Transposition Pattern of the Maize Element *Ds* in *Arabidopsis thaliana*

Ian Bancroft and Caroline Dean

AFRC Institute of Plant Science Research, Cambridge Laboratory, John Innes Centre, Colney, Norwich NR4 7UJ, England

Manuscript received November 30, 1992
Accepted for publication April 12, 1993

ABSTRACT

As part of establishing an efficient transposon tagging system in Arabidopsis using the maize elements *Ac* and *Ds*, we have analyzed the inheritance and pattern of *Ds* transposition in four independent Arabidopsis transformants. A low proportion (33%) of plants inheriting the marker used to monitor excision contained a transposed *Ds*. Selection for the transposed *Ds* increased this to at least 49%. Overall, 68% of *Ds* transpositions inherited with the excision marker were to genetically linked sites; however, the distribution of transposed elements varied around the different donor sites. Mapping of transposed *Ds* elements that were genetically unlinked to the donor site showed that a proportion (3 of 11 tested) integrated into sites which were still physically linked.

THE small dicotyledonous plant *Arabidopsis thaliana* has become an important model system for many aspects of plant science (MEYEROWITZ 1987). An insertional mutagenesis system based on the Agrobacterium T-DNA has been developed and used to clone a number of genes (FELDMANN *et al.* 1989). The availability of an efficient transposon tagging system in Arabidopsis would extend the use of insertional mutagenesis. Although transposable elements have recently been identified in Arabidopsis (KONIECZNY *et al.* 1991; PELEMAN *et al.* 1991; TSAY *et al.* 1993), they are not sufficiently well characterized for use in transposon tagging experiments. Many groups have therefore introduced heterologous transposon systems into Arabidopsis and most success to date has been with the maize *Ac/Ds* transposons (VAN SLUYS, TEMPE and FEDOROFF 1987; SCHMIDT and WILLMITZER 1989; MASTERSON *et al.* 1989; BALCELLS, SWINBURNE and COUPLAND 1991; BANCROFT *et al.* 1992; SWINBURNE *et al.* 1992; GREVELDING *et al.* 1992). We have used a *Ds* element cloned into the 5′-untranslated leader of a streptomycin phosphotransferase fusion (SPT) that confers resistance to streptomycin (Sm). Somatic excision of *Ds* is visualized as green sectors on bleached cotyledons after plating seedlings on Sm-containing medium (JONES *et al.* 1989). Plants inheriting a germinal excision event are fully resistant to streptomycin and have a fully green (FG) phenotype on streptomycin-containing media. The *Ds* carries a hygromycin phosphotransferase fusion, conferring resistance to hygromycin (Hm). The *Ds* element has been transactivated by a stabilized *Ac* (*sAc*) carrying a 537-bp *Nae*I deletion in the 5′-untranslated leader of the transposase. In Arabidopsis, this modification significantly increases the excision frequency of an autonomous *Ac* element (E. LAWSON, C. SJODIN, S. SCOFIELD, J. D. G. JONES and C. DEAN, in preparation) and *Ds* transactivation frequency (BANCROFT *et al.* 1992). The transposase source was linked to a β-glucuronidase (GUS) fusion so that progeny carrying the transposase source could be readily identified. This two element system has been analyzed with respect to position effect of different transformants, dosage effect of the different elements and environmental effects on transposition (BANCROFT and DEAN 1993) and has recently been used to tag a gene required for normal Arabidopsis development (BANCROFT, JONES and DEAN 1993).

To extend and maximize the efficiency of this tagging system, we have investigated the inheritance of transposed *Ds* elements and the pattern of transposition. In maize, tobacco and Arabidopsis, a transposed *Ac* element was inherited with the donor locus at frequencies of 42%, 58% and 53%, respectively (DOONER and BELACHEW 1989; DOONER *et al.* 1991; KELLER *et al.* 1992). Preliminary investigations of the inheritance of *Ds* in Arabidopsis suggested that a large proportion of progeny inheriting the donor locus did not carry transposed *Ds* elements (MASTERSON *et al.* 1989; BANCROFT *et al.* 1992; ALTMANN, SCHMIDT and WILLMITZER 1992). This analysis clearly needed to be extended before large scale tagging experiments involving *Ds* were initiated. In maize and tobacco, germinal transposition of *Ac* was found to be preferentially to closely linked sites (GREENBLATT 1984; DOONER and BELACHEW 1989; DOONER *et al.* 1991). The pattern of transposition varied from locus to locus. In contrast, many transposition events in tomato were to unlinked sites (OSBORNE *et al.* 1991). We wished to determine if there were any receptor site preferences in Arabidopsis, as this would have an impact on the success of different tagging strategies. If *Ac/Ds* transposes preferentially to linked sites, targeted tagging (from a linked *Ds*-containing insertion) would be feasible. For nontargeted tagging strategies,

activation of elements from many sites across the genome would be required to efficiently tag genes from all over the genome.

In this study, the proportion of plant lines inheriting the excision marker and at least one copy of *Ds* has been determined. Identification of the *Ds* allele present (a transposed *Ds*, a nonexcised *Ds*, or both) allowed us to assess the proportion of plants containing a transposed *Ds* (*tDs*) and hence a potential mutagenic event. We then assessed the distribution of genetic linkage of transposed *Ds* relative to the excision marker for four *Ds* transformants. In addition, we mapped three of the four *Ds* donor sites and ten transposed *Ds* elements from one of these, relative to phenotypic markers.

MATERIALS AND METHODS

Plant lines: *sAc* and *Ds* elements were introduced into *A. thaliana* ecotype Landsberg *erecta* using Agrobacterium T-DNA transformation, as described previously (BANCROFT *et al.* 1992). The transformants used in this investigation were Δ*Nae*I *sAc* transformant-1 (used in all transactivation experiments) and -4 (mapping of site of integration of T-DNA only) and Hm^R *Ds* transformants -B1, -A3, -E1 and -C12b (BANCROFT *et al.* 1992; BANCROFT and DEAN 1993). The transformant reference for *Ds*-containing lines is also used to denote the T-DNA insertion locus in those lines.

Plant growth and selection: Plants were grown and antibiotic resistance analysis was performed as described previously (BANCROFT *et al.* 1992).

Genetic analysis: Genetic analysis of the linkage relationships of transposition donor and receptor sites was performed on populations of seedlings segregating for both hygromycin (Hm) (*tDs* locus) and streptomycin (Sm) (T-DNA locus) resistance. These populations were either the progeny of self-pollinated (F_2) FGs that were heterozygous for activated SPT, heterozygous for a *tDs* and did not contain a nonexcised *Ds*, or were the F_2 progeny resulting from a back cross of the progeny of a FG with Arabidopsis ecotype Landsberg *erecta*, the F_1 of which was selected for resistance to both Hm and Sm. These populations of seeds were plated on medium containing both Hm and Sm. Three phenotypes were scored: Hm^RSm^R, fully green and expanded; Hm^RSm^S, expanded, but bleached; Hm^S, stunted and usually green. We observed that Sm resistant seedlings frequently showed some bleaching of the cotyledons, but never the hypocotyl. Several populations showed substantial numbers of seedling which showed this phenotype. They were considered unreliable for mapping purposes and were not scored. The populations used for mapping contained very few seedlings (<1%) for which there was any ambiguity in scoring for Sm resistance. Scoring for Hm resistance was unambiguous.

The genetic map positions of T-DNA integrations carrying *Ds* and *sAc* constructs and of transposed *Ds* elements were determined by linkage analysis relative to some of the phenotypic markers in the multiply marked tester line W100 (KOORNNEEF *et al.* 1987). The lines to be tested were crossed to male sterile W100 individuals. The F_1 seedlings were selected on appropriate antibiotics and allowed to self-pollinate to provide the segregating F_2 populations. The F_2 seeds were sown in Petri dishes on medium containing Km (*sAc* T-DNAs) or Hm (all others). Resistant plants were scored for the mutant phenotypes *hy2* (long hypocotyl), *gl1* (*glabrous*, no trichomes) and *an* (*angustifolia*, narrow leaves) while still in the Petri dishes and for *ap2* (*apetala*, reduced petals), *bp* (*brevipedicellus*) and *ms1* (male sterile) after transplanting and growth on soil. The other markers (*cer2*, *py*, and *tt3*) were found difficult to score reliably and so were not included in the analysis.

For both sets of linkage analysis, markers were considered to be significantly linked if χ^2 tests indicated the segregation was significantly different at the 5% level to that resulting from unlinked markers. Where significant linkage was observed, recombination frequencies were calculated by the method of ALLARD (1956). These were converted to map distances using the genetic mapping function of KOSAMBI (1944).

TABLE 1

Proportions of seedlings showing germinal excision of *Ds* that have retained Hm resistance and proportion that have retained Hm resistance but have not inherited *sAc*, after transactivation of Hm^R *Ds* at different loci by Δ*Nae*I *sAc*-1

Ds line	No. of FGs investigated	Percent of FGs that are Hm^R	Percent of Hm^R FGs that are GUS^-
Hm^R *Ds*-B1	673	68	22
Hm^R *Ds*-A3	108	74	33
Hm^R *Ds*-E1	59	80	17
Hm^R *Ds*-C12b	164	38	26
Overall	1004	68	23

RESULTS

Inheritance of *Ds* with excision marker: Studies have shown that transposed *Ac* elements can be inherited with a marker used to monitor their excision in several plants including maize, tobacco and tomato (DOONER and BELACHEW 1989; DOONER *et al.* 1991; YODER *et al* 1988). Small scale studies have shown the same for *Ac* and *Ds* in Arabidopsis (MASTERSON *et al.* 1989; DEAN *et al.* 1992; BANCROFT *et al.* 1992; KELLER *et al.* 1992). A more detailed analysis was required in order to quantify the extent of inheritance of transposed elements in Arabidopsis. Plant lines containing Hm^R *Ds* at four different loci were crossed to the plant line containing Δ*Nae*I *sAc*-1 and 1004 FG F_2 seedlings (each inheriting a germinal excision event that we expect to be independent; BANCROFT and DEAN 1993) were generated among the progeny of self-pollinated F_1 plants. To determine the proportion that had inherited a *Ds* element, these seedlings were transferred to medium containing Hm and were scored for resistance or sensitivity. In order to confirm that the *sAc* (which was linked to the GUS fusion) was segregating as expected (*i.e.*, *ca.* 25% of F_2 plants should lack *sAc* and therefore be GUS^-), all Hm-resistant FG (Hm^R FG) seedlings were tested for GUS activity. The results are summarized in Table 1. They show that the FG seedlings (*i.e.*, the seedlings inheriting an active SPT fusion) from transactivation of *Ds* from loci B1, A3 and E1 all show similar proportions of inheritance of Hm resistance and hence *Ds*. The inheritance of *Ds*

from locus C12b, however, shows a much lower frequency, about half that of the other three. This demonstrates that there are differences between the proportions of *Ds* elements inherited with the excision marker for different *Ds* loci. Both the somatic and germinal excision frequencies of *Ds* from C12b have previously been shown to differ from those of the other three loci in that excision occurs at about a fourfold higher frequency (BANCROFT and DEAN 1993). The proportions of Hm^R FG seedlings inheriting no *sAc* are close to those expected. A slightly higher proportion of FG seedlings from locus A3 were GUS^- as that locus is weakly linked (in repulsion in F_1 plants) to the transposase source in the line Δ*Nae*I *sAc*-1 (detailed in "Genetic mapping of T-DNAs relative to phenotypic markers" section below).

Analysis of *Ds* alleles present in Hm^R FG seedlings: Although the test for Hm resistance of the F_2 FG plants shows the proportion of seedlings that inherit no *Ds*, the Hm resistance scored could come from either a *tDs* or a nonexcised *Ds* still in the SPT donor locus. In order to determine the proportions of *tDs* elements that were inherited along with the excision marker, we progeny tested the Hm-resistant FG plants. Tests were performed on the progeny of plants that were GUS^- (*i.e.*, lack *sAc*) for the segregation ratios of the markers, Sm resistance (to show whether active SPT is heterozygous or homozygous), Km resistance (the marker carried by both the *sAc*- and *Ds*-containing T-DNAs) and Hm resistance (the marker carried by *Ds*). In the progeny of plants that were GUS^+ (*i.e.*, contain *sAc*), tests were performed for the segregation of Sm and Hm resistance and for the presence of variegation in their progeny (variegation would indicate the presence of a nonexcised *Ds*). Combining data from these segregation ratios and tests, as summarized in Figure 1, allowed the identification of the alleles present at the SPT locus, *i.e.*, whether there was a nonexcised *Ds* present at that locus, and hence whether there was a *tDs* present at another locus. The results are summarized in Table 2.

The balance of *Ds* alleles present in the Hm^R FG seedlings from each locus is similar. The overall proportion of Hm^R FG seedlings that contain a *tDs* is at least 49% (24% *tDs* only plus 25% *tDs* and nonexcised *Ds*). We have not determined the proportion of plants showing anomalous segregations that contain *tDs* in detail, but the few we have investigated (using molecular techniques) generally do not contain a *tDs* (data not shown). As 68% of FG seedlings inherited at least one *Ds*, 33% (49% × 68%) of all *tDs* elements were inherited along with the excision marker at the donor locus. This compares with proportions of 42%, 58% and 53% for equivalent figures for *Ac* in maize (DOONER and BELACHEW 1989), tobacco (DOONER *et al.* 1991) and Arabidopsis (KELLER *et al.* 1992), respectively. We can therefore confirm the previous finding (from a sample size of 21 plants analyzed in detail, BANCROFT *et al.* 1992), that the frequency of inheritance of *tDs* with the excision marker is somewhat lower in Arabidopsis than is the frequency of inheritance of *Ac* in maize, tobacco and Arabidopsis. With the aid of selection for Hm resistance, the proportion of plants containing *tDs* can be increased to at least 49%. There seems to be very little difference between the four *Ds* loci in the proportions of Hm resistant FG seedlings that contain *tDs*, despite the overall inheritance of Hm resistance being substantially lower from C12b.

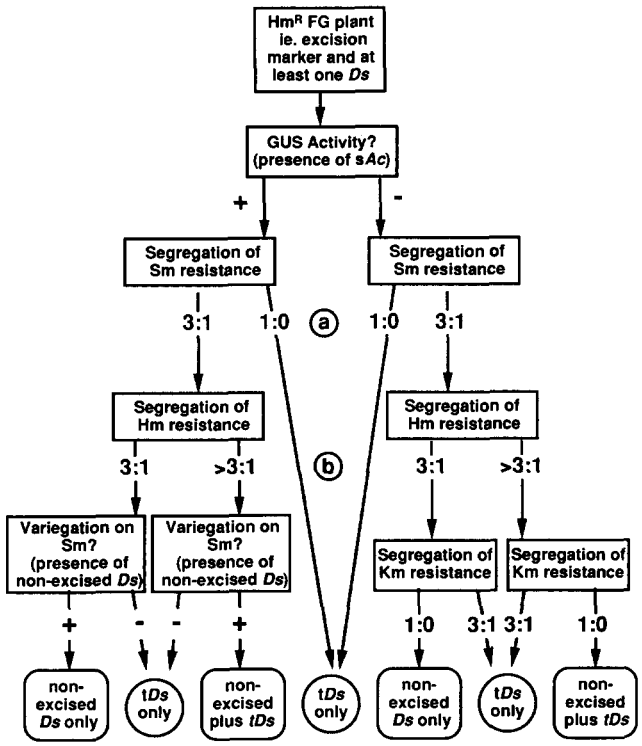

FIGURE 1.—*Ds* allele determination by progeny testing of Hm^R FG plants. Segregation ratios are shown resistant:sensitive; **a**, 1:0 = homozygous for excision (nonexcised *Ds* cannot be present), 3:1 = heterozygous for excision (nonexcised *Ds* may be present); **b**, 3:1 = one copy of *Ds* present, >3:1 = more than one copy of *Ds* present.

Linkage analysis of transposed *Ds* elements and their donor T-DNA loci: In both maize and tobacco, *Ac* transposes preferentially to linked sites [61%, 59% in maize (GREENBLATT 1984; DOONER and BELACHEW 1989; respectively); 72% in tobacco (DOONER *et al.* 1991)], but there are variable patterns of transposition from different loci. In tomato, a lower proportion (25%) of *Ac* elements were found to have transposed to linked sites (OSBORNE *et al.* 1991). In order to define the patterns of *Ds* transposition in Arabidopsis, we undertook a detailed analysis of the linkage of 111 *tDs* elements to their original T-DNA donor loci in four different transformants by analyzing the segregation of streptomycin and hygromycin resistance (de-

TABLE 2

Summary of *Ds* alleles present in plants showing germinal excision of *Ds* and inheritance of Hm resistance

Ds line	No. of FGs investigated	Percent transposed *Ds* only	Percent nonexcised *Ds* only	Percent transposed *and* nonexcised *Ds*	Percent anomalous segregations
Hm^R *Ds*-B1	337	21	28	26	25
Hm^R *Ds*-A3	49	29	29	33	10
Hm^R *Ds*-E1	54	31	30	20	19
Hm^R *Ds*-C12b	47	30	40	21	9
Overall	487	24	30	25	21

tailed in "Genetic analysis" section of METHODS AND MATERIALS). The segregation data are shown in Table 3. They are analysed in terms of the proportions showing significant linkage in Table 4 and the distribution of linkage values of transposed elements (for those showing significant linkage) in Figure 2.

As with previous studies, only those elements inherited with the excision marker were analyzed. This underestimates the number of elements transposing to weakly linked or unlinked sites as a proportion of them will not have been inherited with the excision marker. The low excision frequency of *Ac/Ds* in Arabidopsis, however, means that a preselection for excision is a desirable step in a tagging strategy to significantly reduce the number of plants that need to be screened. Our analysis, therefore, concentrates on the linkage of those *tDs* elements inherited with the excision marker.

The overall proportion of *tDs* elements showing significant linkage to their original loci is 68%. This is similar to the values found in maize and tobacco. Transposed *Ds* elements from C12b are again exceptional in that a larger proportion (82%) show linkage. In maize, most of the "linked" transposed *Ac* elements showed very tight linkage to their original locus (GREENBLATT 1984; DOONER and BELACHEW 1989). In tobacco it appeared that there may be different patterns of transposition, to all tightly linked ("clustered") or more loosely linked ("dispersed") receptor sites (DOONER *et al.* 1991). In Arabidopsis we find that the patterns from all four loci investigated differ in detail. They form a range of distributions from that shown by C12b (which shows mostly clustered, though not as tightly as for most of the "clustered" class of loci in tobacco or for those loci studied in maize), through A3 and B1, to E1 (which shows mostly dispersed).

Genetic mapping of T-DNAs relative to phenotypic markers: In order to map the T-DNAs carrying Hm^R *Ds* and Δ*Nae*I *sAc*, a genetic analysis of the segregation of the antibiotic resistance carried by the T-DNAs relative to phenotypic markers was undertaken (detailed in "Genetic analysis" section of MATERIALS AND METHODS). Linkage between a T-DNA and a phenotypic marker would result in the proportion of mutant plants scored being reduced from 25%, as they would be preferentially included in the antibiotic sensitive fraction. The segregation data are summarized in Table 5 and the approximate genomic locations of the T-DNAs are indicated in Figure 3. Analysis of the segregation of *bp* was not possible as almost all lines showed apparent weak linkage. The T-DNA in line Hm^R *Ds*-A3 shows weak, but significant (χ^2 = 7.2, $P < 0.01$ for 3:1) apparent linkage to *ms1* in addition to the much tighter linkage to *hy2* and *gl1*. The reason for this is unclear, but we have assigned the T-DNA in Hm^R *Ds*-A3 to the chromosome *3* location. We have not assigned the T-DNA in Hm^R *Ds*-C12b to any location as its weak, but significant (χ^2 = 4.4, $P = 0.04$ for a 3:1) apparent linkage to *ms1* is also doubtful. In order to accurately determine the map locations, further crosses and analysis will need to be performed with lines carrying specific mutations; the analysis presented here is intended to show approximate locations only.

Genetic mapping of transposed *Ds* elements relative to phenotypic markers: Although *tDs* elements may show no genetic linkage to their original loci they may still be physically linked, but more than 50 cM away. This would reduce the efficiency of mutagenesis of loci on other chromosomes. It is also possible that elements may show a directional bias in their transposition (GREENBLATT 1984), which could lead to a reduction in the efficiency of tagging genes in some genomic regions. To investigate these possibilities, we mapped 18 *tDs* insertions, originating from the B1 locus, 11 that were found not to show significant linkage to the B1 locus (20.1e/1, 11.ST15/2, 11.SP4/1, 11.SB2/4, 11.ST13/1, 11.GP17/11, 11.GP10/3, 11.SB15/1, 11.SB17/1, 11.GB18/2 and 11.GP20/3) and 7 that were found to be moderately or weakly linked (11.ST2/3, 11.GP19/1, 11.Gh3/1, 11.Gh1/3, 11.SP5/3, 11.GP1/3 and 11.GP1/6). The procedure was similar to that used for the Hm^R *Ds*-containing T-DNAs, except that F_1 plants were selected for resistance to both Hm and Sm (to ensure that we were not mapping *Ds* elements still at the donor, SPT locus). The data are summarized in Table 5 and the approx-

TABLE 3

Segregation of Hm resistance (carried by *Ds*) relative to Sm resistance (the excision marker at the original locus)

Ds transformant	F_2 FG	Self (s) or back cross (b)	Hm^R Sm^R	Hm^R Sm^S	Hm^S	cM	SE
B1	20.1e/1	s	261	97	84	—[a]	
	20.2c/1	s	349	11	167	4.7	1.6
	20.3e/1	s	243	18	85	11	3.3
	20.5b/1	s	326	3	146	1.4	0.93
	20.15/1	s	207	3	53	2.2	1.5
	20.6b/1	s	443	5	102	1.7	0.89
	20.4b/1	s	335	84	139	48	12
	20.10a/1	s	223	2	42	1.3	1.1
	1.LV3/1	s	353	70	148	33	6.6
	1.S2/10	s	98	7	49	11	5.0
	1.S2/13	b	230	59	84	50	16
	10.20/1	s	121	38	36	—	
	10.28/2	s	171	33	51	32	9.0
	10.37/1	s	145	23	35	25	7.5
	10.2/1	b	342	102	135	—	
	10.40/1	b	313	7	113	3.3	1.5
	10.56/2	b	470	17	144	5.4	1.5
	11.GP20/3	s	168	66	49	—	
	11.GP1/6	s	236	51	74	38	9.8
	11.GP1/3	s	202	15	51	11	3.6
	11.GP19/1	s	325	36	80	17	3.6
	11.GP21/1	s	353	5	132	2.1	1.1
	11.GP10/3	s	205	55	72	—	
	11.GP17/11	s	178	82	168	—	
	11.GP17/12	s	334	31	81	14	3.3
	11.GP18/9	s	254	25	60	15	3.8
	11.GT17/3	s	270	13	81	7.1	2.3
	11.GT18/4	s	107	26	28	—	
	11.ST2/3	s	221	27	72	19	4.8
	11.ST13/1	s	100	28	38	—	
	11.ST15/2	s	129	38	49	—	
	11.ST17/8	s	357	17	156	7.1	2.1
	11.ST19/3	s	204	18	46	13	3.9
	11.SP4/1	s	108	34	39	—	
	11.SP5/3	s	333	76	95	40	8.7
	11.SP16/1	s	267	3	97	1.7	1.1
	11.SB2/4	s	100	29	33	—	
	11.SB15/1	s	109	42	46	—	
	11.SB17/1	s	43	17	14	—	
	11.GB9/2	s	208	19	63	13	3.9
	11.GB18/2	s	175	56	55	—	
	11.Gh1/3	s	225	31	79	21	5.2
	11.Gh3/1	s	234	23	73	14	3.8
	11.Gh13/1	s	536	48	184	13	2.4
A3	32.67/2	s	224	39	75	29	7.1
	32.39/3	s	292	8	94	4.1	1.7
	32.29/1	s	322	15	106	6.9	2.1
	32.25/1	s	127	5	52	5.9	3.1
	32.41/1	s	109	40	47	—	
	32.30/1	s	290	124	142	—	
	32.41/3	s	198	49	77	—	
	32.42/6	s	93	10	27	17	6.7
	32.38/2	s	147	59	27	—	
	32.10/3	b	131	32	59	—	
	32.23/1	b	128	25	47	33	11
	32.35/4	b	148	21	54	22	6.8
	32.36/1	b	181	8	48	6.6	2.7
	32.40/1	b	170	36	55	36	11
	32.49/1	b	155	33	49	36	11
	32.6/1	b	737	19	230	3.8	1.0
	32.7/1	b	551	51	187	14	2.5
	32.24/1	b	428	65	159	24	4.2
	32.38/1	b	194	61	94	—	
	32.39/1	b	424	14	158	4.9	1.5
	32.40/2	b	257	3	87	1.8	1.2
	32.52/1	b	252	28	89	17	4.1
	32.60/4	b	208	93	110	—	
	32.76/1	b	163	49	76	—	
E1	26.19/3	s	107	37	57	—	
	26.15/3	s	281	46	87	26	5.7
	26.26/3	s	113	5	38	6.6	3.6
	26.26/2	s	109	36	38	—	
	26.22/2	s	233	40	83	27	6.5
	26.23/1	s	320	28	96	13	3.1
	26.24/4	s	322	17	110	7.9	2.4
	26.6/2	s	284	25	85	13	3.3
	26.9/1	s	142	40	55	—	
	26.8/4	s	161	52	32	—	
	26.3/7	b	128	54	63	—	
	26.7/1	b	168	22	73	20	5.7
	26.8/1	b	178	32	74	29	7.9
	26.9/3	b	201	36	81	29	7.4
	26.10/2	b	228	63	90	—	
	26.18/1	b	111	41	47	—	
	26.22/1	b	142	35	58	—	
	26.22/3	b	125	13	56	15	5.5
	26.25/1	b	161	39	61	45	16
	26.27/4	b	92	36	33	—	
	26.6/1	b	645	93	269	22	3.3
	26.15/1	b	392	26	160	9.9	2.4
	26.26/1	b	470	35	186	11	2.3
	26.26/8	b	315	29	104	14	3.4
	26.7/2	b	157	27	72	27	8.0
	26.10/1	b	196	68	95	—	
C12b	40.TC1/1	s	306	119	89	—	
	40.MVGh1/2	s	45	1	16	3.3	3.8
	40.TC2/3	b	806	34	288	6.3	1.3
	40.MVGh6/1	b	289	15	122	7.8	2.4
	40.VHGh2/6	b	333	47	135	22	4.5
	40.VHGh3/3	b	457	31	156	10	2.2
	40.VHGh6/4	b	323	32	103	15	3.4
	40.VHGh7/1	b	383	16	132	6.2	1.8
	40.VHTC6/5	b	399	89	137	38	8.3
	40.VHTC6/2	b	337	2	118	0.89	0.73
	41.3/3	s	39	10	12	—	
	41.26/1	s	176	61	44	—	
	41.29/2	s	519	15	178	4.3	1.2
	41.33/4	s	376	11	99	4.4	1.5
	41.39/3	s	189	1	52	0.79	0.92
	41.40/2	s	129	5	56	5.8	3.0
	41.45/5	s	213	35	70	26	6.5

[a] — = no significant linkage.

TABLE 4

Proportions of transposed *Ds* elements showing significant linkage to their original loci

Locus	No. of linked transposed *Ds* elements	No. of *Ds* elements showing no significant linkage	Percent linked transposed *Ds* elements
B1	30	14	68
A3	16	8	67
E1	16	10	62
C12b	14	3	82
Overall	76	35	68

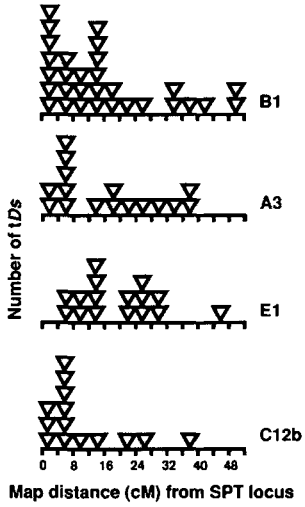

FIGURE 2.—Linkage values for *tDs* showing significant linkage to SPT locus of origin. Genetic analysis was performed as described in MATERIALS AND METHODS.

imate genomic locations are indicated in Figure 3. Three of the 11 unlinked *tDs* insertions map to the same chromosome as the B1 locus, chromosome *1*. Of these, one had transposed toward *an*, while two had transposed toward *ap 1*. The other eight insertions (only one of which could be mapped using the phenotypic markers scored) appear to have transposed to other chromosomes. This shows that although some transposition events occur to "unlinked" (*i.e.*, greater than 50 cM distant) sites on the same chromosome as the donor locus, the majority transpose into other chromosomes. Of the transpositions to sites linked to the B1 locus, two transposed toward *an*, four toward *ap1* and one (11.Gh1/3) showed anomalous segregation (*i.e.*, it showed linkage to the B1 locus, but not *an* or *ap1*, and it showed linkage to *hy2*, but not *gl1*). These results show there to be no obvious polarity in the direction of transposition.

DISCUSSION

Establishment of the inheritance and transposition pattern of *Ds* elements in Arabidopsis was a prerequisite before efficient large scale targeted tagging experiments could be performed. Our investigation of the inheritance of *Ds* in 1004 plants showing germinal excision of *Ds* confirms our previous finding (based on the analysis of 21 plants) that a substantial proportion of plants inheriting an excision marker do not carry a *tDs* in Arabidopsis. Sixty eight percent of FG seedlings inherited Hm resistance, demonstrating the presence of either a *tDs* or a nonexcised *Ds* or both. Progeny testing of 487 Hm resistant FG seedlings showed that at least 49% of these contained a *tDs*. Therefore, at least 33% (68% × 49%) of the FG plants contained a *tDs* (in an earlier experiment this figure was 29%). This proportion is somewhat lower than was found for transposed *Ac* elements in Arabidopsis (53%), which was similar to that observed for transposed *Ac* in maize (42%) and tobacco (58%). It is not known whether failure to inherit a transposed *Ds* is caused by failure of an excised element to reinsert into the genome or be due to preferential transposition into a newly replicated sister chromatid (as transposition is thought to occur soon after DNA replication; GREENBLATT 1984). In the latter case, the transposed element would segregate away from the excision marker. A more extensive analysis will be required to determine the reasons for the difference in efficiency of inheritance of *Ac* and the *Ds* we have constructed and transactivated with Δ*Nae*I *sAc*-1.

In Arabidopsis, *tDs* elements inherited with the donor locus show a similar preference for transposition to linked sites (68%) as that shown by *Ac* in maize (59%) and tobacco (72%). This differs from the situation in tomato in which *Ac* has been shown to transpose predominantly to unlinked sites. In tobacco, the distribution of linkage values lay in one of two classes, clustered or dispersed (DOONER *et al.* 1991). In Arabidopsis four different distributions, representing a range of linkage distributions are observed, but none are as tightly clustered as those observed in maize and tobacco. We conclude that, for Arabidopsis, there is most likely a continuum of distributions. The different distributions observed are measured in terms of amount of recombination between donor and acceptor sites. It may be that the distribution of physical distances between donor and acceptor sites is more uniform and the differences observed are due to differences in rates of recombination in different genomic regions. Alternatively, as proposed by DOONER *et al.* (1991), the pattern of transposition may reflect the timing of DNA replication in different regions of the chromosome.

For targeted transposon tagging strategies, it will clearly be advantageous to transactivate *Ds* from T-DNAs close to the target locus. In order to be able to mutagenize the whole genome with reasonable efficiency in nontargeted tagging strategies, it will be advantageous to start from T-DNAs inserted at many sites distributed throughout the genome. It will also

TABLE 5

Linkage analysis of T-DNA insertion sites and transposed *Ds* acceptor sites relative to phenotypic genetic markers in segregating populations after selection for antibiotic resistance

Plant line		*HY2*	*hy2*	cM	SE	*GL1*	*gl1*	cM	SE	*AN*	*an*	cM	SE	*AP1*	*ap1*	cM	SE	*MS1*	*ms1*	cM	SE
Δ*Nae*I *sAc*	(GUS)-1	261	65	48	13	240	29	19	4.5	198	71	—[d]		50	17	—					
Δ*Nae*I *sAc*	(GUS)-4	293	24	12	3.0	217	28	20	5.1	187	58	—		79	33	—		41	9	—	
Hm^R*Ds*-A3		247	0	<0.61	<0.70	196	20	15	4.4	160	56	—		123	40	—		118	21	29	9.7
Hm^R*Ds*-B1		240	77	—		275	81	—		287	69	43	11	119	46	—		117	32	—	
Hm^R*Ds*-C12b		158	54	—		151	58	—		157	52	—		126	38	—		92	18	33	13
Hm^R*Ds*E1		156	48	—		153	47	—		148	52	—		132	35	—		131	9	10	4.2
20.1e/1	(U)[b]	237	12	7.6	2.6	255	34	21	4.9	218	71	—		112	37	—		24	8	—	
11.ST15/2	(U)	175	63	—		177	54	—		131	36	—		122	31	—		66	16	—	
11.SP4/1	(U)	175	64	—		170	55	—		155	40	—		129	32	—		38	7	—	
11.SB2/4	(U)	174	52	—		168	57	—		178	47	—		126	7	8.3	3.7	23	6	—	
11.ST13/1	(U)	191	60	—		160	42	—		51	16	—		122	36	—		30	9	—	
11.GT17/11	(U)	202	61	—		153	60	—		151	49	—		112	35	—		31	7	—	
11.GP10/3	(U)	190	61	—		165	53	—		109	33	—		108	35	—		29	7	—	
11.SB15/1	(U)	178	60	—		152	50	—		143	6	6.3	3.0	103	36	—		21	8	—	
11.SB17/1	(U)	170	52	—		153	53	—		166	40	—		161	10	9.3	3.5				
11.GB18/2	(U)	167	51	—		155	60	—		67	12	—		128	38	—		31	10	—	
11.GP20/3	(U)	238	78	—		155	52	—		117	30	—		128	34	—					
11.ST2/3	(L, 19 cM)[c]	208	71	—		173	60	—		179	20	17	4.8	114	40	—		29	6	—	
11.GP19/1	(L, 17 cM)	181	65	—		188	77	—		116	36	—		81	26	—		26	6	—	
11.Gh3/1	(L, 14 cM)	252	76	—		166	53	—		131	50	—		95	38	—		27	9	—	
11.Gh1/3	(L, 21 cM)	279	12	6.4	2.2	158	58	—		132	48	—		124	38	—		22	9	—	
11.SP5/3	(L, 40 cM)	199	76	—		150	58	—		220	61	—		138	26	32	10				
11.GP1/3	(L, 11 cM)	148	51	—		158	64	—		233	44	32	7.7	132	37	—		43	9	—	
11.GP1/6	(L, 38 cM)	165	50	—		159	64	—		191	61	—		138	27	33	11				

[a] — = no significant linkage.
[b] (U) = *tDs* unlinked to B1.
[c] (L, *x* cM) = *tDs* linked to B1, *x* cM (from Table 4.).

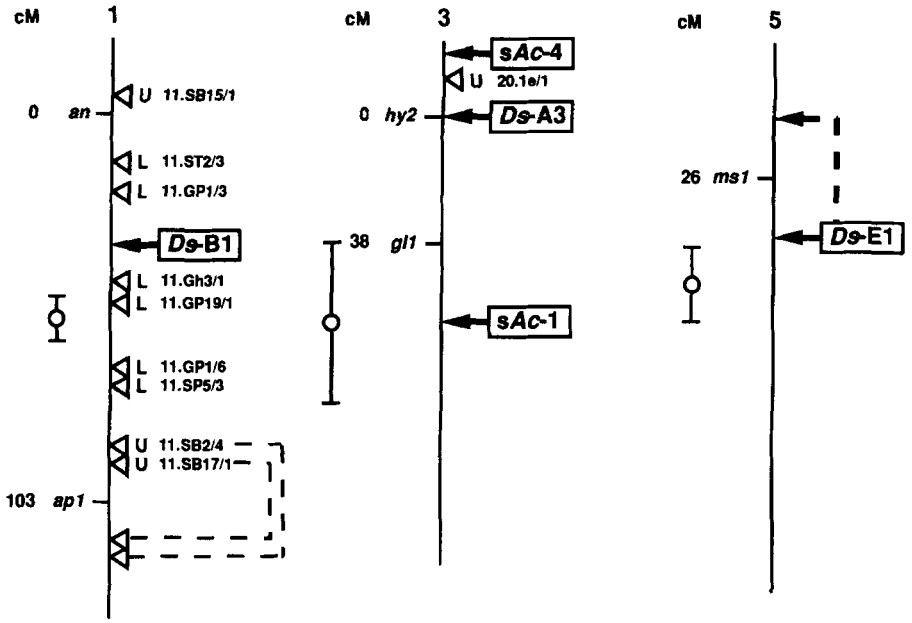

FIGURE 3.—Approximate genomic locations of T-DNAs and *tDs* elements from the Hm^R *Ds*-B1 locus. Boxed, T-DNA insertions; L, *tDs* elements shown to be linked to the Hm^R *Ds*-B1 locus; U, *tDs* elements showing no significant linkage to the B1 locus; broken lines indicate alternative positions; circle with range, centromere position. Map coordinates of phenotypic markers and positions of centromeres are from KOORNNEEF 1987.

be advisable to conduct a preliminary analysis of these to identify lines where the transposition pattern of the *Ds* elements is to tightly linked sites (such as C12b). This could result in lower than expected efficiency of mutagenesis of certain regions of the genome.

I.B. was supported by a fellowship from the Sainsbury Laboratory, John Innes Centre, which is supported by the Gatsby Charitable Foundation. We would like to thank DAVID FLANDERS for the preparation of the figures. This work was conducted under Ministry of Agriculture Food and Fisheries license no. PHF 1418/8/22.

LITERATURE CITED

ALLARD, R. W., 1956 Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia **24:** 235–278.

ALTMANN, T., R. SCHMIDT and L. WILLMITZER, 1992 Establishment of a gene tagging system in *Arabidopsis thaliana* based on the maize transposable element *Ac*. Theor. Appl. Genet. **84:** 371–383.

BALCELLS, L., J. SWINBURNE, G. COUPLAND, 1991 Transposons as tools for the isolation of plant genes. Trends Biotechnol. **9:** 31–37.

BANCROFT, I., and C. DEAN, 1993 Factors affecting the excision frequency of the maize transposable element *Ds* in *Arabidopsis thaliana*. Mol. Gen. Genet. (in press).

BANCROFT, I., J. D. JONES and C. DEAN, 1993 Heterologous tagging of the *DRL1* locus in Arabidopsis. Plant Cell (in press).

BANCROFT, I., A. M. BHATT, C. SJODIN, S. SCOFIELD, J. D. G. JONES and C. DEAN, 1992 Development of an efficient two-element transposon tagging system in *Arabidopsis thaliana*. Mol. Gen. Genet. **233:** 449–461.

DEAN, C., C. SJODIN, T. PAGE, J. D. G. JONES and C. LISTER, 1992 Behaviour of the maize transposable element *Ac* in *Arabidopsis thaliana*. Plant J. **2:** 69–81.

DOONER, H. K., and A. BELACHEW, 1989 Transposition pattern of the maize element *Ac* from the *bz-m2* (*Ac*) allele. Genetics **122:** 447–457.

DOONER, H. K., J. KELLER, E. HARPER and E. RALSTON, 1991 Variable patterns of transposition of the Maize element *Activator* in tobacco. Plant Cell **3:** 473–482.

FELDMANN, K. A., M. D. MARKS, M. L. CHRISTIANSON and R. S. QUATRANO, 1989 A dwarf mutant of *Arabidopsis* generated by T-DNA insertional mutagenesis. Science **243:** 1351–1354.

GREENBLATT, I. M., 1984 A chromosome replication pattern deduced from pericarp phenotypes resulting from movements of the transposable element *Modulator* in maize. Genetics **108:** 471–485.

GREVELDING, C., D. BECKER, R. KUNZE, A. VON MENGES, V. FANTES, J. SCHELL and R. MASTERSON, 1992 High rates of *Ac/Ds* germinal transposition in *Arabidopsis* suitable for gene isolation by insertional mutagenesis. Proc. Natl. Acad. Sci. USA **89:** 6085–6089.

JONES, J. D. G., F. M. CARLAND, P. MALIGA and H. K. DOONER, 1989 Visual detection of transposition of the maize element *Activator* (*Ac*) in tobacco seedlings. Science **244:** 204–207.

KELLER, J., E. LIM, D. W. JAMES, JR., and H. K. DOONER, 1992 Germinal and somatic activity of the maize element *Activator* (*Ac*) in Arabidopsis. Genetics **131:** 449–459.

KONIECZNY, A., D. F. VOYTAS, M. P. CUMMINGS and F. M. AUSUBEL, 1991 A superfamily of *Arabidopsis thaliana* retrotransposons. Genetics **127:** 801–809.

KOORNNEEF, M., 1987 Linkage map of *Arabidopsis thaliana* (2*n* = 10), pp. 742–745 in *Genetic Maps*, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

KOORNNEEFF, M., C. J. HANHART, E. P. VAN LOENEN-MARTINEL and J. H. VAN DER VEEN, 1987 A marker line, that allows the detection of linkage on all Arabidopsis chromosomes. Arabidopsis Inform. Serv. **23:** 46–50.

KOSAMBI, D. D., 1944 The estimation of map distances from recombination values. Ann. Eugen. **12:** 172–175.

MASTERSON, R. V., D. B. FURTEK, C. GREVELDING and J. SCHELL, 1989 A maize *Ds* transposable element containing a dihydrofolate reductase gene transposes in *Nicotiana tabacum* and *Arabidopsis thaliana*. Mol. Gen. Genet. **219:** 461–466.

MEYEROWITZ, E. M., 1987 *Arabidopsis thaliana*. Annu. Rev. Genet. **21:** 93–111.

OSBORNE, B. I., C. A. CORR, J. P. PRINCE, R. HEHL, S. D. TANKSLEY, S. MCCORMICK and B. BAKER, 1991 *Ac* transposition from a T-DNA can generate linked and unlinked clusters of insertions in the tomato genome. Genetics **129:** 833–844.

PELEMAN, J., B. COTTYN, W. VAN CAMP, M. VAN MONTAGU and D. INZE, 1991 Transient occurrence of extrachromosomal DNA of an *Arabidopsis thaliana* transposon-like element, *Tat1*. Proc. Natl. Acad. Sci. USA **88:** 3618–3622.

SCHMIDT, R., and L. WILLMITZER, 1989 The maize autonomous element *activator* (*Ac*) shows a minimal germinal excision frequency of 0.2%-0.5% in transgenic *Arabidopsis* plants. Mol. Gen. Genet. **220:** 11–24.

SWINBURNE, J., L. BALCELLS, S. R. SCOFIELD, J. D. G. JONES and G. COUPLAND, 1992 Elevated levels of *Ac* transposase mRNA are associated with high frequencies of *Ds* excision in Arabidopsis. Plant Cell **4:** 583–595.

TSAY, Y.-F., M. J. FRANK, T. PAGE, C. DEAN and N. M. CRAWFORD, 1993 A mutable allele of the Arabidopsis *CHL 1* gene contains an active transposable element. Science **260:** 342–344.

VAN SLUYS, M. A., J. TEMPE and N. FEDOROFF, 1987 Studies on the introduction and motility of the Maize *activator* element in *Arabidopsis thaliana* and *Daucus carota*. EMBO J. **6:** 3881–3889.

YODER, J. I., J. PALYS, K. ALPERT and M. LASSNER, 1988 *Ac* transposition in transgenic tomato plants. Mol. Gen. Genet. **213:** 291–296.

Communicating editor: M. R. HANSON