Distribution of Unlinked Transpositions of a Ds Element
From a T-DNA Locus on Tomato Chromosome 4

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ABSTRACT

In maize, receptor sites for unlinked transpositions of Activator (Ac) elements are not distributed randomly. To test whether the same is true in tomato, the receptor sites for a Dissociation (Ds) element derived from Ac, were mapped for 26 transpositions unlinked to a donor T-DNA locus on chromosome 4. Four independent transposed Ds mapped to sites on chromosome 4 genetically unlinked to the donor T-DNA, consistent with a preference for transposition to unlinked sites on the same chromosome as opposed to sites on other chromosomes. There was little preference among the nonnondonor chromosomes, except perhaps for chromosome 2, which carried seven transposed Ds, but these could not be proven to be independent. However, these data, when combined with those from other studies in tomato examining the distribution of transposed Ac or Ds among nonnondonor chromosomes, suggest there may be absolute preferences for transposition irrespective of the chromosomal location of the donor site. If true, transposition to nonnondonor chromosomes in tomato would differ from that in maize, where the preference seems to be determined by the spatial arrangement of chromosomes in the interphase nucleus. The tomato lines carrying Ds elements at known locations are available for targeted transposon tagging experiments.

TRANSPOSABLE genetic elements were first described by McClintock (1947, 1951). When such elements insert into genes, mutant alleles are induced and the corresponding gene can be isolated using the transposable element as a probe. Transposable elements of maize (Schwarz-Sommer et al. 1985) and Antirrhinum majus (Coen et al. 1989) are well characterized and have been used for the isolation of genes mutated by transposon insertion (Fedoroff et al. 1984; Martin et al. 1985; O'Reilly et al. 1985; Theres et al. 1987; Hake et al. 1989; Coen et al. 1989).

In many plants, endogenous transposable elements have not been sufficiently well characterized for use in gene isolation. To establish gene isolation systems based on tagging, transposons such as the maize Activator (Ac) or Dissociation (Ds) elements have been introduced into heterologous plant species and shown to transpose (Baker et al. 1986; Vansluijs et al. 1987; Knapp et al. 1988; Yoder et al. 1988; Zhou and Atherly 1990; Zhang et al. 1991; Dean et al. 1992) and mutagenise plant genes (Chuck et al. 1993; Jones et al. 1994; Whittam et al. 1994). For several species, transposon systems have been established in which the transposable element and transposase gene are provided as separate components (Hehl and Baker 1989; Lassner et al. 1989; Swinburne et al. 1992). In maize (Greenblatt 1984; Dooner and Belachew 1989) and other species (Jones et al. 1990; Dooner et al. 1991; Bancroft and Dean 1993; Keller et al. 1993; Carroll et al. 1995), Ac and Ds transpose preferentially to linked receptor sites.

Dooner et al. (1994) studied the distribution of unlinked receptor sites after transposition of Ac elements from the maize bronze (bz) gene on chromosome 9 and found it to be nonrandom with an apparent preference for sites on the same chromosome as the donor site. It was suggested that the nonrandom pattern of transpositions to the remaining chromosomes (preferentially to chromosomes 5 and 7) might reflect higher order spatial organization of the chromosomes in the interphase nucleus. Thus, unlinked receptor site distribution could provide an interesting insight into nuclear organization and raises the issue of whether this nonrandom distribution might also prevail for transposition from other maize loci and in other species.

We set out to test if there is also a nonrandom pattern to the unlinked transpositions of Ds in tomato, and we report here on the distribution of unlinked transposed Ds (trDs) from the tomato chromosome 4 T-DNA locus designated 1561E. Of 103 families analyzed that harbored germinally trDs elements, 36 carried trDs genetically unlinked to the T-DNA donor site. Using inverse PCR (Triglia et al. 1988) we have amplified tomato DNA sequences adjacent to unlinked trDs for 33 families, and we have determined the RFLP map.

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FIGURE 1.—Experimental strategy for the generation, re-
covery and mapping of transposed Ds elements unlinked to
the donor T-DNA 1561E located on chromosome
of tomato.

locations of 26 trDs. Our results, like those for maize
(Dooner et al. 1994) are consistent with preferential
transposition to unlinked sites on the same chromo-
some as the donor locus vs. sites on other chromo-
somes. However, unlike the results for maize, our results
suggest little preference among the other chromo-
somes, with the possible exception of chromosome 2.

MATERIALS AND METHODS
Isolation of trDs unlinked to the donor T-DNA: All of the
DNA constructs and transgenic tomato lines used in this study
were generated and described previously (Carroll et al.
1995). The donor T-DNA 1561E (derived from construct
SLJ1561), located on chromosome 4, contains a 35S:NPT
transformation marker conferring kanamycin resistance, a
nos:SPEC excision marker conferring spectinomycin resis-
tance and a Ds element in the 5' untranslated leader of the
nos:SPEC gene (Figure 1). Transposition of the Ds was induced
by either 10512A or 105121 T-DNAs (derived from construct
SLJ10512) carrying stabilized Ac (sAc); this provided a source
of transposase and a 2'-GUS marker conferring β-glucuron-
idase activity that was used as a histochemical marker for the
presence of sAc (Figure 1).

The crossing and screening strategy for the generation and
recovery of trDs unlinked to the donor T-DNA is outlined in
Figure 1. Individuals carrying sAc and 1561E were crossed
either individually, as seed parents, or bulked, as pollen par-
ents, to wild-type plants to generate several TC1 populations.
TC1 seed was germinated on spectinomycin-containing me-
dium, and individuals carrying a trDs and lacking
GUS were identified by carrying out PCR (to detect the Ds) on spectin-
mycin resistant individuals that lacked a
GUS gene as deter-
mined by staining plant tissue for β-glucuronidase activity.
Each TC1 plant of this phenotype was allowed to self-pollinate
to generate TC1F2 seed. To assay for the presence of the donor
T-DNA, seed of each family was germinated on MS medium
containing 300 µg ml\(^{-1}\) kanamycin. To distinguish unlinked
and linked trDs elements, 10 kanamycin sensitive (kanS) seed-
ings of each family (i.e., 20 gametes) were assayed for the
presence of the Ds by PCR. PCR analyses were carried out as
described by Klumyuk et al. (1993) and four oligonucleotide
primers were added to each PCR. Two (D60 and D75, Table
1) amplified a 334-bp fragment composed of the 3' end of
Ds and the other two (2995AR and 2995AL) (see Klumyuk et
al. 1993) amplified a 141-bp fragment from tomato chromo-
some 11 as a positive control.

Upon transposition of the Ds to a site closely linked to the
T-DNA, little or no recombination will occur between the T-
DNA and the trDs, so the majority or all of the kanS TC2F2
individuals should lack Ds. In contrast, upon transposition of
the Ds to an unlinked site, the T-DNA and the trDs should
 assort independently, and 75% of the kanS plants will inherit
the Ds element. The proportion of kanS plants lacking a trDs
was used to identify families likely to carry a trDs unlinked to
the T-DNA. Families with five or more kanS individuals car-
thereafter, hybridization was conducted essentially with kanamycin solution (Weide were subjected to Southern analysis buffer (SAMRROOK). Individuals carrying a single unlinked trDs. Presumed to carry a linked trDs (for four Ds+:six Ds progeny P = 0.02 for a fit to a 3:1 ratio).

Fifteen of the remaining kanR plants in each family carrying a presumed unlinked trDs were used to determine Ds copy number. Plants were bulked to make DNA that was digested with BsM. Southern blotted and probed with the 334-bp 3' end of Ds, which contains no BsM site. In families harbouring more than one trDs, TC3F2 seedlings were grown in the greenhouse and sprayed at the two-to three-leaf stage with kanamycin solution (Weide et al. 1989). A week later kanR plants could be distinguished and seven to eight of these were subjected to Southern analysis as above, to identify individuals carrying a single unlinked trDs.

DNA isolation and Southern hybridization analysis: The procedure for isolation of Lycopersicon esculentum DNA was essentially as described previously (CARROLL et al. 1995). For isolation of DNA from L. pennellii and L. esculentum × L. pennellii F2 plants, the method of TAI and TANKSELY (1991) was used. To perform Southern analyses, 15 μg of each DNA were digested with the appropriate restriction enzyme, then resolved in 1% agarose gels containing Tris-borate/EDTA buffer (SAMBROOK et al. 1989) and blotted onto Hybond-N membranes as described by the manufacturer (Amersham). Thereafter, hybridization was conducted essentially as described by CHURCH and GILBERT (1984) and the blots were autoradiographed using a Bio-Imaging Analyzer BAS 1000 (Fuji Photo Film, Japan).

Mapping of trDs: Inverse PCR (IPCR), to amplify tomato DNA flanking trDs, was carried out essentially as described by THOMAS et al. (1994), using the restriction endonuclease and primer combinations shown in Table 2. Primer sequences are shown in Table 1. IPCR products obtained from nested primer reactions were purified by agarose gel electrophoresis, electroeluted from gel slices and labeled to high specific activity with α32P dCTP (3000 Ci/mmol) using a random priming kit (Pharmacia LKB). Labeled PCR fragments were hybridized to Southern blots of L. esculentum and L. pennellii DNA digested with EcoRI, EcoRV, Dnl or HindIII to identify restriction fragment length polymorphisms (RFLPs). The probes were then hybridized to blots of DNA from a mapping population of L. esculentum × L. pennellii F2 plants (TANKSELY et al. 1992) digested with the appropriate restriction enzyme. The position of each trDs element was then determined using the program MAPMAKER (LANDER et al. 1987) and segregation data for RFLPs covering the 12 tomato chromosomes (TANKSELY et al. 1992).

RESULTS

Isolation of trDs unlinked to the donor T-DNA: Of 103 families assayed, 45 harbored a trDs closely linked to the donor T-DNA, with 0 recombinants in 20 gametes (Figure 2). Fifteen families showed an intermediate recombination frequency, with between one and four kanR progeny carrying a Ds. Forty-three families were classified as carrying trDs elements unlinked to the T-DNA and were used for mapping of trDs (Figure 1). Eighteen of these 43 families (42%) were found to carry two or more trDs (examples are shown in Figure 3). Individuals carrying a single unlinked trDs were isolated from 11 of these 18 families so that unlinked trDs were isolated from 36 families in total. Seven of the 18 families containing multiple trDs were found to have one copy linked to the donor T-DNA. Taking these families into
account 67 (65%) of the families studied carried a Ds element transposed to sites linked to the donor T-DNA.

**Mapping of trDs**: IPCR products were obtained for 33 out of the 36 trDs recovered. Labeled IPCR fragments were hybridized to Southern blots of *L. esculentum* and *L. pennellii* DNA digested with EcoRI, EcoRV, Dral or HindIII. The IPCR products from 19 trDs hybridized to single copy sequences and were mapped. Seven hybridized to low (two to three) copy number sequences, and all of the copies mapped to the same location in each case. Seven could not be mapped because they hybridized to repeated sequences. The positions of the 26 mapped trDs elements on the tomato RFLP map are presented in Figure 4. Transpositions of
the Ds element from the 1561E locus on chromosome 4 occurred onto 10 out of 12 tomato chromosomes (chromosomes 5 and 8 did not receive a trDs). A statistical comparison of the observed and expected number of unlinked receptor sites based on the physical length of the chromosome (SHERMAN and STACK 1992) was performed for each chromosome (Table 3). The prediction for chromosome 4 takes into account the fact that much of it is linked to 1561E. The results suggest that the distribution of receptor sites for unlinked trDs was not random because significantly more transpositions occurred onto chromosomes 2 (P = 0.009) and 4 (P = 0.027) than expected.

**DISCUSSION**

Transpositions of Ac tend to occur into genetically linked receptor sites in maize (DOONER and BELACHEW 1989), tobacco (JONES et al. 1990; DOONER et al. 1991) and Arabidopsis (KELLER et al. 1993). This tendency has also been reported previously for transposition of Ds elements originating from three different donor T-DNA loci in tomato, (CARROLL et al. 1995), but only 15 transposition events from each locus were examined. In the present study, 103 families harboring trDs from one of these loci were analyzed, and 67 (i.e. 65%) of them were shown to carry a trDs linked to the T-DNA; 45 receptor sites were shown to cosegregate with the donor site. Other reports suggest that transpositions of Ac or Ds to sites linked to the donor T-DNA are much less frequent in tomato (OSBORNE et al. 1991; BELZILE and YODER 1992; HEALY et al. 1993). However, these authors studied somatic events and acknowledged that the real pattern could have been masked by secondary or even subsequent transpositions. They concluded that the distribution of unlinked trAa in dispersed clusters was probably the result of primary transposition from the T-DNA to unlinked sites followed by linked transpositions around these new positions.

In maize, transpositions of Ac tend to occur into genetically unlinked receptor sites on the donor chromosome in preference to sites on nondonor chromosomes (DOONER et al. 1994). This tendency has been observed previously in tomato for Ac or Ds elements originating from three different donor T-DNA loci, (OSBORNE et al. 1991; HEALY et al. 1993), but only a limited number of transpositions were examined for each locus. In the present study, four independent transpositions of Ds (Table 4) out of 26 trDs genetically unlinked to the donor locus, were shown to be on the donor chromosome (Figure 4), confirming this preference in tomato. However, reports for other donor T-DNAs indicate no

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**TABLE 3**

Chromosomal locations of tranposed Ds and the nature of the populations from which they were isolated

<table>
<thead>
<tr>
<th>TCi population*</th>
<th>No. of F1 plants used</th>
<th>Contribution to TCi</th>
<th>No. of TCi seed</th>
<th>trDs</th>
<th>Chromosomal location</th>
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* As described in Tables 1 and 2 of CARROLL et al. (1995).
TABLE 4
Testing the chromosomal distribution of 26 transposed Ds elements, for departure from randomness

<table>
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<tr>
<th>Chromosome number</th>
<th>Observed no. of trDs</th>
<th>Expected no. of trDs</th>
<th>Probability*</th>
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<td>3</td>
<td>1.84</td>
<td>0.28</td>
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</table>

Ds elements were genetically unlinked to the donor T-DNA locus on chromosome 4.

* Asterisks denote statistically significant (**P < 0.01; *P < 0.05) differences between the observed and expected number of trDs.

The number of trDs expected for chromosome 4 is about half that for the whole chromosome because only sites unlinked to the donor T-DNA could serve as receptor sites for unlinked trDs.

apparent preference for transpositions of Ac or Ds to genetically unlinked sites on the donor chromosome (Osborne et al. 1991; Belzile and Yoder 1992; Rommens et al. 1992; Rommens et al. 1992; Knapp et al. 1994; Thomas et al. 1994). These differences may be consistent with the observation by Dooner et al. (1991) that different T-DNA loci can give rise to different patterns of Ac transposition in tobacco.

This study has also examined the distribution of trDs among the nondenor chromosomes. The remaining 22 trDs are distributed over nine of the 11 nondenor chromosomes, but chromosome 2 appears to carry significantly more trDs than would be expected on the basis of a random distribution (Table 3). There are several possible explanations for this apparent preference for transposition to chromosome 2.

First, chromosome 2 may comprise a site for preferential insertion of both T-DNAs and Ds or Ac elements. This might be consistent with the fact that many genes map to this chromosome, even though one arm consists primarily of the nucleolus organizing region (Tanksley et al. 1992). Chromosome 2 may be particularly active, carrying a higher proportion of "open" chromatin that might predispose the DNA to receive transpositions or T-DNA insertions. Chromosome 2 does appear to be a frequent site for T-DNA insertions (Thomas et al. 1994).

Second, the presence of seven trDs on chromosome 2 near RFLP marker TG191 (Figure 4) could have occurred because of an early transposition to chromosome 2 that gave rise to a large sector from which subsequent transpositions to linked sites occurred. This is possible because all seven of the transpositions to chromosome 2 arose from TC1 population 1 (Table 3), so they are not necessarily independent. It seems unlikely that they are all secondary transpositions for several reasons. A large sector should, on average, have contributed to no more than 1/5 of the TC1 progeny, since pollen was bulked from 5 F1 plants (Table 3), yet 1/5 of these plants carry trDs on chromosome 2. The chance of seven or more trDs arising from the same F1 plant is 0.018. Some of the other populations described by Carroll et al. (1995) did show evidence for large sectors of early transposition, but these populations were avoided in this analysis and there was no evidence for a common Ds hybridizing band among any of the individuals examined from population 1 (Figure 3). The currently accepted model for Ac or Ds transposition in maize is for transposition from replicated to either unreplicated or replicated DNA (Chen et al. 1992). Assuming the mechanism of transposition in tomato is similar to that in maize, many of the individuals with a trDs on chromosome 2 should, if they were in fact secondary transpositions, have shown a common band comprising the primary transposed Ds. No common band was observed among the individuals carrying a trDs on chromosome 2 (Figure 3). Based on these arguments, it would seem unlikely that all the transpositions on chromosome 2 could be secondary transpositions. A mixture of primary and secondary transpositions seems more plausible. The four trDs clustered in a 5.6-cM interval around RFLP marker TG191 are the most likely to have arisen by secondary transposition. The chance of four or more trDs arising from the same F1 plant is 0.35.

Third, the excess of trDs on chromosome 2 might be a consequence of close proximity between chromosome 2 and donor chromosome 4 at the time of transposition during interphase. The number of transpositions to genetically linked and unlinked sites on the same chromosome, strongly suggest that physical proximity increases the probability of a site receiving a transposition. In maize, a nonrandom distribution of unlinked trAs was interpreted as possibly due to an ordering of the chromosomes in interphase nuclei (Dooner et al. 1994). These authors reported preferential Ac transpositions to chromosomes 5 and 7 and using the models of Bennett (1984) for associations of chromosomes by arm length, predicted that in maize chromosomes 3 and 7 would be most frequently next to the donor 9S and chromosome 5 to 9L. A model of nuclear architecture that puts chromosomes with most similar arm lengths together indicates that tomato chromosome 4 is most likely to be near to tomato chromosomes 6 and 7, not chromosome 2 (J. S. Heslop-Harrison, personal communication). However, it is not clear whether such packing models can be applied widely, particularly in species with smaller genome sizes and for which there
is little independent evidence regarding the chromosomal organization of interphase nuclei. The spatial organization and relative positions of decondensed chromosomes within interphase nuclei can now be studied using in situ hybridization with chromosome painting probes and low copy probes in both animals (Gremer et al. 1993) and plants (Heslop-Harrison et al. 1995). It will be interesting to use this approach to study interphase chromosomal associations in tomato to establish whether there is a correlation with unlinked trDs receptor site distributions.

It will also be valuable to study the transposition patterns of more unlinked trDs from additional T-DNA loci in tomato. A number of such studies already exist, but these are limited in extent and by lack of independence between transpositions. Nevertheless, a clear trend for recovery of dispersed clusters of transpositions, consistent with linked secondary transpositions arising from sites of primary transposition, has emerged. The distribution of presumptive primary transpositions among nondonor chromosomes is shown in Table 5 for each of these studies and for the present study, based on the assumption that each cluster observed represents a single primary transposition. In total, these studies suggest a preference for unlinked transpositions to some nondonor chromosomes e.g. chromosomes 1, 2, 6, 7 and 12, but not for others e.g. chromosomes 4, 8 and 9, irrespective of donor chromosome. This raises the possibility that there are absolute site preferences for transposition among nondonor chromosomes in tomato and that transposition beyond the donor chromosome may depend more on chromosome composition than proximity. Any such preferences could be important for transposon tagging. Clearly, many independent nondonor chromosome transpositions need to be analysed for each of the 12 possible donor chromosomes of tomato to test this possibility rigorously.

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LITERATURE CITED


### TABLE 5

Summary of the reported chromosomal distributions of transposed Ac or Ds elements genetically unlinked to their donor T-DNA loci

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</table>

Donor chromosomes are indicated by asterisks. The number of single transpositions or clusters of transpositions presumed to have arisen by secondary transposition (i.e., nonindependent transpositions clustered within an interval of 15 cM) are indicated.

References: B. Belzile and Yoder (1992); H. Healy et al. (1993); K. Knapp et al. (1994); O. Osborne et al. (1991); R. Rommens (1992) and Rommens et al. (1992); T. Thomas et al. (1994).

The donor site was a site of primary transposition rather than a T-DNA.


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