels of standard normal (wild type), *sh1* and *sh1*^{B} (Bombay allele) stocks were harvested 10, 14, 16 and 22 days after pollination. Crude extract was prepared as described by Chourey (1981). The enzyme was assayed for sucrose synthesis as described by Chourey (1981).

3. Results

3.1 *Self-pollination of dotted kernels*

The selfed progenies of dotted kernels for 4 successive generations are shown in table 3. In general, these kernels show a segregation pattern approximately in the

Table 3. Self pollinated progenies of dotted kernels.

Pedigree No.	No. of coloured kernels	No. of dotted kernels	No. of colourless kernels	X^2 (1:2:1)	P
4: 12–7	45	104	50	0·6583	0·7–0·8
4: 13–7	44	105	45	1·3299	0·5–0·7
4: 14–6	22	55	20	1·8247	0·3–0·5
4: 15–5	15	34	18	0·2836	0·8–0·9
4: 16–9	21	51	27	0·8182	0·5–0·7
5: 17–2	32	62	22	2·2759	0·3–0·5
5: 18–4	55	120	56	0·3593	0·8–0·9
5: 19–17	20	46	25	0·5604	0·7–0·8
5: 21–4	66	118	61	0·5343	0·7–0·8
5: 24–75	58	102	50	0·7800	0·3–0·5
6: 24–29	32	60	27	0·4280	0·5–0·7
6: 129–15	60	126	68	0·5178	0·7–0·8
6: 135–22	54	94	52	0·7600	0·5–0·7
6: 22–4	50	112	60	0·9189	0·5–0·7
6: 135–13	68	130	70	0·2673	0·8–0·9
7: 137–4	48	106	40	2·3299	0·3–0·5
7: 129–13	70	126	59	0·9827	0·5–0·7
7: 135–17	48	95	45	0·1170	0·9–0·95
7: 129–11	38	78	48	1·6098	0·3–0·5
7: 140–11	58	102	52	0·6415	0·7–0·8

Table 4. Test crosses of dotted kernels with recessive anthocyanin marker stocks.

♀	Total no. of cobs	No. of coloured kernels	No. of kernels showing dots	No. of colourless kernels	X^2 (1:1)	P
a1	10	1010	1022	—	0·0700	0·7–0·8
a2	6	674	680	—	0·0266	0·8–0·9
c1	20	1075	—	1098	0·2434	0·5–0·7
c2	20	1350	1338	—	0·2934	0·5–0·7
r	15	1304	1322	—	0·1234	0·9–0·95

ratio of 1 (colourless):2 (coloured dots on a colourless aleurone background):1 (coloured). A close-up of one of the cobs from the 4th generation is shown in figure 1.

3.2 *Test-crosses with dotted aleurone kernels*

Phenotypic segregations of plants obtained from crossing dotted kernel stocks to *a1*, *a2*, *c1*, *c2* and *r* testers are shown in table 4. A ratio of 1 (coloured):1(coloured dots on a colourless background) was observed in the kernels of all crosses except the one with *c1*. A ratio of 1 (coloured):1 (colourless) was observed in the kernels when *c1* was used as one parent in the test cross.

Plants grown from dotted kernels when crossed to local *A*, *C*, *R* stock gave segregation ratios as shown in table 5. A ratio of 1 coloured:1 dotted (coloured dots on colourless background) kernels in general suggests that the *C* and *I* (inhibitor) allele is involved.

Segregation patterns in the selfed progeny of dotted kernels and the test cross data indicate that the *C* locus on chromosome 9 and its allele C^I, the dominant inhibitor of anthocyanin pigmentation in aleurone tissue, are involved in the somatic instability. They also suggest the possibility of an insertion of a mobile element into the C^I allele. Transposition of the element away from C^I in C^{Im}/C during kernel development would restore anthocyanin synthesis giving rise to coloured dots on a colourless aleurone background.

3.3 *Analysis of the presence of a transposable element in stocks showing instability*

Plants raised from shrunken kernels carrying *C-I*^{m} were crossed with plants having homozygous *Ds* at *Sh1* (*Ac* tester). The kernels produced by this cross did not show

Table 5. Test crosses of dotted kernels with *ACR* (*local*) stocks.

Pedigree no.	No. of dotted kernels	No. of coloured kernels	X^2 (1:1)	P
7: 2–47 × 7: 24–75	70	75	0·1724	0·5–0·7
7: 2–7 × 7: 24–72	56	60	0·1379	0·7–0·8
7: 2–117 × 7: 24–39	65	72	0·3577	0·5–0·7
7: 2–32 × 7: 24–85	42	45	0·1034	0·7–0·8
7: 2–51 × 7: 24–10	82	78	0·1000	0·7–0·8

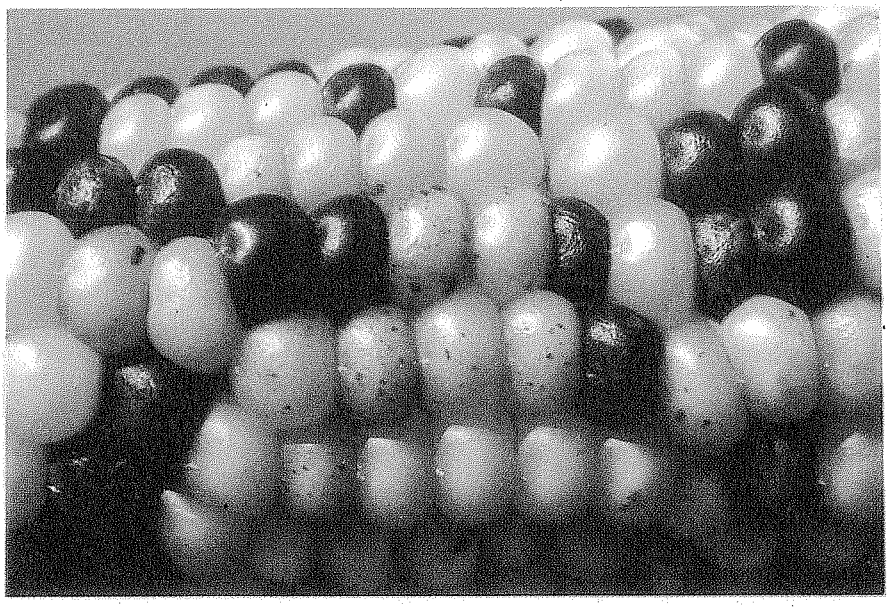

Figure 1. Expression and segregation of somatic instability of a *C-I*^{m} case.

any mutability at the *sh1* locus. This indicates the absence of the *Ac* element in the stock. Its absence was also confirmed by DNA hybridization with an *Ac* DNA probe. The presence or absence of *En* could not be confirmed unequivocally on the basis of genetic analysis. Molecular hybridization was carried out to resolve this problem.

DNA from the leaves of plants with dotted kernels was cut with *Eco* R1 for preparing Southern blots. The plasmid-containing *En* insert was also cut with *Eco* R1 and an internal 3 kb fragment was isolated and used as a probe. Southern hybridization revealed an intense hybridizing signal at 3 kb position (figure 2). This shows that there are *En*-like sequences present in the maize genomic DNA. The above results indicate that the somatic instability at the aleurone layer in the PRAN I stock is due to the presence of an *En* element at C^{1} allele.

3.4 *Genetical studies of shrunken mutants*

PRAN I stock showing somatic instability had 4 out of 152 kernels with a *shrunken* phenotype varying from near-shrunken to non-shrunken (table 6). Very few clear-cut fully-shrunken kernels were present on the cob (figure 3). However, when non-shrunken kernels were dissected, all the kernels showed hollow cavities of varying sizes. To test the allelism of *sh1*^{B}, the plants from mutant kernels were crossed to standard *sh1*. All the kernels were completely *shrunken*. This test showed that *sh1*^{B} is allelic to standard *sh1*.

3.5 *Electrophoretic analysis of sh1*

Endosperm extracts were taken from normal, *sh1*^{B} and standard *sh1* kernels. A

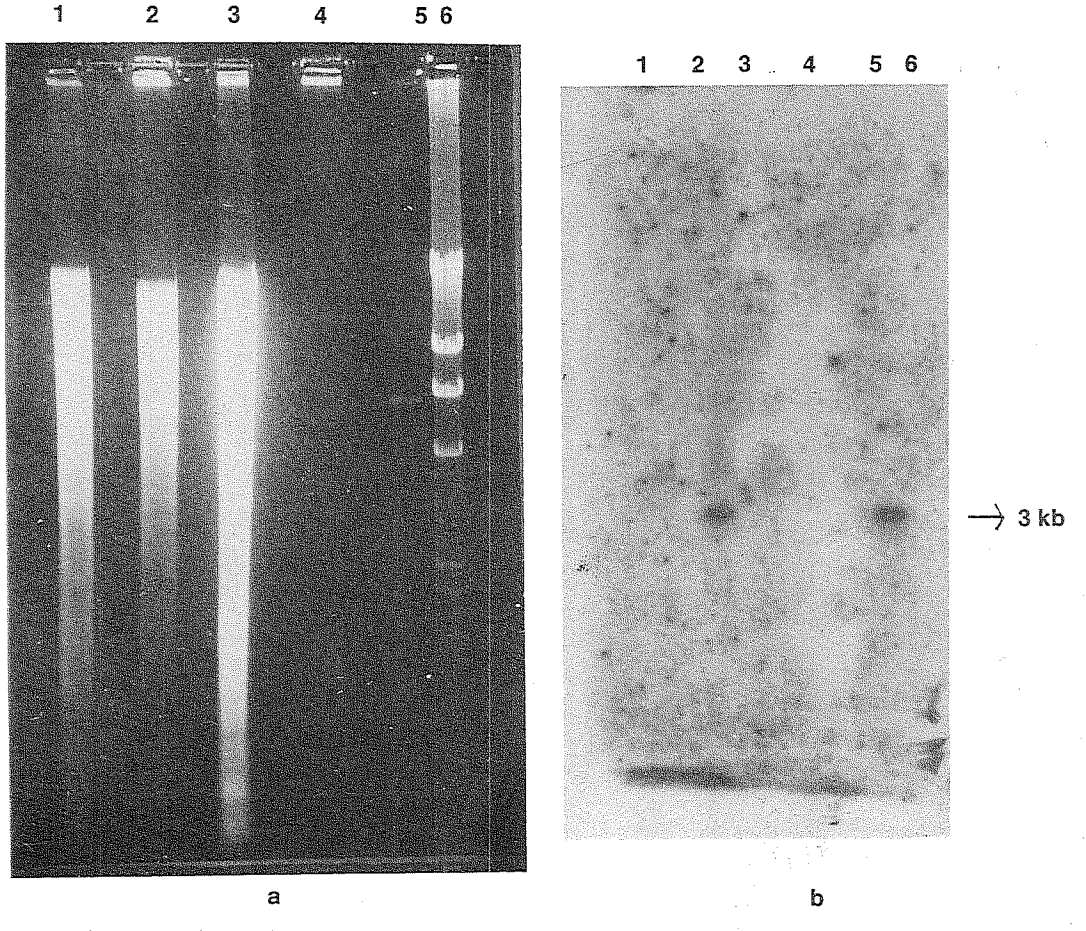

Figure 2. (**a**) Agarose gel electrophorogram of genomic DNA from various genotypes. *Lane 1*: without *En*; *Lane 2*: dotted kernels; *Lane 3*; colourless kernels; *Lane 4*: Blank; *Lane 5*: *En* Plasmid; *Lane 6*: molecular weight marker: λ DNA digested with Hind III. (**b**) Autoradiograph depicting Southern hybridization of the blot prepared from agarose gel as described in figure 2a. The probe used was an internal 3 kb fragment from the *En* clone.

Table 6. Kernels showing varying degrees of shrunkenness.

Pedigree no.	No. of shrunken kernels	No. of kernels with depression	No. of normal (non-shrunken) kernels
4: 76–13	2	85	63
4: 77–25	6	78	58
4: 80–12	8	92	85
4: 80–15	3	86	72
4: 81–4	5	62	48

number of protein bands were seen under non-denaturing conditions, out of which one band was identified as that corresponding to *sucrose synthetase*. Visual differences were observed in the intensity of sucrose synthetase protein in $sh1^{B}$, normal and standard *sh1*. The *sh1* protein band was most intense in the normal, followed by $sh1^{B}$ and lightest for the standard *sh1* (figure 4).

3.6 *Sucrose synthetase assay*

Developing kernels of normal, $sh1^{B}$ and standard *sh1* were assayed for sucrose synthetase activity 10, 14, 16 and 22 days after pollination. The enzyme activity was assayed in the direction of sucrose synthesis. The results are expressed in μg of sucrose synthesized/g of endosperm (average of 3 readings) (table 7). Maximum sucrose synthesis was observed 16 days after pollination. The amount of sucrose synthesized in $sh1^{B}$ was larger (almost double) that in the standard *sh1* allele but it showed definite lower activity as compared to normal wild-type kernels.

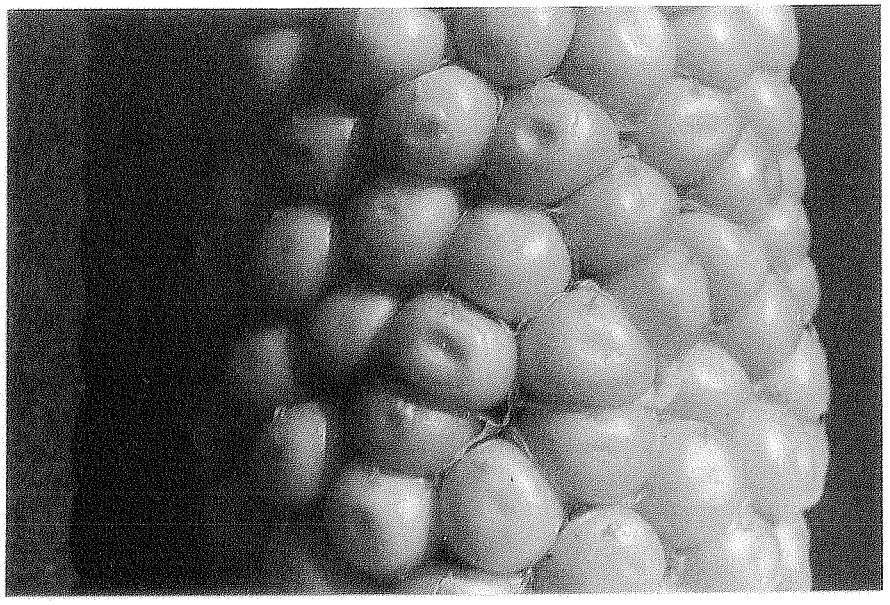

Figure 3. A cob from a self-pollinated plant grown from a *shrunken* kernel.

Table 7. Sucrose synthetase activity in developing endosperm.

Days after pollination	Amt. of sucrose synthesized / Amt. of endosperm (μg/g)		
	Normal	*sh1*^{B}	Standard *sh1*
10	70	25	20
14	175	60	40
16	280	85	40
22	200	90	50

4. Discussion

Our evidence is consistent with the interpretation that an *En* (*Spm*)-like element (Peterson 1960) is conjoined with the C^{I} allele and is responsible for the somatic instability. The relationship amongst the three known alleles viz. C^{I}, C, and c at the locus is somewhat enigmatic. Dominance of the C^{I} allele over C which in turn is dominant over c is at least superficially reminiscent of the dominance of super-repressed allele I^{S} (I is the regulatory gene of beta-galactosidase) over its wild type allele in *E. coli* (Jacob and Monod 1961). At the molecular level the gene product of I is the repressor, an allosteric protein, one site of which binds to the operator region and the other to the inducer molecule. The defect in the inducer-binding site leaves the beta-galactosidase gene repressed forever because the repressor molecule is impervious to the inducer signal and continues to bind to the operator. A similar explanation could hold for C^{I}. Even so, insertion of a transposable element into C^{I} may be expected to yield kernels with colourless dots on a coloured aleurone

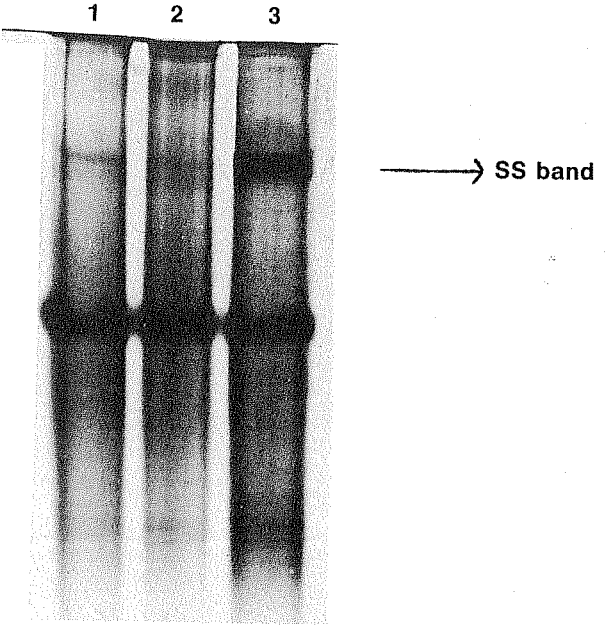

Figure 4. Polyacrylamide gel showing the sucrose synthetase band under non-denaturing conditions of various genotypes, *Lane 1:sh1*^{B}; *Lane 2:* standard *sh*; *Lane 3:* normal kernels.

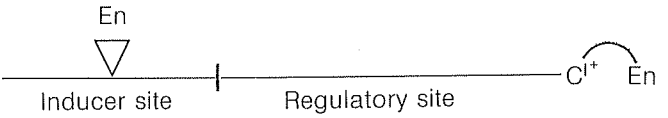

Figure 5. A model for the structure of the C^I allele and the instability due to the insertion of the *En* element in the 'inducer' site.

background. The phenotype observed is, on the contrary, coloured dots on a colourless background. Our explanation and the model are shown in figure 5.

This model envisages that the C^I allele is regulatory in nature and could in fact be structurally separate from *C* (the two genes *I* and *C* could be in tandem). The product of *I* would be an allosteric protein with a regulatory site and an inducer site. The two sites would be reflected in the gene structure. Insertion of an *En/Spm*-like element in the inducer site would, in spite of the induction signal, make the kernel colourless to begin with. When the transposable element moves away, pigment is produced and the aleurone manifests itself as coloured dots.

The behaviour of $sh1^B$ is puzzling in a different way – upon test-crossing to standard American *sh1* stock, all the kernels are shrunken but upon self-pollination, the phenotype is variable. Somehow these phenotypes appear to be more reminiscent of activation and inactivation of an allele by methylation. There is no direct evidence on this point, except that it is suggested from the look of the phenotypes. Speculatively, there is a possibility that when the $sh1^B$ allele enters the 'dent' background of the American tester, it is restricted in expression.

The natural occurrence of *En/Spm*-like elements in Indian stocks would suggest that such elements have been in maize for long. That they contribute to variability is apparent but whether they do it only under stress conditions is somewhat less clear.

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