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Activation of the maize transposable element *Suppressor-mutator* (*Spm*) in tissue culture

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Summary. Previous experiments have revealed that the maize transposable element *Activator (Ac)* may become active during tissue culture. The objective of the present study was to determine whether a second transposable element, *Suppressor-mutator (Spm)*, could also be activated in tissue culture and detected in regenerated maize plants. Approximately 500 R_1 progeny of 143 regenerated plants (derived from 49 embryo cell lines) were crossed as males onto an *Spm*-responsive tester stock. *Spm* activity was observed in two R_1 progeny of a single regenerated plant. This plant had been regenerated from Type II (friable embryogenic) callus of an A188 × B73 genetic background after 8 months in culture; the absence of *Spm* activity in four other plants regenerated from this same callus demonstrates that *Spm* activity was not present before culturing. Approximately 20 *Spm*-homologous DNA sequences were detected in each of the inbreds used to initiate the tissue cultures; it is presumed that one of these became active to give rise to *Spm* activity.

Key words: Somaclonal variation – Transposable elements – *Suppressor-mutator (Spm)* – *Enhancer (En)* – *Zea mays* L.

Introduction

The various genetic and cytological alterations produced by plant tissue culture [collectively termed "somaclonal variation" (Larkin and Scowcroft 1981)] have been described in numerous reports to date. In regenerated plants of a single species (*Zea mays* L.), one can detect morphological and biochemical mutants controlled by single loci (Edallo et al. 1981; Benzion 1984; Lee and Phillips 1987b; Armstrong and Phillips 1988; Brettell et al. 1986; Dennis et al. 1987), quantitative trait variation (Lee et al. 1988; Zehr et al. 1987), and mitochondrial mutants (Brettell et al. 1980; Umbeck and Gengenbach 1983). Changes in chromosome structure, usually involving chromosome breakage, have been detected at a high frequency (Rhodes et al. 1986; Lee and Phillips 1987a; Armstrong and Phillips 1988; Benzion and Phillips 1988). At the DNA level, point mutations (Brettell et al. 1986; Dennis et al. 1987) and methylation changes (Brown and Lorz 1986; Brown 1989; Phillips et al. 1990) have been reported.

Despite the abundance of information on the effects of tissue culture, very little is understood about the mechanisms involved in producing this variability. For example, researchers who have looked for direct relationships between chromosomal abnormalities and morphological mutants have been unable to establish a clear association between the two (Benzion 1984; Lee and Phillips 1987b; Armstrong and Phillips 1988). While it is likely that a number of mechanisms are involved in producing somaclonal variation, one or a few basic processes may still be responsible for initiating "chains of events" that eventually lead to the various outcomes which have been observed.

Several authors (Larkin and Scowcroft 1981; Burr and Burr 1981) have suggested that the tisue culture environment may cause the release or activation of previously silent transposable elements, which would then be capable of producing genetic and cytological change in the cultures and in regenerated plants. McClintock (1950) first observed newly active transposable elements in the progeny of maize plants that had undergone a cycle of

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chromosome breakage, joining, and rebreakage. She proposed (1978, 1984) that the presence of a broken chromosome within a cell causes a "genomic stress", to which the cell may respond by activating transposable elements. A variety of experiments with chromosome-breaking maize stocks (Doerschug 1973; Rhoades and Dempsey 1982) and irradiation (Peterson 1953; Neuffer 1966; Bianchi et al. 1969; Walbot 1988) have all produced transposable element activation. The high frequency of chromosome breakage observed in regenerated maize plants (Rhodes et al. 1986; Lee and Phillips 1987a; Armstrong and Phillips 1988; Benzion and Phillips 1988) indicated that transposable elements were likely to become active in such plants as well.

We have reported that the maize transposable element *Activator (Ac)* (McClintock 1950) could be detected in 2–3% of the regenerated plants tested, even though no *Ac* activity had been present in the materials before tissue culture (Peschke et al. 1987). In at least one case, this activity is correlated with the presence of an *Ac*-homologous DNA sequence (Peschke et al., in press). The objective of the present study was to determine whether a second maize transposable element, *Suppressor-mutator/Enhancer (Spm/En)*, could also become active during the tissue culture process. The *Spm/En* element is an autonomous (self-transposing) element, discovered and named independently by Peterson (*En;* 1953) and McClintock (*Spm;* 1954). *En* and *Spm* were found to be genetically equivalent (Peterson 1965) and to differ at the DNA level by only a few base changes (Pereira et al. 1986; Masson et al. 1987). Several recent reviews of the *Spm/En* transposable element system are available (e.g., Fedoroff 1989; Gierl and Saedler 1989).

The ability to "activate" transposable elements by chromosome breakage and other mechanisms implies that these elements are present in the genome in an inactive form. DNA sequences homologous to the *Ac* (Fedoroff et al. 1983), *Spm* (Cone et al. 1986), *Mutator* (Chandler et al. 1988), and *Bs1* (Johns et al. 1985) elements have been found in every line examined by the respective investigators, despite the lack of transposable element activity in those lines. A second objective of the present study was to examine whether *Spm*-homologous DNA sequences were present in noncultured control materials.

Materials and methods

Source of regenerated plant materials

First-generation progeny of regenerated plants (designated generation R_1) were obtained from previous studies (Lee and Phillips 1987a, b; Armstrong and Phillips 1988). As indicated in Table 1, the materials were derived from Oh43, A188, and B73 genetic backgrounds, and from both Type I (organogenic) and Type II (friable embryogenic) callus. Cultures had been initiated using immature embryos 1–2 mm in length as the explant source, and plants were regenerated 4–22 months after culture initiation. Many of the regenerants and their progeny were examined cytologically and genetically by the initial investigators. Most of these materials were also tested for *Ac* activity (Peschke et al. 1987).

Table 1. Sources of regenerated plants tested for *Spm* activity

	Culture type	Inbred background	No. embryo cell lines	No. regenerated plants
C. L. Armstrong	I, II	B73, A188	30	55
M. Lee	I	Oh43 × A188	19	88
Total			49	143

Spm-responsive tester lines

The *c-m(r)* allele, obtained from P. Peterson (Iowa State University, Ames), was used to test for *Spm* activity. This allele was produced by the insertion of a nonautonomous, *Spm*-responding element (called *dSpm*) into an otherwise functional *C* allele. In the absence of *Spm* activity, *c-m(r)* behaves as a stable recessive allele and results in a colorless aleurone. As diagrammed in Fig. 1, testcrosses of plants without *Spm* activity will produce colorless kernels with aleurone genotype *c-m(r)/c-m(r)/c*. The aleurone tissue obtains two copies of each chromosome from the female parent and one from the male; hence, a triploid genetic constitution is indicated. If *Spm* activity is present in the plant being tested, the *dSpm* can transpose out of the *C* locus, restoring gene function; the phenotype thus produced is a colorless background with colored spots.

Four to six R_1 progeny from each regenerated plant were crossed onto the *c-m(r)* tester stock. These same plants were also selfed to provide progeny seed for further testing. In 1987, approximately 500 scorable testcross ears were harvested and scanned with a hand-held lens. Families that gave evidence of *Spm* activity were retested in 1988 using the *c-m(r)* tester.

A second *Spm*-responsive allele, *brittle-mutable (bt-m)*, was used as an additional test of *Spm* activity (Phillips et al. 1986). This allele contains an insertion of a defective *Spm* element into the *Brittle* locus, necessary for normal endosperm constitution and texture. This test is described in Fig. 2. Since the plants being tested contained the wild-type allele (*Bt*), the testcrosses (*Bt/bt-m*) were selfed to produce homozygous *bt-m* progeny kernels in which the presence or absence of *Spm* activity would be visible. Kernels homozygous for *bt-m* but without *Spm* activity are collapsed in appearance, as is characteristic of stable *bt* alleles. In the presence of *Spm* activity, the *dSpm* element will occasionally transpose, restoring the normal endosperm texture in small sections of the kernel. The resulting mosaic of normal (*Bt*) and *brittle* tissue gives the endosperm a blistered appearance (Phillips et al. 1986).

DNA analysis

Three inbreds (B73, Oh43 *ms13,* and A188) that were used to initiate the majority of tissue cultures represented in this study were tested for *Spm*-homologous DNA sequences, using a clone of *dSpm-13* (Fedoroff et al. 1984) provided by T. Sullivan (University of Wisconsin-Madison). A 454-bp DraI fragment, which includes a complete *Spm* exon as well as surrounding intron sequences, was isolated from the vector by cleavage with DraI (BRL), electrophoresis, and isolation from the agarose via repeated freezing and thawing. The probe was labelled by the

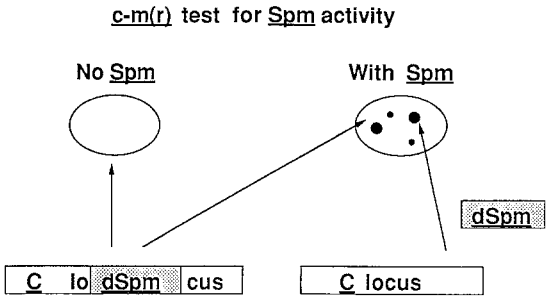

Fig. 1. Schematic drawing of the testcross for *Spm* activity using *c-m(r)*. In the absence of *Spm*, kernels are colorless due to insertion of a defective *Spm* in the *C* locus (*left*). When *Spm* activity is present, the *dSpm* element will occasionally transpose, restoring color in small sectors of the aleurone (*right*)

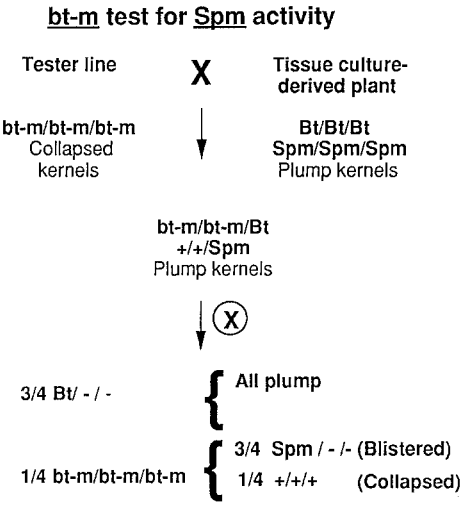

Fig. 2. Illustration of the testcross for *Spm* activity using *bt-m*. The plant being tested in this example is predicted to be homozygous for *Spm* activity based on the previous results of a *c-m(r)* test. Two generations of crosses are necessary since the plants being tested carry a wild-type (*Bt*) allele. Triploid endosperm genotypes are indicated along with the corresponding phenotypes

"random primer" method of Feinberg and Vogelstein (1983). Approximately 100 ng of isolated insert was labelled to a specific activity of 1×10^9 cpm in an 8-h reaction.

Total DNA was isolated from individual seedlings approximately 3 weeks after planting (Shure et al. 1983). The restriction enzyme SstI was obtained from Bethesda Research Laboratories and used in accordance with their recommendations. Overnight digests were electrophoresed in 0.6% agarose in 1 × TBE buffer. After electrophoresis, the gel was soaked with agitation in 0.25 *N* HCl for ca. 30 min, rinsed with water, and blotted onto Zetabind (AMF Products) nylon membrane for 18–24 h using 0.4 *M* NaOH (Reed and Mann 1985). Southern hybridization was done as described by Benner et al. (1989). Filters were hybridized at 65 °C for 18–24 h with constant agitation. After hybridization, filters were rinsed twice using 2 × SCP, 1% SDS, for 20 min at 65 °C, followed by a final rinse (0.2 × SCP, 1% SDS) for 45 min at 65 °C. Blots were exposed to X-Omat X-ray film, using one Lightning Plus intensifying screen, at −70 °C for 10 days.

Fig. 3. A homozygous *c-m(r)* tester kernel without *Spm* activity (*left*). In the absence of *Spm* activity the *c-m(r)* allele behaves as a stable recessive gene. A positive testcross for tissue-culture-derived *Spm* activity (*right*). The colored spots represent transposition of a defective *Spm* element out of the *C* locus, restoring its function in small sectors

Table 2. Testcrosses of plants regenerated from cell line that produced *Spm* activity

Regenerated plant[a]	Progeny tests	Culture type	Months in culture
	Positive : Negative		
283 (1)	2:2	II	8
283 (2) Triplet 1	0:3	II	8
283 (3)	0:7	II	8
283 (4) Twin 2	0:11	II	8
219 (4)	0:4	I	8

[a] The notations "twin" and "triplet" indicate that the regenerated plant was one of a set (of two or three, respectively) which regenerated from the same small piece of callus and was difficult to separate. This distinction does not bear on the present study, but the notation is included to be consistent with that of Armstrong (1986)

Results

Spm activity in progeny of regenerated plants

Approximately 500 R_1 progeny of 143 regenerated plants were crossed as males onto the *c-m(r)* tester stock. The regenerated plants were derived from 49 embryo cell lines, which are described in Table 1. Based on the first set of testcrosses, *Spm* activity was observed in two R_1 progeny of a single regenerated plant [designated 283(1); Armstrong and Phillips 1988]. This plant had been regenerated from Type II (friable embryogenic) callus of an A188 × B73 genetic background after approximately 8 months in culture. Tests of two other R_1 progeny plants were negative, indicating that plant 283(1) was heterozygous for *Spm* activity. A positive testcross is pictured in Fig. 3.

Twenty-five tests of four other regenerated plants from the same embryo cell line were negative for *Spm* activity (Table 2), providing evidence that *Spm* activity was not present in the original materials before culturing. Thirteen tests of the inbreds A188 and B73 were also

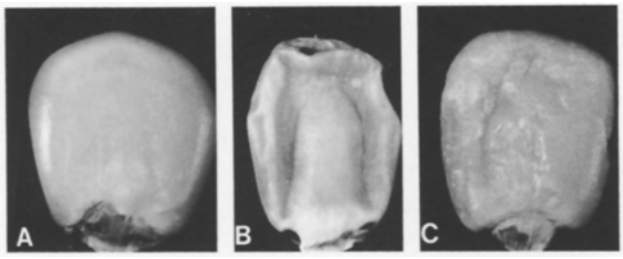

Fig. 4 A–C. Kernels from a single ear produced by the test diagrammed in Fig. 2. **A** A plump (*Bt--*) kernel, which may or may not contain *Spm* activity; **B** A collapsed (*bt-m/bt-m/bt-m*) kernel without *Spm* activity; **C** a blistered-appearing kernel, indicating the presence of *Spm* activity (genotype *bt-m/bt-m/bt-m; Spm--*). The endosperm tissue of this kernel is a mosaic of normal (*Bt*) and *brittle* tissue

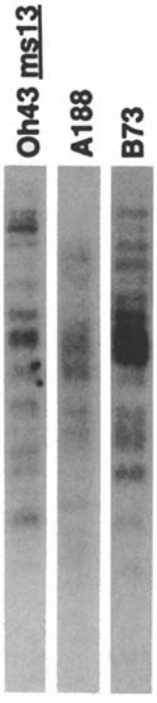

Fig. 5. Analysis of noncultured control materials with the 454-bp DraI fragment from *Spm*. The DNA was cut with SstI, which has no sites within the probe sequence, so the number of bands should approximate the number of *Spm*-homologous sequences in the genome

negative for *Spm* activity. Control kernels (from the same ear as the original embryo) were planted but did not germinate.

A number of plants homozygous for tissue-culture-derived *Spm* activity (based on the *c-m(r)* testcrosses) were crossed onto plants containing the *bt-m* allele; the progeny were then selfed to produce homozygous *bt-m* kernels (Fig. 2). As predicted, approximately three-fourths of the homozygous *bt-m* kernels had the blistered endosperm phenotype characteristic of the interaction of *bt-m* and an active *Spm* element (Fig. 4).

Tests for Spm-homologous sequences

DNA from the inbreds A188, Oh43 *ms13*, and B73 was cleaved with SstI and examined for *Spm*-homologous sequences using the 454-bp DraI probe. SstI does not cut within the portion of *Spm* covered by the probe, so the number of bands detected on a Southern blot should approximate the number of homologous sequences in the genome. About 20 hybridizing bands could be counted in each lane, although more may be hidden behind other bands (Fig. 5). It is presumed that at least some of these bands represent sequences with structure capable of being "activated" to give *Spm* activity.

Discussion

This report describes the finding that the *Spm* transposable element, like *Ac*, may become active during the tissue culture process. Several other researchers have also obtained evidence for activation of transposable elements in tissue culture. Evola et al. (1984, 1985) have reported the activation of the elements *Ac* and *Spm* in tissue-culture-derived maize plants. Culley (1986 and personal communication) has obtained phenotypic and molecular evidence for the activation of *Ac* in maize endosperm cultures initiated from materials containing the *Ac*-responsive aleurone color allele *c-m2*, but without active *Ac* elements. An unstable flower color allele produced by tissue culture of alfalfa (*Medicago sativa* L.) appears to be due to the presence of an autonomous transposable element in the *C2* locus (Groose and Bingham 1986). Its frequency of reversion (from white to purple color) is at least 20 times greater *in vitro* than *in planta* (Bingham et al. 1988).

How transposable elements may cause somaclonal variants

As discussed previously, it has been suggested that transposable elements might be responsible for some of the mutants observed in plants regenerated from tissue culture. Active *Mutator* elements put into tissue culture will transpose *in vitro* (James and Stadler 1989; Planckaert and Walbot 1989), which implies that such elements would be capable of causing mutations and rearrangements. However, there is no apparent association between the transposable element activity we have observed (Peschke et al. 1987; this report) and the mutant occurrence and cytological variation detected by the initial investigators (Lee and Phillips 1987a, b; Armstrong and Phillips 1988). Mutant alleles produced by a transposable element insertion are often characterized by instability; however, few unstable tissue-culture-derived mutants

have been recorded in the literature. The mutable alfalfa allele described above (Groose and Bingham 1986; Bingham et al. 1988) is one notable case of such a variant. An unstable maize cob color allele has also been reported (Woodman and Kramer 1986).

Though instability is often considered to be a hallmark of transposable element activity, genetically stable mutants are often produced by transposable elements. For example, mutants caused by the insertion of a non-autonomous transposable element (e.g., *Ds, dSpm*) are stable in the absence of their corresponding autonomous element. At the DNA level, these events would be apparent as insertions of up to several kilobases in length. More subtle changes are often effected when a transposable element enters and then excises from a locus. Most plant transposable elements create a small duplication in the host DNA at their site of insertion (the "footprint"), which is sometimes, but not always, repaired upon excision of the element (Sachs et al. 1983; Saedler and Nevers 1985; Chen et al. 1986). The genetic effects of these sequence alterations depend on their extent and location in the gene. While some footprints apparently do not interfere with full gene expression (Sachs et al. 1983; Dooner 1980), a number of stable null (Dooner and Nelson 1977) and functional but altered (Dooner and Nelson 1977, 1979; Echt and Schwartz 1981; Chen et al. 1986) genes have been produced by transposable element excision. Some transposable element insertions have produced large deletions in the adjacent host DNA, permanently inactivating the gene into which they have inserted (Peacock et al. 1984; Taylor and Walbot 1985; Dooner et al. 1988). Certain *Ds* elements have long been recognized to break chromosomes (McClintock 1951 a); more recently, *Mu*-induced chromosome breakage has been reported (Robertson and Stinard 1987).

Nevertheless, the few tissue-culture-derived variants at specific loci that have been studied at the DNA level are not obvious results of a transposable element insertion. In two studies involving variants at the maize *Adh1* locus, single-base changes have been detected (Brettell et al. 1986; Dennis et al. 1987). Evola et al. (1985) examined five tissue-culture-derived mutants at the *shrunken* locus using restriction mapping; these mutations apparently occurred late in regeneration because only single pollen grains carried the mutant alleles. Four mutants did not differ from the progenitor allele, while a fifth contained a 50-bp deletion within the transcription unit. Additional such studies would possibly reveal transposable element insertions if they are occurring, as well as other types of rearrangements and mutations. However, as noted above, some transposable element-mediated genetic or chromosomal changes might be unusual enough not to be recognized as such, even at the DNA level.

Mechanisms for activation of transposable elements

Although it is not clear what role tissue-culture-derived transposable elements play in generating genetic variability in regenerated plants, the elements themselves may be regarded as genes that have been altered in a significant way by the tissue culture process. It is probable that the mechanism(s) responsible for their activation in tissue culture are similar to changes affecting other loci and chromosome regions as well. The two major hypotheses regarding the activation of transposable elements involve (1) chromosome breakage and (2) changes in methylation.

Chromosome breakage per se may allow transposable elements to become active by "freeing" them from surrounding heterochromatin. This phenomenon, known as "position effect," has been documented for *Oenothera* (Catcheside 1939) and *Drosophila* (Sturtevant 1925). Stadler (1941) found no evidence for position effect in maize, based on his studies of translocation stocks. However, McClintock's early studies of the break-fusion-bridge cycle demonstrated that most rearrangements initiated by the cycle involved the nucleolus organizer region (NOR), knobs, and/or centromeres, all of which are heterochromatic regions (McClintock 1951 a, 1978). It is not known whether the same frequency of transposable element activation would have occurred had the rearrangements involved only euchromatin.

The role of DNA methylation in the activation of transposable elements has been the subject of intense study in recent years. Many researchers have observed correlations between transposable element activity and hypomethylation at specific sites within the elements (Schwartz and Dennis 1986; Chandler and Walbot 1986; Chomet et al. 1987; Bennetzen 1987, Banks et al. 1988; Schwartz 1989; Dennis and Brettell 1990). *Ac* elements activated in tissue culture are often highly unmethylated at internal PvuII and HpaII sites, while *Ac*-homologous sequences in sibling plants without *Ac* activity are highly methylated (Peschke et al., in press). A few reports on other plant genetic systems also correlate hypomethylation with gene activity (Hepburn et al. 1983; Bianchi and Viotti 1988), although cases where no apparent correlation exists can also be found (Nick et al. 1986).

It should be noted that chromosome breakage and methylation changes are not necessarily independent events. For example, chromosome breakage events could cause perturbations in the normal methylation pattern of a gene. Methylation is a signal for DNA mismatch repair (Hare and Taylor 1985), and chromosome breakage can lead to hypo- or hypermethylation (Grafstrom et al. 1984). Possibly the transient hypomethylation associated with newly repaired DNA regions can cause the activation of a transposable element, especially if the DNA

methyltransferase does not "keep up" with repair and if hemimethylated DNA is replicated (Walbot 1988).

A third hypothesis (Burr and Burr 1988) is that transposable elements are activated through point mutations produced by an "SOS-type" response similar to that observed in bacteria. As a first step in examining this mechanism, Burr and Burr (1988) tested whether maize transposable elements could be activated via point mutations produced by low doses of ethyl methanesulfonate (EMS). *Spm* activation was observed at a frequency 10 × over background in plants from EMS-treated seeds. In addition, the events occurred later in development after the EMS treatment had been applied, indicating that activation had occurred several cell generations after treatment. This type of delayed reaction would be consistent with an SOS-type response (Burr and Burr 1988).

Frequency of transposable element activation in tissue culture

The finding that *Spm* as well as *Ac* activity can be detected in our materials indicates that activation of transposable elements in tissue culture may be a common event. While each of these two elements has been detected in only a small percentage of the plants tested, at least 12 other transposable element systems have been identified in maize (Peterson 1986). Given that some of these others are known to be "activated" by stresses such as chromosome breakage or viral infection (Johns et al. 1985; Doerschug 1973; McClintock 1951 b; Rhoades and Dempsey 1982), it is probable that they could be affected by tissue culture as well. In addition, the *Ac* and *Spm* activities were detected in plants from separate experiments that used different culture types (Type I versus Type II), different culture media (modified MS versus N6 + proline), and different genetic backgrounds (Oh43 × A188 versus A188 × B73) (Armstrong and Phillips 1988; Lee and Phillips 1987a, b). This result demonstrates that activation of transposable elements in tissue culture is not limited to a specific set of conditions, although it is likely that certain genotypes and culture conditions would cause different frequencies of activation. In our studies, this frequency was not obviously related to the number of transposable element-homologous DNA sequences in the lines used to initiate the tissue cultures, since all of the lines had similar numbers of such cryptic sequences (this report; Peschke et al., in press). However, a number of these sequences may be defective and incapable of genetic activity.

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References

Armstrong CL (1986) Genetic and cytogenetic stability of maize tissue cultures: a comparative study of organogenic and embryogenic cultures. PhD thesis, University of Minnesota-St. Paul

Armstrong CL, Phillips RL (1988) Genetic and cytogenetic variation in plants regenerated from organogenic and friable, embryogenic tissue cultures of maize. Crop Sci 28:363–369

Banks JA, Masson P, Fedoroff N (1988) Molecular mechanisms in the developmental regulation of the maize *Suppressor-mutator* transposable element. Genes Dev 2:1364–1380

Benner MS, Phillips RL, Kirihara JA, Messing JW (1989) Genetic analysis of methionine-rich storage protein genes in maize. Theor Appl Genet 78:761–767

Bennetzen JL (1987) Covalent DNA modification and the regulation of *Mutator* element transposition in maize. Mol Gen Genet 208:45–51

Benzion G (1984) Genetic and cytogenetic analysis of maize tissue cultures: a cell line pedigree analysis. PhD thesis, University of Minnesota-St. Paul

Benzion G, Phillips RL (1988) Cytogenetic stability of maize tissue cultures: a cell line pedigree analysis. Genome 30:318–325

Bianchi MW, Viotti A (1988) DNA methylation and tissue-specific transcription of the storage protein genes of maize. Plant Mol Biol 11:203–214

Bianchi A, Salamini F, Parlavecchio R (1969) On the origin of controlling elements in maize. Genet Agrar 22:335–344

Bingham ET, Groose RW, Ray IM (1988) Activation of a mutable allele in alfalfa tissue culture. In: Nelson OE (ed) Plant transposable elements. Plenum Press, New York, pp 325–338

Brettell RIS, Thomas E, Ingram DS (1980) Reversion of Texas male-sterile cytoplasm maize in culture to give fertile, T-toxin resistant plants. Theor Appl Genet 58:55–58

Brettell RIS, Dennis ES, Scowcroft WR, Peacock WJ (1986) Molecular analysis of a somaclonal mutant of maize alcohol dehydrogenase. Mol Gen Genet 202:235–239

Brown PTH (1989) DNA methylation in plants and its role in tissue culture. Genome 31:717–729

Brown PTH, Lorz H (1986) Molecular changes and possible origins of somaclonal variation. In: Semal J (ed) Somaclonal variation and crop improvement. Martinus Nijhoff, Dordrecht, The Netherlands, pp 148–159

Burr B, Burr F (1981) Transposable elements and genetic instabilities in crop plants. Stadler Genet Symp 13:115–128

Burr B, Burr FA (1988) Activation of silent transposable elements. In: Nelson OE (ed) Plant transposable elements. Plenum Press, New York, pp 317–324

Catcheside DG (1939) A position effect in *Oenothera*. J Genet 38:345–352

Chandler VL, Walbot V (1986) DNA modification of a maize transposable element correlates with loss of activity. Proc Natl Acad Sci USA 83:1767–1771

Chandler VL, Talbert LE, Raymond F (1988) Sequence, genomic distribution, and DNA modification of a *Mu1* element from non-mutator maize stocks. Genetics 119:951–958

Chen CH, Freeling ML, Merckelbach A (1986) Enzymatic and morphological consequences of *Ds* excisions from maize *Adh1*. Maydica 31:93–108

Chomet PS, Wessler S, Dellaporta SL (1987) Inactivation of the maize transposable element *Activator (Ac)* is associated with its DNA modification. EMBO J 6:295–302

Cone KC, Burr FA, Burr B (1986) Molecular analysis of the maize anthocyanin regulatory locus *C1*. Proc Natl Acad Sci USA 83:9631–9635

Culley DE (1986) Evidence for the activation of a cryptic transposable element *Ac* in maize endosperm cultures. 6th Int Congr Plant Tiss Cell Cult, Minneapolis/MN. Abstracts, p 220

Dennis ES, Brettell RIS (1990) DNA methylation of maize transposable elements is correlated with activity. Philos Trans R Soc Lond Ser B 326:217–229

Dennis ES, Brettell RIS, Peacock WJ (1987) A tissue-culture-induced *Adh1* null mutant of maize results from a single base change. Mol Gen Genet 210:181–183

Doerschug EB (1973) Studies of *Dotted,* a regulatory element in maize. 1. Induction of *Dotted* by chromosome breaks. 2. Phase variation of *Dotted.* Theor Appl Genet 43:182–189

Dooner HK (1980) Regulation of the enzyme UFGT by the controlling element *Ds* in *bz-m4,* an unstable mutant in maize. Cold Spring Harbor Symp Quant Biol 45:457–462

Dooner HK, Nelson OE Jr (1977) Controlling element-induced alterations in UDP glucose: flavonoid glucosyltransferase, the enzyme specified by the *bronze* locus in maize. Proc Natl Acad Sci USA 74:5623–5627

Dooner HK, Nelson OE Jr (1979) Heterogeneous flavonoid glucosyltransferases in purple derivatives from a controlling element-suppressed *bronze* mutant in maize. Proc Natl Acad Sci USA 76:2369–2371

Dooner HK, Ralston E, English J (1988) Deletions and breaks involving the borders of the *Ac* element in the *bz-m2(Ac)* allele of maize In: Nelson OE (ed) Plant transposable elements. Plenum Press, New York, pp 213–226

Echt CS, Schwartz D (1981) Evidence for the inclusion of controlling elements within the structural gene at the waxy locus in maize. Genetics 99:275–284

Edallo S, Zucchinali C, Perenzin M, Salamini F (1981) Chromosome variation and frequency of spontaneous mutation associated with *in vitro* culture and plant regeneration in maize. Maydica 26:39–56

Evola SV, Burr FA, Burr B (1984) The nature of tissue-culture-induced mutations in maize. 11 th Annu Aharon Katzir-Katchalsky Conf, 8–13 January, 1984, Jerusalem. Abstract

Evola SV, Tuttle A, Burr F, Burr B (1985) Tissue-culture-associated variability in maize: molecular and genetic studies. 1st Int Congr Plant Mol Biol, Savannah/GA. Abstracts, p 10

Fedoroff NV (1989) About maize transposable elements and development. Cell 56:181–191

Fedoroff N, Wessler S, Shure M (1983) Isolation of the transposable maize controlling elements *Ac* and *Ds*. Cell 35:235–242

Fedoroff N, Shure M, Kelley S, Johns M, Furtek D, Schiefelbein J, Nelson O (1984) Isolation of *Spm* controlling elements from maize. Cold Spring Harbor Symp Quant Biol 49:339–345

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Gierl A, Saedler H (1989) The *En/Spm* transposable element of *Zea mays*. Plant Mol Biol 13:261–266

Grafstrom RH, Hamilton DL, Yuan R (1984) DNA methylation: DNA replication and repair. In: Razin A, Cedar H, Riggs AD (eds) DNA methylation: biochemistry and biological significance. Springer, New York, pp 111–126

Groose RW, Bingham ET (1986) An unstable anthocyanin mutation recovered from tissue culture of alfalfa (*Medicago sativa*). 1. High frequency of reversion upon reculture. 2. Stable nonrevertants derived from reculture. Plant Cell Rep 5:104–110

Hare JT, Taylor JH (1985) One role for DNA methylation in vertebrate cells is strand discrimination in mismatch repair. Proc Natl Acad Sci USA 82:7350–7354

Hepburn AG, Clark LE, Pearson L, White J (1983) The role of cytosine methylation in the control of nopaline synthase gene expression in a plant tumor. J Mol Appl Genet 2:315–329

James MG, Stadler J (1989) Molecular characterization of *Mutator* systems in maize embryogenic callus cultures indicates *Mu* activity in vitro. Theor Appl Genet 77:383–393

Johns MA, Mottinger J, Freeling M (1985) A low copy number, *copia*-like transposon in maize. EMBO J 4:1093–1102

Larkin PJ, Scowcroft WR (1981) Somaclonal variation – a novel source of variability from cell cultures for plant improvement. Theor Appl Genet 60:197–214

Lee M, Phillips RL (1987a) Genomic rearrangements in maize induced by tissue culture. Genome 29:122–128

Lee M, Phillips RL (1987b) Genetic variants in progeny of regenerated maize plants. Genome 29:834–838

Lee M, Geadelmann JL, Phillips RL (1988) Agronomic evaluation of inbred lines derived from tissue cultures of maize. Theor Appl Genet 75:841–849

Masson P, Surosky R, Kingsbury JA, Fedoroff NV (1987) Genetic and molecular analysis of the *Spm*-dependent *a-m2* alleles of the maize *a* locus. Genetics 117:117–137

McClintock B (1950) The origin and behavior of mutable loci in maize. Proc Natl Acad Sci USA 36:344–355

McClintock B (1951a) Chromosome organization and genic expression. Cold Spring Harbor Symp Quant Biol 16:13–47

McClintock B (1951b) Mutable loci in maize. Carnegie Inst Wash Yearbk 50:174–181

McClintock B (1954) Mutations in maize and chromosomal aberrations in Neurospora. Carnegie Inst Wash Yearbk 53:254–260

McClintock B (1978) Mechanisms that rapidly reorganize the genome. Stadler Genet Symp 10:25–47

McClintock B (1984) The significance of responses of the genome to challenge. Science 226:792–801

Neuffer MG (1966) Stability of the suppressor element in two mutator systems of the *A1* locus in maize. Genetics 53:541–549

Nick H, Bowen B, Ferl RJ, Gilbert W (1986) Detection of cytosine methylation in the maize alcohol dehydrogenase gene by genomic sequencing. Nature 319:243–246

Peacock WJ, Dennis ES, Gerlach WL, Sachs MM, Schwartz D (1984) Insertion and excision of *Ds* controlling elements in maize. Cold Spring Harbor Symp Quant Biol 49:347–354

Pereira A, Cuypers H, Gierl A, Schwarz-Sommer Zs, Saedler H (1986) Molecular analysis of the *En/Spm* transposable element system of *Zea mays*. EMBO J 5:835–841

Peschke VM, Phillips RL, Gengenbach BG (1987) Discovery of transposable element activity among progeny of tissue-culture-derived maize plants. Science 238:804–807

Peschke VM, Phillips RL, Gengenbach BG. Genetic and molecular analysis of tissue culture-derived *Ac* elements. Theor Appl Genet, in press

Peterson PA (1953) A mutable pale-green locus in maize. Genetics 38:682–683

Peterson PA (1965) A relationship between the *Spm* and *En* control systems in maize. Am Nat 99:391–398

Peterson PA (1986) Mobile elements in maize. Plant Breed Rev 4:81–122

Phillips RL, Block LG, Peschke VM, Burnham CR (1986) A suppressor-mutator transposable element system of independent origin. Maize Genet Coop Lett 60:115–117

Phillips RL, Kaeppler SM, Peschke VM (1990) Do we understand somaclonal variation? In: Nijkamp HJJ, Van Der Plas LHW, Van Aartrijk J (eds) Progress in Plant Cellular and Molecular Biology: Proceedings, 7th International Congress on Plant Tissue and Cell Culture. Kluwer Acad Publ, Dordrecht, The Netherlands, pp 131–141

Planckaert F, Walbot V (1989) Molecular and genetic characterization of *Mu* transposable elements in *Zea mays:* behavior in callus culture and regenerated plants. Genetics 123:567–578

Reed KC, Mann DA (1985) Rapid transfer of DNA from agarose gels to nylon membranes. Nucleic Acids Res 13:7207–7221

Rhoades MM, Dempsey E (1982) The induction of mutable systems in plants with the high-loss mechanism. Maize Genet Coop Lett 56:21–26

Rhodes CA, Phillips RL, Green CE (1986) Cytogenetic stability of aneuploid maize tissue cultures. Can J Genet Cytol 28:374–384

Robertson DS, Stinard PS (1987) Genetic evidence of *Mutator*-induced deletions in the short arm of chromosome nine of maize. Genetics 115:353–361

Sachs MM, Peacock WJ, Dennis ES, Gerlach WL (1983) Maize *Ac/Ds* controlling elements – a molecular viewpoint. Maydica 28:289–301

Saedler H, Nevers P (1985) Transposition in plants: a molecular model. EMBO J 4:585–590

Schwartz D (1989) Gene-controlled cytosine demethylation in the promoter region of the *Ac* transposable element in maize. Proc Natl Acad Sci USA 86:2789–2793

Schwartz D, Dennis E (1986) Transposase activity of the *Ac* controlling element in maize is regulated by its degree of methylation. Mol Gen Genet 205:476–482

Shure M, Wessler S, Fedoroff N (1983) Molecular identification and isolation of the *waxy* locus in maize. Cell 35:225–233

Stadler LJ (1941) The comparison of ultraviolet and X-ray effects on mutation. Cold Spring Harbor Symp Quant Biol 9:168–178

Sturtevant AH (1925) The effects of unequal crossing-over at the *Bar* locus in *Drosophila.* Genetics 10:117–147

Taylor LP, Walbot V (1985) A deletion adjacent to the maize transposable element *Mu1* accompanies loss of *Adh1* expression. EMBO J 4:869–876

Umbeck P, Gengenbach BG (1983) Reversion of male-sterile T-cytoplasm to male fertility in tissue culture. Crop Sci 23:584–588

Walbot V (1988) Reactivation of the *Mutator* transposable element system following gamma irradiation of seed. Mol Gen Genet 212:259–264

Woodman JC, Kramer DA (1986) The recovery of somaclonal variants from tissue cultures of B73, an elite enbred line of maize. 6th Int Congr Plant Tiss Cell Cult, Minneapolis/MN. Abstracts, p 215

Zehr BE, Williams ME, Duncan DR, Widholm JM (1987) Somaclonal variation in the progeny of plants regenerated from callus cultures of seven inbred lines of maize. Can J Bot 65:491–499