Variable Transposition of Eight Maize Activator (Ac) Elements Located on the Short Arm of Chromosome 1

William F. Sheridan
Department of Biology, University of North Dakota, Grand Forks, North Dakota 58202

ABSTRACT Eight Activator (Ac) transposable elements mapped to the maize chromosome arm 1S were assessed for Ac transposition rates. For each of the Ac stocks, plants homozygous for the single Ac element and the Ds reporter r1-sc:m3 on chromosome 10 were crossed as females by a homozygous r1-sc:m3 tester color-converted W22 line. The resulting ears produced mostly coarsely spotted kernels and a low frequency of either near-colorless fine-spotted kernels or nonspotted kernels. The relative frequency of these two types of near-colorless kernels differed among the eight Ac stocks. The extent to which increased Ac dosage results in nonspotted kernels may be Ac-specific. Although all of the Ac elements are in near-isogenic inbred W22 lines, they varied to a large extent in their transposition frequency. These differences might possibly result from structural differences among the Ac elements. Because one pair of Ac elements derived from Ac33 on chromosome arm 5S differed about 13-fold in transposition frequency and a second pair of Ac elements derived from Ac12 on chromosome arm 1S differed about 3-fold in transposition frequency, this is not a likely explanation for all eight Ac elements. The data presented here support the notion that the differences in transposition frequency of the eight Ac elements may be a reflection of variability in Ac transcription or accessibility of the transposase to the Ac element, resulting from differences in the chromatin environments wherein the Ac elements are located. This is the first report of variability in transposition rates among different Ac donor lines.

KEYWORDS

maize
Ac elements
transposition rate
variability

Transposon tagging with the maize Ac element is a useful tool for regional mutagenesis (Federoff et al. 1984; Brutnell and Conrad 2003; Singh et al. 2003). Two features of the Ac element make it a tractable system for gene tagging. There are several genes controlling anthocyanin synthesis in the aleurone and embryo that contain Ds element insertions and can serve as reporter loci for the presence of an Ac element (McClintock 1955; Dooner et al. 1994). In addition, the delayed timing of Ac transposition in tissues containing increased Ac copy number provides a means of assessing the occurrence of an Ac transposition event by observing the size of pigmented sectors in the aleurone and embryo tissues of kernels.

MATERIALS AND METHODS

A collection of Ac-containing, near-isogenic, color-converted W22 inbred lines was produced by Kolkman et al. (2005); these included 41 precisely mapped Ac elements. The present report concerns 8 of these lines, all containing Ac elements mapped to the short arm of chromosome 1. The results presented here concern the variability in transposition frequency of these Ac elements observed while pursuing a regional mutagenesis program on this chromosome arm. For each of the Ac stocks, plants homozygous for the single Ac element and the Ds reporter r1-sc:m3 on chromosome 10 were crossed as females by a homozygous r1-sc:m3 tester color-converted W22 line (Figure 1).

Scoring of ears for Ac transposition events

The ears borne on plants homozygous for the Ac element produce mostly coarsely spotted kernels and a low frequency of near-colorless kernels. The coarsely spotted kernels display the pattern of colored sectors expected when the nuclei of the female gametophyte contain...
96-99% coarsely spotted kernels 1-4% finely spotted kernels

Figure 1 Crossing scheme to produce finely spotted kernels. The chromosome constitutions of the embryos are shown. In most cases, the Ac element does not transpose and the embryo contains a single copy of the Ac element, whereas the aleurone cells of the endosperm contain two copies (one Ac from each of the two polar nuclei of the embryo sac). This dosage results in coarse spotting of the kernel aleurones. In a few cases, the Ac element replicates, and subsequently, one copy transposes to a new site. When both copies are transmitted to the embryo sac (whether they are linked as shown in the figure or are on different chromosomes), the embryo will have two copies of the Ac element and the aleurone will have four copies, resulting in a finely spotted aleurone.

Initially, the transposition frequencies were calculated on the basis of transposition events per ear. The number of ears scored for each of the Ac stocks ranged from 24 to 77. Among the eight Ac stocks, the mean values ranged from a high frequency of 9.67 fine-spotted kernels and 6.34 nonspotted kernels per ear (bti00252::Ac) to a low frequency of 0.58 fine-spotted kernels and 0.03 nonspotted kernels per ear (mon00106::Ac) (supporting information, Table S1, Figure S1). Subsequently, all of the kernels on the scored ears were weighed to provide an estimate of the total number of kernels per ear. The total estimated number of kernels examined was approximately 108,000. These data were used to calculate an estimated frequency of fine-spotted and nonspotted kernels per 1000 kernels for each of the eight Ac elements. The mean values ranged from a high frequency of 38.80 fine-spotted kernels and 26.28 nonspotted kernels per 1000 kernels (bti00252::Ac) to a low frequency of 2.15 fine-spotted kernels and 0.15 nonspotted kernels per 1000 kernels (mon00106::Ac) (Table 1). The frequency of transposition of Ac elements per 1000 kernels for the individual families of the eight Ac elements is shown in Table S2.

Variation in Ac dosage effects
An average transposition frequency of 2 to 4% (20 to 40 per 1000 kernels) was reported by Kolkman et al. (2005) based on their examination of approximately 12,400 kernels generated from 10 different Ac lines. No data were provided for any of the individual lines. In this report, the Ac transposition frequency as evidenced by the frequency of near-colorless, fine-spotted kernels ranged from 0.215% to 3.880%. When the near-colorless nonspotted kernels are included in the

Table 1 Frequency of transposition of Ac elements per 1000 kernels from eight sites on maize chromosome arm 1S

| Donor Ac element | Source of Aca | Bin location | Number of ears scored | Total number of kernels scored | Number of fine spotted kernels per 1000 kernels | Number of nonspotted kernels per 1000 kernels |
|------------------|---------------|--------------|-----------------------|-----------------------------|-----------------------------------------------|-----------------------------------------------|
| mon03080         | r-nj:m1 10L   | 1.02         | 31                    | 9411                        | 11.66 ± 2.3 bc                            | 24.35 ± 2.96 ac                              |
| bti95004         | P1-vv 1S      | 1.02/0.03    | 51                    | 13774                       | 13.57 ± 1.48 bc                           | 20.85 ± 1.79 abc                             |
| mon00106         | Ac33 5S       | 1.02/0.03    | 77                    | 21257                       | 2.15 ± 0.37 e                            | 0.15 ± 0.15 f                                |
| bto00228         | P1-vv 1S      | 1.03         | 71                    | 19032                       | 9.67 ± 0.97 b                            | 7.43 ± 1.26 d                                |
| mon00192         | Ac12 1S       | 1.03         | 58                    | 16629                       | 12.74 ± 1.17 bc                           | 10.71 ± 1.25 de                              |
| bti95006         | P1-vv 1S      | 1.03         | 25                    | 6470                        | 18.85 ± 1.96 c                           | 15.22 ± 2.75 cde                             |
| bto00252         | Ac12 1S       | 1.04/0.05    | 24                    | 6121                        | 38.80 ± 3.84 a                           | 26.28 ± 3.28 a                               |
| mon00068         | Ac33 5S       | 1.05         | 57                    | 15100                       | 27.81 ± 1.96 d                           | 16.49 ± 1.69 be                              |

a The source of the Ac elements that transposed into the mapped sites on chromosome arm 1S are presented together with the chromosome arm location of the source Ac element (Kevin Ahern, personal communication).

b Mean values with the same letter are not significantly different at the 0.05 level. See Table S3 and Table S4 for ANOVA and Tukey comparisons.

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calculations, then the frequency of Ac transposition ranges from 0.230% to 6.508%. Kolkman et al. (2005) noted differences in the degree of variation in Ac-mediated Ds variegation patterns (the size of sectors or spots) in kernels homozygous for independent Ac insertions. These researchers further noted that, inasmuch as all of the Ac elements they studied were in near-isogenic lines using the same Ds reporter, it was not likely that the variations they observed resulted from differences in the reporter gene or segregating modifier loci.

RESULTS

The size of spots on self-pollinated kernels on ears of plants homozygous for the eight different Ac stocks differed only slightly in size, and there was no obvious relationship with their Ac transposition frequency. The same degree of similarity of spotting size was observed on kernels of plants homozygous for Ac elements that were crossed as females by the reporter stock. When plants homozygous for Ac elements were crossed by pollen of the reporter stock, the relative frequency of fine-spotted to nonspotted kernels differed among the eight Ac elements (Table 1, Figure S1). In the case of the two most distally located Ac elements (mon03080::Ac and bti95004::Ac), the number of nonspotted kernels per 1000 kernels was greater than the number of spotted kernels per 1000 kernels. However, for the six most proximally located Ac elements, the frequency of spotted kernels was greater than the frequency of nonspotted kernels. The relative frequency of nonspotted kernels may depend on the level of transposase transcription by the Ac elements, and this level may vary among the Ac elements at both their original sites on chromosome arm 1S and at the target sites of the tr-Ac elements. Where the sum of the resulting transposase levels is high enough, then the negative dosage effect (Heinlein 1996; Kunze and Weil 2002) exerted upon transposition of Ac elements, the frequency of nonspotted kernels per 1000 kernels is likely a result of differences in the transcriptional activity of the Ac elements. However, this is not a likely explanation for all eight cases. An examination of Table 1 reveals that the source of both mon00106::Ac and mon00068::Ac was Ac33 on chromosome arm 5S, yet their transposition frequencies differed about 13-fold. Likewise, the source of both mon00192::Ac and bti00252::Ac was Ac12 on chromosome arm 1S, yet their transposition frequencies differed about 3-fold. The data presented here support the notion that the differences in transposition frequency of these eight mapped Ac elements may be a reflection of variability in Ac transcription or accessibility of transposase to the Ac element, resulting from differences in the chromatin environments wherein the Ac elements are located.

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