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Transgenic tomato lines containing *Ds* elements at defined genomic positions as tools for targeted transposon tagging

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Abstract We have introduced a genetically marked *Dissociation* transposable element (Ds^{HPT}) into tomato (*Lycopersicon esculentum*) by *Agrobacterium tumefaciens*-mediated transformation. Probes for the flanking regions of the T-DNA and transposed Ds^{HPT} elements were obtained with the inverse polymerase chain reaction (IPCR) technique and used in RFLP linkage analyses. The RFLP map location of 11 T-DNAs carrying Ds^{HPT} was determined. The T-DNAs are distributed on 7 of the 12 tomato chromosomes. To explore the feasibility of gene tagging strategies in tomato using Ds^{HPT}, we examined the genomic distribution of Ds^{HPT} receptor sites relative to the location of two different, but very closely linked, T-DNA insertion sites. After crosses with plants expressing *Ac* transposase, the hygromycin phosphotransferase (HPT) marker on the *Ds* element and the excision markers β-glucuronidase (GUS) and Basta resistance (BAR) facilitated the identification of plants bearing germinally transposed Ds^{HPT} elements. RFLP mapping of 21 transposed Ds^{HPT} elements originating from the two different T-DNA insertions revealed distinct patterns of reintegration sites.

Key words *Lycopersicon esculentum* · *Ac/Ds* Transposon tagging · Inverse PCR RFLP – linkage analysis

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Introduction

In tomato (*Lycopersicon esculentum*), a large collection of mutant phenotypes, many of them involving agronomically important characters, have been described (Stevens and Rick 1986). However, the majority of corresponding genes are not amenable to the classical method of gene isolation via protein characterization and cDNA cloning, because the respective gene products are not known. In maize and *Anthirrhinum*, transposon tagging has become an efficient method for isolating genes whose products are not known or are synthesized only in minute amounts (e.g. Fedoroff et al. 1984; Martin et al. 1985; Theres et al. 1987). To extend the technique of transposon tagging to other species, the maize transposable element *Activator* (*Ac*), which has proven to be very effective in maize (Fedoroff et al. 1984), has been introduced into heterologous plant species including tobacco (Baker et al. 1986), potato (Knapp et al. 1988), *Arabidopsis* (Van Sluys et al. 1987), and tomato (Yoder et al. 1988). Recently, the first successful tagging experiment using the maize transposable element *Ac* in a heterologous system was reported (Chuck et al. 1993).

In maize (Greenblatt 1984; Dooner and Belachew 1989), tobacco (Jones et al. 1990; Dooner et al. 1991) and *Arabidopsis* (Keller et al. 1993), the *Ac* element shows a tendency to transpose preferentially to linked positions on the same chromosome. In tomato, clusters of transposed *Ac* elements, either linked or unlinked to the donor locus, were observed (Osborne et al. 1991; Belzile and Yoder 1992). Although the actual distribution of reinsertion sites seems to be donor site specific (Dooner et al. 1991), the preference for short-range transpositions on the same chromosome was successful-

ly exploited to tag the maize *R* locus (Dellaporta et al. 1988).

A drawback of the autonomous *Ac* element for tagging experiments in tomato is the accumulation of transposon footprints resulting from the high frequency of transposition and a tendency to amplify the element to give high copy numbers in a few generations (Yoder 1990a). This may explain why many mutations observed in the presence of *Ac* do not cosegregate with the transposable element (Yoder 1990b). To overcome these difficulties, several groups have developed two-component systems consisting of a *Ds* element transactivated by a stabilized *Ac* element which itself cannot transpose (e.g. Lassner et al. 1989; Scofield et al. 1992; Healy et al. 1993). The transposase source can be separated from the *Ds* component by genetic segregation, allowing for a limitation of transposition events to one generation and a stabilization of *Ds*-induced mutations. In addition, *Ds* elements have been engineered by insertion of selectable or scorable markers to facilitate the recovery of plants harbouring the respective element (e.g. Lassner et al. 1989; Masterson et al. 1989; Rommens et al. 1992; Healy et al. 1993). Recently, a two-component system was successfully used in *A. thaliana* to tag the *DRL1* locus (Bancroft et al. 1993).

With the intention of exploiting the preference for transpositions to linked positions of the *Ac*/*Ds* transposable element system for targeted transposon tagging experiments in tomato, we have established a collection of transgenic tomato plants, each harbouring a genetically marked *Ds* element (Ds^{HPT}). Eleven T-DNA insertions and 21 transposed Ds^{HPT} elements originating from two independent, but closely linked, T-DNAs have been mapped. This set of 32 transgenic tomato lines harbouring Ds^{HPT} elements at defined positions on 11 chromosomes is a valuable tool for targeted as well as for untargeted transposon tagging experiments. The observed patterns of transposition show that the actual distribution of reinsertion sites can differ strikingly from donor site to donor site.

Materials and methods

Construction of Ds^{HPT} vectors

A Ds^{HPT} element was constructed by replacing the internal 1.6 kb *Hin*dIII fragment of *Ac* (Müller-Neumann et al. 1984) with a 2 kb P_{nos}-hygromycin phosphotransferase (HPT)-A4 3' fusion (Koncz et al. 1989). The marked *Ds* element was inserted between the 1' promoter (Velten et al. 1984) and the β-glucuronidase (GUS) gene (Jefferson et al. 1987) in pKU304, and between the CaMV 35S promoter and a Basta resistance (BAR) gene (De Block et al. 1987) in pKU306 and pKU310. These constructs were obtained by using the binary vectors pBI101 (Jefferson et al. 1987), pBIR (Meissner 1990) and pBI121 (Jefferson et al. 1987), respectively. The binary vector constructs were transformed into *Agrobacterium tumefaciens* strain LBA4404 (Hoekema et al. 1983). Schematic representations of the vector constructs pKU304 and pKU310 are shown in Fig. 1. Details of the vector constructions can be obtained from the corresponding author.

Nucleic acid manipulations

Restriction enzyme analysis, gel electrophoresis and DNA cloning were done according to standard procedures (Sambrook et al. 1989). DNA sequencing was performed using the fmol DNA sequencing system (Promega) following he manufacturer's protocol.

Plant material and transformation

The DNA constructs were introduced into tomato (*Lycopersicon esculentum*) cultivars *VF*36 and *Moneymaker* by *Agrobacterium tumefaciens*-mediated transformation as described by Fillatti et al. (1987), with minor modifications. Instead of cotyledons, leaves were used for preparation of explants. Transformed shoots were regenerated in the presence of 100 μg/ml kanamycin.

Transgenic tomato plants containing a stabilized *Ac* element, SLJ10512, were crossed as transposase source to Ds^{HPT} transformants. A 177 bp deletion at the 3' end of *Ac* prevents transposition of the element (Scofield et al. 1992). Seeds of this tomato line were kindly provided by J.D.G. Jones (Norwich).

Tests for hygromycin resistance, Basta resistance and GUS activity

To select seedlings harbouring the Ds^{HPT} element, seeds were germinated on medium containing Murashige-Skoog (MS) salts, 3% sucrose and 20 mg/l hygromycin. The phenotypes were best assayed 10–20 days after plating. To test for BAR activity, seedlings were sprayed with a 1:200 dilution of the commercial formulation Basta (Hoechst), when they were about 5–10 cm in height. The phenotypes were assayed after about one week. To test for GUS activity, leaf pieces were vacuum-infiltrated with a solution containing 0.5 mM 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid in 50 mM sodium phosphate pH 7.0, 0.5% Triton-X-100 and incubated overnight at 37° C. After staining, plant pigments were partially removed in 70% ethanol.

DNA preparation and Southern blot analysis

DNA for Southern blot analysis and the inverse polymerase chain reaction (IPCR) was prepared as described previously (Brandstädter et al. 1993). For Southern blot analysis, approximately 5 μg genomic DNA was subjected to electrophoresis through 0.8% agarose. DNA was blotted onto Hybond N^+ membranes (Amersham) according to the manufacturer's instructions. The presence and copy number of T-DNAs in transgenic plants was determined using the 0.9 kb *Xho*I-*Hin*dIII fragment (probe D, Fig. 1) from plasmid pRT100neo (Töpfer et al. 1987). The presence of the *GUS*, *HPT*, and *BAR* genes was assessed using probes A, C, and E, respectively (Fig. 1). *Ds* elements were detected with the 1.1 kb *Eco*RI-*Hin*dIII fragment of *Ac* (probe B, Müller-Neumann et al. 1984). DNA for PCR analysis was prepared according to Klimyuk et al. (1993).

Isolation of T-DNA flanking regions

Templates for IPCR (Ochman et al. 1988; Triglia et al. 1988) were prepared by digestion of 500 ng genomic DNA with *Taq*I, followed by self-ligation and *Sst*II linearisation. The first PCR reaction was performed using the primers L621 (5'-GGGGATCC-GATTGTCGTTTCCCGCCTTCAG-3', positions 18–39 from the T-DNA right border) and L616 (5'-GGGAATTC-GACTGGGCACAACAGACAATCG-3', positions 414–435). Five μl of a 100-fold dilution from the first reaction was amplified in a second PCR reaction using nested primers L620 (5'-GGGAATTCCCCGCCTTCAGTTTAAACTATC-3', positions 7–28) and L517 (5'GGGAATTCGTTCTTTTTGTCAAGACC-GACC-3', positions 483–504). Each primer is equipped at the 5'

end (bases 1–8) with an *Eco*RI or a *Bam*HI recognition sequence to facilitate cloning.

Isolation of regions flanking transposed Ds^{HPT} elements

Flanking regions of transposed *Ds* elements were isolated using the IPCR technique (Ochman et al. 1988; Triglia et al. 1988). Genomic DNA was cut with *Eae*I (5' end of Ds^{HPT}) or *Apo*I (3' end of Ds^{HPT}), self-ligated, and linearized with *Bam*HI (5' end) or *Pac*I) (3' end), respectively. 5' Flanking regions were amplified with primers LDs1 (5'-GGGAATTCTGAAACGGTCGGGAAACTAG-3' positions 158–177 of *Ac*; Kunze and Starlinger 1989) and LDs2 (5'GGGGATCCATCGGGATGATCCCGTTTCG-3' (positions 195–214) for the first PCR, and LDs3 (5'-GGGAATTCTACGGGATTTTCCCATCCTA-3', positions 13–32) and LDs11 (5'GGGAATTCTTCTAATTCGGGATGACTGC-3', positions 225–244) for the second PCR. For amplification of sequences flanking the 3' end of transposed Ds^{HPT} elements, primers LDs7 (5'-GGGGATCCATAAGTCTTGGGCTCTTGGC-3', positions 4219–4238) and LDs8 (5'-GGGGATCCCGAACAAAAATACCGGTTCC-3', positions 4373–4392) were used for the first PCR and LDs9 (5'-GGGAATTCCTTGCTCACATCTGGATCAC-3', positions 4126–4145) and LDs10 (5'-GGGAATTCTTTTACCGACCGTTACCGAC-3', positions 4532–4551) for the second. IPCR products were cloned into the plasmid vector pGEM4Z.

Assignment of isolated sequences to the tomato RFLP map

Restriction fragments containing sequences flanking the T-DNA or transposed Ds^{HPT} elements were used to probe Southern blots of *L. esculentum* (cv. *Moneymaker*) and *L. pennellii* DNA separately digested with *Bam*HI, *Dra*I, *Hin*dIII, *Xba*I, *Eco*RI, and *Eco*RV. Probes that revealed polymorphisms were hybridized to DNA from 33 F_2 plants from an *L. esculentum* (cv. *VF*36) × *L. pennellii* F_1 hybrid digested with the appropriate enzyme. Segregation data were analyzed using the computer program MapMaker (Lander et al. 1987) and a framework dataset of 335 RFLP markers distributed over the 12 tomato chromosomes (provided by S. Tanksley, Tanksley et al. 1992). Markers were assigned to chromosomes using the "near" command with a LOD value greater than 3 and a recombination fraction smaller than 0.2, and to intervals within the framework using the "try" command.

Results

Characterization of Ds^{HPT}-bearing primary transformants

A genetically marked Ds^{HPT} element was introduced into the tomato cultivars *VF*36 and *Moneymaker* by *Agrobacterium tumefaciens*-mediated transformation. Independent transgenic plants, each arising from an individual leaf disc, were regenerated on kanamycin-containing medium using the P_{nos}-NPTII gene fusion at the right T-DNA border (Fig. 1) as a selectable marker.

Copy number and structural integrity of the introduced T-DNAs were analyzed by Southern hybridization experiments. The T-DNA copy number, as determined by the number of *Eco*RI fragments hybridizing to probe D (Fig. 1), varied between 1 and 6. To facilitate the assignment of T-DNA insertions to specific map positions and to allow for the characterization of transposition patterns from individual donor sites, plants

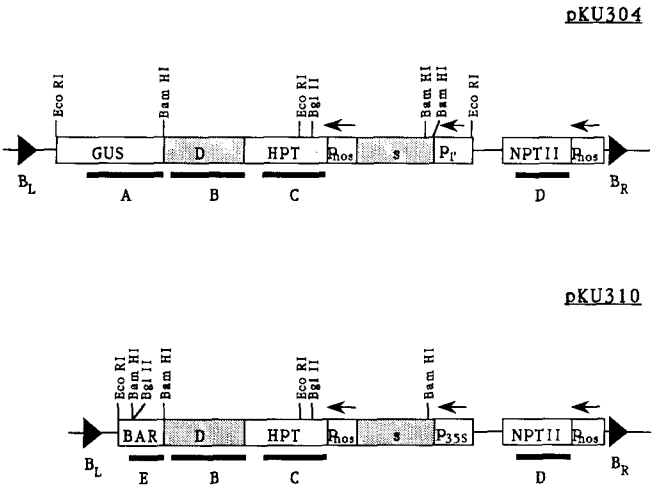

Fig. 1 Schematic representations of the vector constructs pKU304 and pKU310. B_L, left border; B_R, right border; BAR, phosphinotricin resistance gene, Ds, *Dissociation* element; GUS, β-glucuronidase; HPT, hygromycin phosphotransferase; NPT, neomycin phosphotransferase; P_{nos}, nopaline synthase promoter; P_1, 1' promoter of T-DNA; P_{35S}, 35S promoter of cauliflower mosaic virus. The *thick bars* indicate the probes A, B, C, D, and E used for Southern blot analysis

Table 1 Transgenic plants with mapped T-DNA insertions carrying Ds^{HPT}

Plant No.	Tomato Line	Vector	Mapped to chromosome
ET85	VF36	pKU304(Ds^{HPT} GUS)	7
ET240	VF36	pKU304(Ds^{HPT} GUS)	3
ET100	VF36	pKU304(Ds^{HPT} GUS)	1
90504	MM[a]	pKU306(Ds^{HPT} BAR)	9
90530	MM	pKU306(Ds^{HPT} BAR)	11
91570	MM	pKU310(Ds^{HPT} BAR)	3
91590	MM	pKU310(Ds^{HPT} BAR)	7
91655	MM	pKU310(Ds^{HPT} BAR)	4
91699	MM	pKU310(Ds^{HPT} BAR)	4
91672	MM	pKU310(Ds^{HPT} BAR)	1
91714	MM	pKU310(Ds^{HPT} BAR)	6

[a] Moneymaker

containing a single T-DNA insertion were chosen for further experiments. Structural integrity of the introduced T-DNAs was examined using probes A, B, C, D, and E (Fig. 1) in combination with appropriate restriction enzyme digests. With few exceptions, no grossly rearranged T-DNA structures were observed. Only plants with structurally intact T-DNA were further analyzed.

Mapping of T-DNA insertions

Genomic DNA sequences flanking the right border of T-DNA insertions were amplified by IPCR (Ochman et al. 1988; Triglia et al. 1988) and subsequently used in RFLP linkage analyses to map the corresponding T-

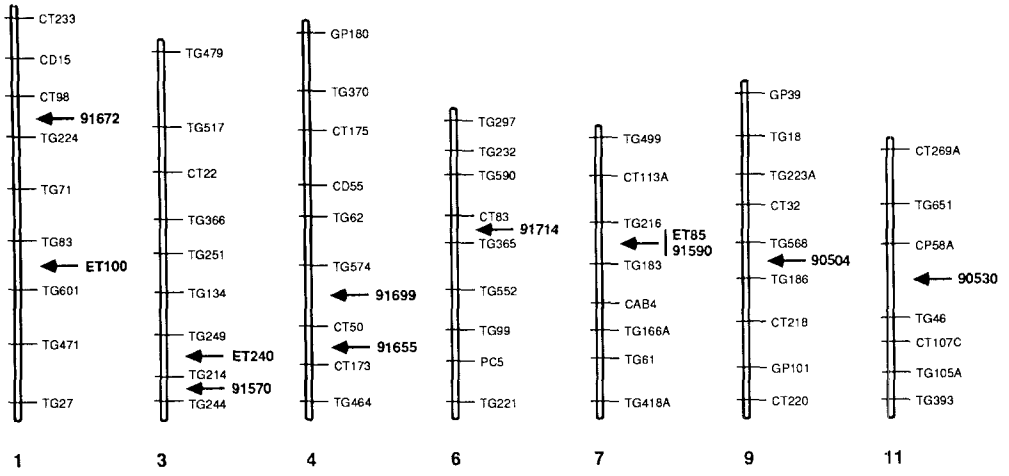

Fig. 2 RFLP map positions of T-DNA insertions harbouring Ds^{HPT} elements. *Arrows* indicate the positions of T-DNA insertions in independent transgenic plants on a partial RFLP map of tomato. A set of framework RFLP markers (Tanksley et al. 1992) is indicated beside tick marks on numbered chromosomes. The Figure shows 7 of the 12 tomato chromosomes

DNA insertions. IPCR products from 11 T-DNA insertions that hybridized to unique sequences in the tomato genome were assigned to the tomato RFLP map (Tanksley et al. 1992). They are distributed on 7 of the 12 tomato chromosomes (Table 1, Fig. 2). In order to exclude PCR artifacts, we have determined the pattern of T-residues (T-tracking) of the IPCR products (data not shown). These experiments revealed that each IPCR product consisted of a piece of known T-DNA sequence flanked by a stretch of unknown DNA. The stretches of unknown DNA showed unique patterns of T-residues, confirming that the IPCR products were derived from independent T-DNA insertions in the tomato genome. Some of the T-DNA insertions were found to be linked (e.g. ET240 and 91570, ET85 and 91590). The insertion sites of the two T-DNAs located on chromosome 7 (ET85 and 91590) are closely linked, as no recombination was observed in a segregating F_2 population of 33 individuals. Since the RFLP patterns of these two probes on the Southern blot of the parental DNAs, as well as the patterns of T-residues in the flanking genomic DNA, are different, the possibility that these probes originated by cross contamination from the same T-DNA insertion can be excluded.

Identification of plants harbouring transposed Ds^{HPT} elements

In order to study the transposition pattern of the introduced Ds^{HPT} elements and to isolate tomato lines with Ds^{HPT} insertions at new chromosomal positions, we have chosen the transgenic plants ET85 and 91590, each of which harbours a single T-DNA insertion in closely linked positions on chromosome 7 (Table 1, Fig. 2). ET85 and 91590 were pollinated by a transgenic tomato line homozygous for the stabilized *Ac* element SLJ10512 (Scofield et al. 1992). F_1 plants containing the Ds^{HPT} element were selected on hygromycin-containing medium and either test-crossed or self-pollinated. PCR analysis for Ds^{HPT} excision revealed somatic excision in different F_1 individuals of both families (data not shown).

Transposed Ds^{HPT} elements inherited from the F_1 generation were identified in the F_2 generation. To exclude early somatic transposition events in the F_2 generation from the analysis, and to prevent further transposition of Ds^{HPT} elements, only F_2 seedlings without the transposase source were evaluated. F_2 seedlings of family 91590 were selected with Basta and hygromycin to enrich for those containing an empty donor site and a Ds^{HPT} element, respectively, in the genome. F_2 plants containing the transposase source were easily identified by the GUS marker present on the T-DNA carrying the stabilized *Ac* element. To identify transposed Ds^{HPT} elements in family 91590, 101 selected F_2 plants were finally analyzed by Southern hybridization experiments using restriction fragment B (Fig. 1) as a probe. These experiments revealed the presence of 207 transposed Ds^{HPT} elements at new genomic locations, 134 of which seem to be independent. A representative Southern blot

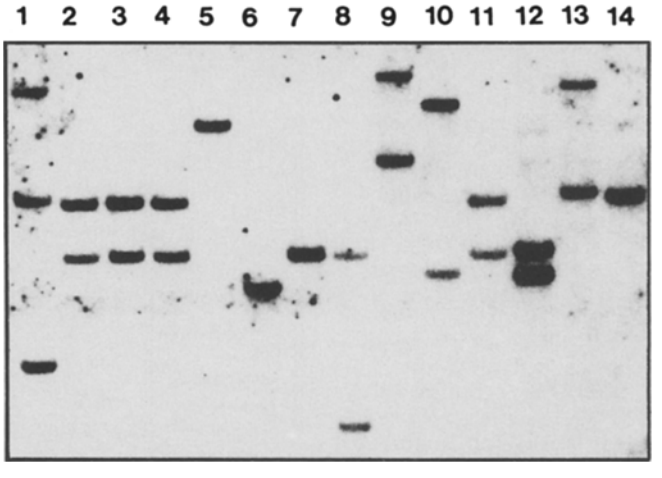

Fig. 3 Southern blot analysis of selected progeny plants from family 91590. Genomic plant DNA from the test-crossed progeny plants 92388-1 (1) to 92388-14 (14) was digested with *Bgl*II and hybridized to probe B (see Fig. 1)

is shown in Fig. 3. The copy number of transposed Ds^{HPT} elements varied between 1 and 6.

In family ET85, expression of a GUS marker on the T-DNA carrying the Ds^{HPT} element indicates excision of the element (Fig. 1, Table 1). Because the GUS gene is also present on the stabilized *Ac* T-DNA, F_2 seedlings of this family were selected only on hygromycin, and tested for the presence of the stabilized *Ac* element by PCR analysis. Southern blot hybridizations of 43 selected F_2 plants without the transposase source revealed the presence of 57 Ds^{HPT} elements in new chromosomal positions, 33 of which seem to be independent. Twenty-four F_2 plants contained a *Ds* border fragment of the same size, probably reflecting a transposition event which occured early in the F_1 plant and was transmitted to many F_2 progeny. The copy number of transposed Ds^{HPT} elements varied between 1 and 4.

Mapping of transposed Ds^{HPT} elements

Genomic DNA flanking either the 5' or the 3' end of transposed Ds^{HPT} elements in selected F_2 plants from the primary transgenic plants ET85 and 91590 was amplified by IPCR and subsequently used to localize the respective Ds^{HPT} elements on the tomato RFLP map. Thirteen transposed Ds^{HPT} elements from family 91590 were mapped (Table 2, Fig. 4). Seven transposed Ds^{HPT} elements (*Ds*20, *Ds*23, *Ds*24, *Ds*117, *Ds*131, *Ds*132, *Ds*137) had reinserted in the immediate vicinity of the T-DNA insertion on chromosome 7. In a mapping population of 33 F_2 plants, no recombination was observed between any of these transposed Ds^{HPT} elements and the 91590 T-DNA. To refine the mapping data, the T-DNA insertion and the transposed Ds^{HPT} element *Ds*20 were mapped again in a larger population of 92 F_2 plants. This experiment revealed a recombination frequency of 0.5 cM between these two markers. Another Ds^{HPT} element (*Ds*135) was mapped to the distal part of chromosome 7, 40 cM from the donor site. The remaining five transposed elements are located on chromosomes 1, 2, 3, 5, and 11 (Fig. 4).

The pattern of mapped Ds^{HPT} elements in family ET85 was strikingly different. Only one out of eight mapped Ds^{HPT} elements had reinserted on chromosome 7 in close proximity to the ET85 T-DNA insertion (Table 3, Fig. 5). The other seven elements had transposed to five different chromosomes. Three of these ele-

Table 2 List of mapped Ds^{HPT} elements in family 91590

F_2 Plant No.	F_1 Plant No.	No. of transposed Ds^{HPT}	IPCR probe	Mapped to chromosome
92374-4	92101-2	1	Ds17	11
92374-10	92101-2	1	Ds23	7
92388-6	92101-2	1	Ds20	7
92388-13	92101-2	2	Ds24	7
92388-19	92101-2	2	Ds25	1
9345-19	92101-2	2	Ds117	7
9345-2	92101-2	1	Ds131	7
9345-4	92101-2	3	Ds132	7
9345-30	92101-2	2	Ds134	2
9345-56	92101-2	1	Ds135	7
9345-49	92101-2	1	Ds136	5
92375-1	92101-5	1	Ds18	3
9398-2	92101-5	1	Ds137	7

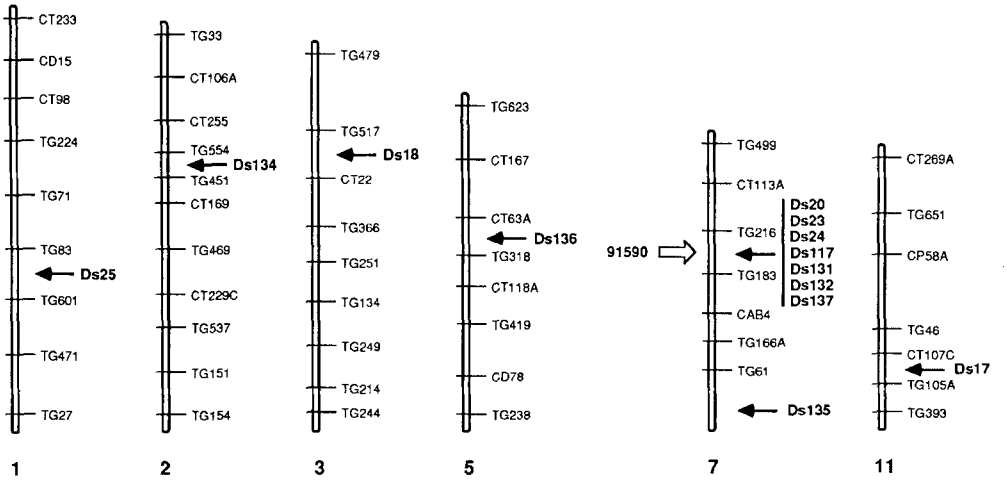

Fig. 4 RFLP map positions of transposed Ds^{HPT} elements in family 91590. The *open arrow* indicates the map position of the single T-DNA insertion in family 91590 on a partial RFLP map of tomato. *Filled arrows* indicate the positions of transposed Ds^{HPT} elements in independent transgenic plants. Framework RFLP markers (Tanksley et al. 1992) are indicated beside tick marks on numbered chromosomes. The Figure shows 6 of the 12 tomato chromosomes

Table 3 List of mapped Ds^{HPT} elements in family ET85

F_2 Plant No.	F_1 Plant No.	No. of transposed Ds^{HPT}	IPCR probe	Mapped to chromosome
92475-7	92327-9	1	Ds22	10
92535-9	92327-9	4	Ds102	3
92535-20	92327-9	1	Ds103	4
92535-40	92327-9	4	Ds104	10
92535-153	92327-9	3	Ds129	10
92536-199	92327-3	1	Ds110	7
92536-186	92327-3	1	Ds124	12
92537-230	92327-2	4	Ds112	2

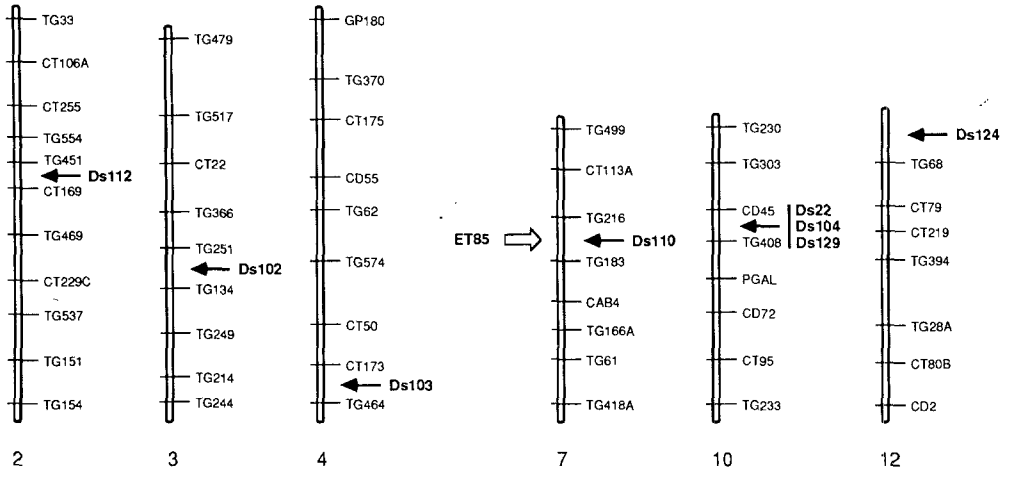

Fig. 5 RFLP map positions of transposed Ds^{HPT} elements in family ET85. The *open arrow* indicates the map position of the single T-DNA insertion in family ET85 on a partial RFLP map of tomato. *Filled arrows* indicate the positions of transposed Ds^{HPT} elements in independent transgenic plants. Framework RFLP markers (Tanksley et al. 1992) are indicated beside tick marks on numbered chromosomes. The Figure shows 6 of the 12 tomato chromosomes

ments (*Ds*22, *Ds*104, and *Ds*129) mapped to closely linked positions on chromosome 10. One of these, *Ds*104, corresponds to the early transposition event uncovered by the previous Southern analysis. Therefore, this cluster of linked elements is probably the result of secondary transposition events of *Ds*104.

To exclude PCR artifacts, we determined the pattern of T-residues (T-tracking) in those IPCR products mapping to the same chromosomal location (see Fig. 4 and 5). Each product revealed a stretch of known DNA sequence derived either from the 5' or the 3' end of the element and an unknown DNA sequence of varying length. The stretches of unknown DNA showed unique patterns of T residues, indicating an independent origin of each product. In addition, each probe detected a unique pattern of restriction fragments when hybridized to genomic DNA of *L. esculentum* and *L. pennellii* digested with seven different restriction enzymes. These results demonstrate that each IPCR probe was derived from a different insertion site of a transposed Ds^{HPT} element in the tomato genome.

Discussion

With the goal of using the maize transposable element system *Ac*/*Ds* for targeted transposon tagging experiments in tomato, we have introduced and mapped a genetically marked Ds^{HPT} element in this species. The introduced constructs are equipped with two selectable or scorable marker genes to reduce the number of progeny plants to be screened in transposon tagging experiments for mutant phenotypes. A hygromycin phosphotransferase gene (*HPT*) inserted inside the *Ds* element allows for the selection of plants harbouring the Ds^{HPT} element. In most transgenic plants the *BAR* gene was used to indicate excision of the element from its donor site in the T-DNA. After crossing to a plant containing a transposase source, this marker was used to select progeny plants that had undergone an excision event. We have demonstrated that both marker genes were active in several transgenic plants over at least three generations, thus it is feasible to use double selection with hygromycin and Basta to enrich for plants harbouring a transposed Ds^{HPT} element.

The T-DNA insertions in 11 independent transgenic plants are distributed over 7 chromosomes (Fig. 2). Surprisingly, two T-DNA insertions map to closely linked positions on chromosome 7. This finding may indicate a hot-spot for T-DNA insertions in this region. Alternatively, and perhaps more likely, this chromosome 7 segment, which is located in the centromere region, may represent a region of suppressed recombination, leading to an underestimation of the physical distance between the two insertion sites (Tanksley et al. 1992).

In addition, transposed Ds^{HPT} elements were mapped in progeny plants lacking a transposase source. These plants were propagated to extend the collection of

transgenic lines bearing mapped Ds^{HPT} insertions. Despite the absence of an excision marker, the use of transposed Ds^{HPT} elements for transposon tagging experiments seems feasible, because the proportion of progeny plants containing germinally transmitted transposed Ds^{HPT} elements is high (e.g. about 52% in family ET85).

Several T-DNA insertions as well as transposed Ds^{HPT} elements are located close to interesting genes. For example, the T-DNA insertions ET85 and 90504 map in the vicinity of the disease resistance genes *I-3* (resistance to *Fusarium oxysporum* race 3) and *Tm-2a* (resistance to tobacco mosaic virus), respectively (Tanksley et al. 1992). The transposed Ds^{HPT} element *Ds*136 maps close to the genes *Pto* (resistance to *Pseudomonas syringae* pv. *tomato*; Martin et al. 1993) and *rin* (ripening inhibitor; Tanksley et al. 1992), and the transposed element *Ds*17 is located close to the *I-2* gene (resistance to *Fusarium oxysporum* race 2; Tanksley et al. 1992). After transactivation by a stable *Ac* element, these insertions can serve as starting-points for targeted transposon tagging experiments aimed at mutating the respective genes. The whole collection of transgenic tomato lines containing Ds^{HPT} insertions at defined genomic locations is a valuable tool for targeted and for untargeted transposon tagging experiments.

Because the efficiency of targeted transposon tagging depends largely on the pattern of transposition, we have studied the pattern of Ds^{HPT} transposition. To test whether transposition events originating from two closely linked loci will result in similar patterns of reinsertion sites, we have chosen the families ET85 and 91590, each containing a T-DNA insertion in closely linked positions on chromosome 7, for these experiments. The observed distributions of transposed Ds^{HPT} elements are strikingly different. In family 91590, transposed Ds^{HPT} elements preferentially reinserted close to the T-DNA (Fig. 4). This result is in contrast to recent observations on the transposition patterns of a *Ds-r* element (Rommens et al. 1993) and a Ds^{neo} element (Healy et al. 1993), which did not reveal a preference for reinsertions close to the T-DNA. However, the extreme clustering of reinsertion sites in family 91590 may be an effect of lower levels of meiotic recombination, because donor site and reinsertion sites are located near the centromere, which is known to exert a negative effect on the frequency of recombination (Tanksley et al. 1992).

In family ET85, we saw no preference for transpositions of Ds^{HPT} to linked positions (Fig. 5). This observation may be explained by the assumption that, from this genomic position, Ds^{HPT} transposes preferentially to unlinked sites, with a hot spot of integration on chromosome 10. Alternatively, the observed pattern of transposed elements may be the result of a combination of primary and subsequent transpositions. In F_1 plant 92327-9, a very early transposition of Ds^{HPT} (e.g. in the zygote) to chromosome 10 may have been followed by secondary transpositions with a preference for linked positions (Table 3). Therefore, the distribution of transposed elements in family ET85 does not necessarily contradict a preference for transpositions to linked positions. We realize the number of F_1 progenies analyzed is too low to prove or disprove this hypothesis. Nevertheless, for targeted transposon tagging it is advisable to use different F_1 plants to exclude this kind of bias from the experiment. To overcome these difficulties, it would be desirable to generate a large collection of transgenic plants with mapped *Ds* insertions. This would allow for the selection of several linked elements for tagging experiments directed towards a particular gene. If one has to rely on a single donor site, the pattern of transposition from the respective donor site should be studied prior to a targeted tagging experiment.

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