Theor Appl Genet (2004) 109:333–341
DOI 10.1007/s00122-004-1629-5

ORIGINAL PAPER

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Mapping of transposable element *Dissociation* inserts in *Brassica oleracea* following plant regeneration from streptomycin selection of callus

Received: 12 June 2003 / Accepted: 9 February 2004 / Published online: 10 March 2004

Abstract To investigate the potential of heterologous transposons as a gene-tagging system in broccoli (*Brassica oleracea* var. *italica*), we have introduced a *Dissociation* (*Ds*)-based two-element transposon system. *Ds* has been cloned into a 35S-SPT excision-marker system, with transposition being driven by an independent 35S-transposase gene construct. In three successive selfed generations of plants, there was no evidence of germinal-excision events. In a previous study, we overcame this apparent inability to produce *B. oleracea* plants with germinal excisions by performing a novel tissue-culture technique to select for fully green shoots from seed with somatic excision events. The results showed a very high efficiency of regeneration of fully green plants (up to 65%), and molecular analysis showed that the plants contained the equivalent of a germinal-excision event. In this study, we followed the previous work by using inverse and nested PCR to generate probes of flanking genomic DNA adjacent to independently reinserted *Ds* elements, and these were hybridised to DNA from a double-haploid mapping population of *B. oleracea.* Seventeen *Ds* insertions and the original *Ds* T-DNA site have been localised, and these are spread over six (out of nine) linkage groups. Distribution of inserts show that 15 were found on a different linkage group to the original 'launch' site, and of these 11 were found to be clustered on two separate groups. Previous studies in other plant species have found that germinal excision of *Ds* predominantly moves to sites linked close to the donor site. However, this study shows a potential to produce plants with *Ds* insertion scattered over many unlinked sites.

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Introduction

The well-characterised maize transposable elements *Activator* (*Ac*) and *Dissociation* (*Ds*) have been shown to transpose in several plant species, for example, tobacco (Baker et al. 1988), tomato (Yoder et al. 1988), *Arabidopsis thaliana* (Van Sluys et al. 1987), potato (Knapp et al. 1988), *Petunia* (Haring et al. 1989), rice (Izawa et al. 1991), soybean (Zhou and Atherly 1988) and the legume *Lotus japonicus* (Thykjaer et al. 1995). The *Ds* element does not contain a functional transposase gene. Therefore, it cannot induce its own transposition (non-autonomous). A *Ds* element transposes only under the influence of transposase produced by an *Ac* element (autonomous) within the same plant. A two-element system using the *Ds* element mobilised by the presence of an *Ac* element derivative, which is itself unable to transpose (*Tpase*), has been found to be effective in transposon tagging experiments in *A. thaliana* (Bancroft et al. 1993; Long et al. 1993; Altmann et al. 1995; Springer et al. 1995), tomato (Jones et al. 1994; van der Der Biezen et al. 1996a) and tobacco (Whitham et al. 1994).

In a previous study we introduced a *Ds*-based two-element transposon system into *Brassica oleracea* var. *italica* (Mckenzie et al. 2002). *Ds* has been cloned into a 35S-SPT excision marker system, to allow identification of excision events by selection on streptomycin (Jones et al. 1989). We anticipated that the behaviour of the system would be similar to that found in previous studies of heterologous plant species, but observed very low activity of the *Ac*/*Ds* system in broccoli.

A crucial requirement for the use of the *Ac*/*Ds* transposon system for gene isolation is to obtain germinal excision events or plants with transposon excision in all cells (Carroll et al. 1995). However we found in three successive selfed generations of plants there was no evidence of germinal excision events.

As previously described (Mckenzie et al. 2002), we performed a novel tissue culture technique to select using streptomycin for fully green shoots from seed with somatic excision events. The results showed a very high

efficiency of regeneration of fully green plants (up to 65%), and molecular analysis showed that the plants were genetically like plants that contain a germinal excision event. Further molecular analysis of these plants showed that 69% exhibited reinsertion of *Ds* into the plant genome.

In this study we aim to characterise the distribution of transposition events. Is it similar to other species, where the *Ac*/*Ds* system transposes predominantly to linked sites following germinal excision events (Jones et al. 1990; Dooner et al. 1991; Bancroft and Dean 1993; Carroll et al. 1995; van der Biezen et al. 1996b)? In tomato it was found that transposable elements exhibiting somatic transposition events have a tendency to move to unlinked sites (Osborne et al. 1991; Belzile and Yoder 1992; Healy et al. 1993). The antibiotic selection method used in our study was based on a seed stock containing somatic transposition events. Therefore, we need to consider whether this produces 'germinal' plants that have a tendency for *Ds* to move further than normally observed for germinal transposition events. This is of importance when devising transposon-tagging strategies.

Independent *Ds* elements and the original *Ds* construct location ('launch' site) were characterised, and we devised an inverse PCR strategy combining 'nested' primers to isolate flanking genomic DNA. The amplified DNA was used as probes in a double-haploid mapping population subset from a cross between two *B. oleracea* var., *alboglabra* (A12DHd) and *italica* (GDDH33), to identify the extent of *Ds* mobility.

Materials and methods

Plasmids

The two binary plasmid constructs used in this study are shown in Fig. 1. The *Ds* construct [*Ds* (HYG)::35S-SPT, Fig. 1A] is composed of a *Ds* element that has been cloned into the untranslated leader of the *SPT* (streptomycin phosphotransferase) gene, which is used as an excision marker to monitor the excision of the *Ds* element by selection on media containing streptomycin. Details of the construction of this construct have been previously described by Mckenzie et al. (2002). The transposase (*Tpase*) source needed for the transposition of *Ds* in trans was provided by the plasmid SLJ1111 (Fig. 1B). This construct consists of a stabilised *Ac* element driven by the CaMV 35S promoter (35S*Tpase*), which has been found to give elevated excision frequencies in tobacco and *Arabidopsis* (Swinburne et al. 1992; Scofield et al. 1994). The construction of this plasmid has previously been described by Scofield et al. (1992).

Plant transformation

The binary plasmids *Ds* (HYG)::35S-SPT and 35S*Tpase* were introduced into *B. oleracea* var. *italica* (Italian green sprouting 577) cotyledonary petioles or hypocotyl segments by *Agrobacterium*-mediated transfer by the methods of Moloney et al. (1989) and De Block et al. (1989), using *A. tumefaciens* strains LBA 4404 or C58C1. Progeny of the crosses were screened on streptomycin to detect evidence of transposition events (Jones et al. 1989; Mckenzie et al. 2002)

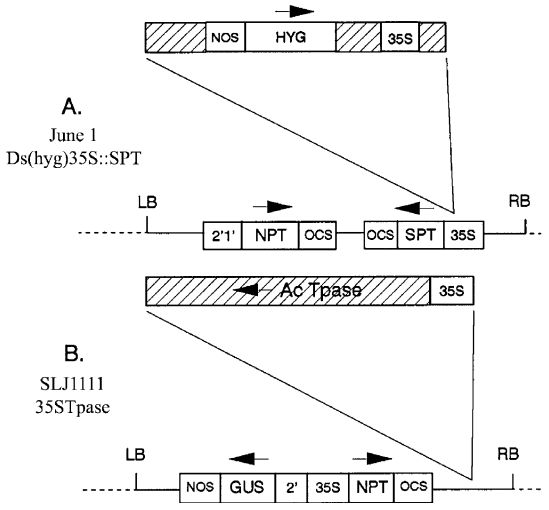

Fig. 1A, B Diagrammatic representation of the binary constructs used in this study. **A** June 1 [*Ds*(HYG)::35S SPT]. The *hatched sections* represent *Activator* (*Ac*) sequences in the constructs. **B** SLJ1111 (35S *Tpase*), a stabilised *Ac* element used to stimulate transposition of the *Dissociation* (*Ds*) element

Callus and shoot regeneration on streptomycin media

A line displaying high somatic excision activity from streptomycin screening (F_3 805-2 with 66% of seedlings displaying excision) was chosen as the seed stock for selection. Cotyledons or hypocotyl explants were excised and plated onto basal Murashige and Skoog media (Imperial Labs) with 0.8% phytoagar (Gibco BRL); inositol 100 mg/l; thiamine 10 mg/l; pyridoxine 1 mg/l; nicotinic acid 1 mg/l; 3% sucrose with the addition of benzyl aminopurine (Sigma) at a concentration of 3.75 mg/l; and streptomycin sulphate at 25, 50, 100, 200 or 300 μg/ml. Explants were subcultured every 2 weeks, and any white leaves or shoots were cut out so that only green shoots were allowed to grow. When plantlets had root initiation they were transferred to peat pots. After a further 2 weeks cultivation plants were repotted and allowed to mature in the glasshouses. A more detailed methodology of the regeneration of plants from streptomycin selection of callus and plant genotypes are described by Mckenzie et al. (2002).

Plant genomic DNA isolation and Southern-hybridisation analysis

Plant genomic DNA was extracted as described by the procedure of Sharp et al. (1988), except that the Kirby mix containing tri-iso propylnaphthalenesulphonate (1%), 4-aminosalycilate (6%), phenol (5%) and 50 mm Tris-HCl pH 8.4 (Covey and Hull 1981) was used for the first extraction step of the milled, freeze-dried leaf material. The genomic DNA was quantified flourometrically following the method of Labarca and Paigen (1980). Ten micrograms genomic DNA was digested to completion using *Eco*R1 restriction endonuclease and run out by electrophoresis on 0.8% TAE agarose gels and blotted onto Hybond-N^+ membranes (Amersham).

A 1.46-kb *Sal*I/*Bgl*II fragment from plasmid pPCV720 [supplied by C. Dean, John Innes Centre (JIC)], corresponding to the reading frame of the hygromycin phosphotransferase gene was used as a probe. This was prepared by the standard methods of Sambrook et al. (2001). Probe DNA was isolated from agarose gels (low-melt point) using the Geneclean II or Geneclean Spin kit (Bio101, La Jolla, Calif.) and labelled using the random primer method (Feinberg and Vogelstein 1984).

Ligation and PCR strategies to produce hybridisation probe

A hybridisation probe was created using inverse and nested PCR strategies (Sambrook et al. 2001). Selected plant DNA (3.3 µg) was digested using *Eco*R1 restriction endonuclease. DNA was cleaned using Geneclean Spin, and the product (approx. 2.5 µg after cleaning) ligated in a 400-µl reaction volume containing 10 Weiss units T4 DNA ligase 1× ligation buffer (Pharmacia) and 40 mM ATP at 10°C for 16 h. The ligation reaction was stopped by heating to 65°C for 10 min. Ten microlitres of ligation reaction (60–70 ng) was used in a 100-μl inverse-PCR reaction containing 250 μM each nucleotide, 250 ng each oligonucleotide primer, 1× reaction buffer and 2.5 U Pfu turbo DNA polymerase (Stratagene). The PCR product was run on 1% to 1.5% TAE low melting point agarose electrophoresis gels (depending on expected fragment size) and the expected PCR fragment isolated from the gel by using Geneclean spin. A second, nested PCR reaction was performed to confirm that the correct band was isolated and to produce product with less *Ds* or construct sequences.

The oligonucleotide sequences used for the first PCR were for the original *Ds* construct position and are from the T-DNA left border of the *Ds* construct, LBP1 5′-ATGGCGAAATCAAGGCATCG, and from the hygromycin gene, HYG5′P1 5′- CGTGGATATGTCCTGCGGGTAAAT. Nested PCR was performed using LBP3 5′-CGACGGATCGTAATTTGTCG or LBP4 5′-TGTAGATTTCCCGGACATGAAGCC and HYG5′P2 5′-CCGATTCCGGAAGTGCTTGACATT.

The oligonucleotide sequences used for the first PCR for the independent *Ds* insertions are from the *Ac*/*Ds* 3′-end Ac3′P1 5′-GCCCAAGACTTATCACTTATGTGC and HYG5′P1 as above. Nested PCR was performed using Ac3′P2 5′-TCCGTCCCGCAAGTTAAATATGAA or Ac3′P3 5′-TTTACCGACCGTTACCGACCGTTT and HYG5′P2.

The thermal cycler programme parameters were: 4 min at 95°C followed by 30 cycles of 1 min at 95°C, 1 min at 55°C, and 2 min per kb of expected PCR product size at 72°C.

To calculate the expected fragment size for the inverse and nested PCR reactions, the oligonucleotide primers are located at the following distances from the genomic DNA: LBP1, 318 bp; LBP3, 192 bp; LBP4, 48 bp; HYG5′P1, 120 bp; HYG5′P2, 33 bp; Ac3′P1, 422 bp; Ac3′P2, 159 bp; and Ac3′P3, 114 bp.

Nested PCR product was isolated from low-melt gel, cleaned with Geneclean spin, then radiolabelled using the random primer method (Feinberg and Vogelstein 1984).

Production of mapping population and hybridisation of inverse and nested PCR products

The mapping population was created from a microspore-derived double-haploid (DH) line of *B. oleracea* var. *italica* (GDDH33) pollinated with a DH line of *B. oleracea* ssp. *alboglabra* (A12DHd) which produced a set of uniform F_1 offspring as described by Bohuon et al. (1996).

A subset of 55 progeny (from a total population of 210 individuals) covering nine linkage groups was used. Plant DNA was isolated and restriction digested with *Eco*RI. DNA was Southern blotted and hybridised with the flanking genomic *Ds* PCR products by the methods described by Sharpe et al. (1995). The resulting restriction fragment length polymorphisms (RFLPs) detected were scored and compared with the genetic linkage data from a 124 RFLP marker subset from 310 RFLP loci previously compiled by Bohuon et al. (1996). Data was analysed using MAPMAKER version 3 (Lander et al. 1987) using a minimum LOD score of 4.0.

Results

Characterisation of *Ds* inserts

The objective of the streptomycin selection method was to regenerate plants from a tissue culture system, similar to that used for transformation, to obtain plants made up entirely of streptomycin-resistant cells. Throughout this report we refer to these regenerant plants, which are completely resistant to streptomycin, as ‘germinals’. Regenerant plants that were not fully streptomycin resistant, where there are still *Ds* elements present within the donor site, are termed ‘somatics’. A detailed account of production of these plants was described by Mckenzie et al. (2002). To summarise, a total of 90 regenerant plants were produced, of which 58 were found to be germinals, and 41 of these showed reinsertion of *Ds* into the plant genome. Overall there were 73 plants that showed *Ds* reinsertion events that could be used for further analysis.

Plant DNA was extracted from the regenerated plants and was digested with *Eco*RI restriction endonuclease, Southern blotted, then hybridised with a DNA probe corresponding to the hygromycin gene which is located within the *Ds* element. The autoradiograph in Fig. 2 shows the presence of independent *Ds* insertions from a selected number of regenerated plants. Plant samples from lanes 6, 8, 9, 12, 14, 16, 20, 23 and 24 all were characterised as ‘somatic’ where the *Ds* donor site is visualised by a fragment of about 11 kb (band A). Plant samples from lanes 18 and 22 are examples of a germinal plant that has not had *Ds* reinsertion.

The *Ds* element and construct have been fully characterised and sequenced, and the approximate length of flanking genomic DNA specific for each *Ds* insertion can be calculated. For the *Ds* construct or non-excised *Ds* element, the length of known sequences, from the *Eco*RI site in the hygromycin gene to the left border insertion site, is 7,683 bp, which results in approximately 3.3 kb of genomic DNA from the construct to the nearest *Eco*RI site in the genome. The length of genomic DNA for the independent *Ds* inserts can be approximated by subtracting 1,883 bp (*Eco*RI site to end of *Ds* element) from the fragment size for each specific *Ds* insertion event. To know this is very important when performing the inverse and nested PCR, especially for plant samples with more than one *Ds* insert, as the expected fragment size can be pre-determined so that isolating the correct band for the specific *Ds* element can be accurately performed.

Inverse and nested PCR strategy to isolate flanking genomic DNA

Twenty-six plant samples were used for probe production by inverse and nested PCR. A total of 42 probes were produced, of which 37 represent independent *Ds* insertions (‘landing’ sites) from 24 individual regenerant plants. The additional five probes that were produced correspond to original *Ds* location (launch site); these

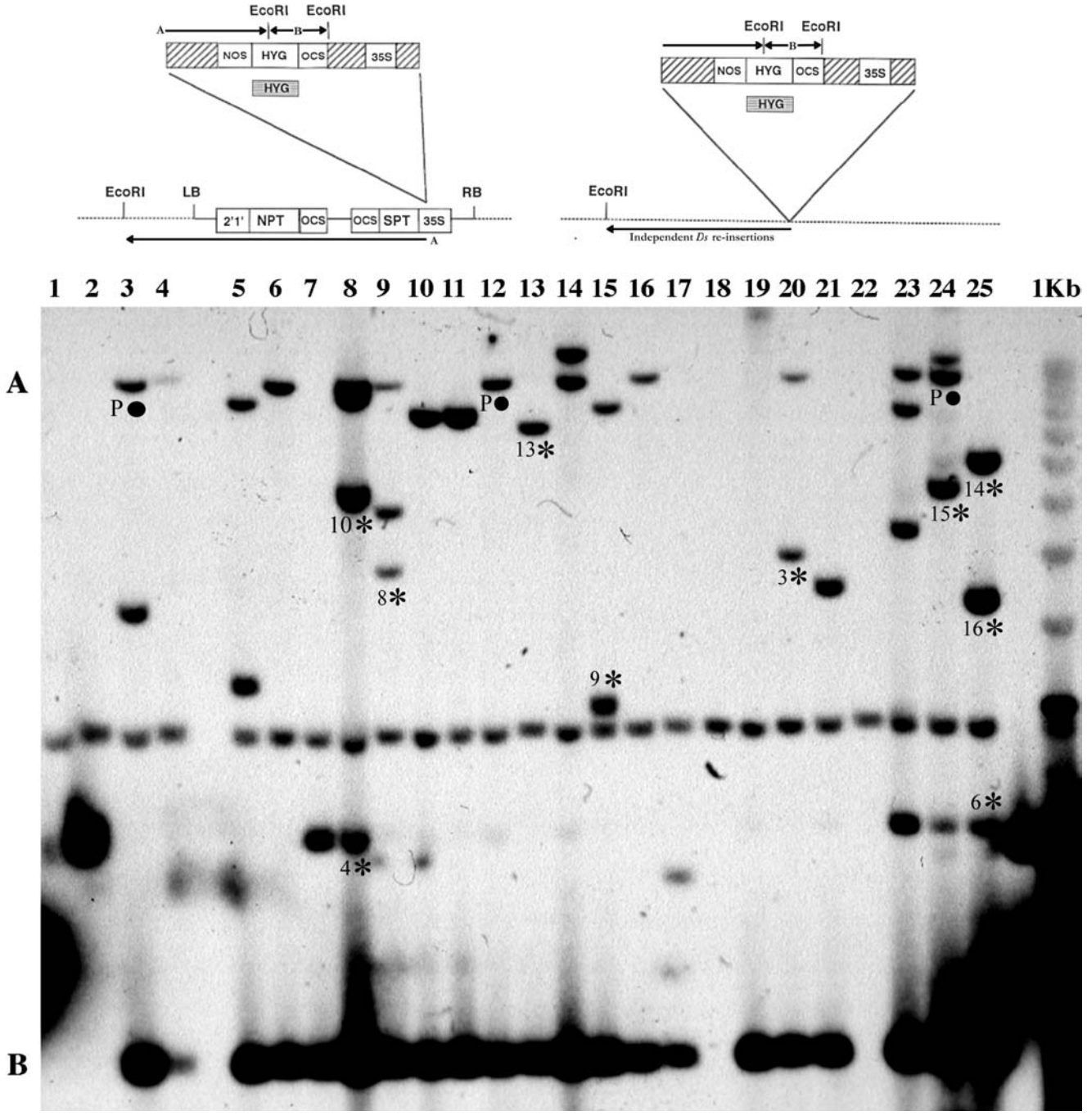

Fig. 2 Schematic representation (*top panel*) of the *Ds* construct showing the approximate position of the *Eco*RI restriction sites. The *Eco*RI site on the *dashed line* adjacent to the left border sequences (*LB*) for the *Ds* construct (*left*) represents the nearest *Eco*RI site to the T-DNA insertion site within the plant genomic DNA and is represented on the autoradiograph as band *A*. The diagram on the *right* represents the re-insertion of the *Ds* element after excision. Independent re-insertions of *Ds* are visualised by variable-sized bands depending on the nearest *Eco*RI site.

*Eco*RI Southern blot hybridised with a probe corresponding to the reading frame of the hygromycin phosphotransferase gene (*bottom panel*). *Band A* Non-excision of *Ds* (~11 kb). *Band B* Internal *Eco*RI sites in the *Ds* element. *Lane 1* Non-transformed *Brassica oleracea*, *lane 2* 35S *Tpase* parent, *lane 3 Ds*(HYG)::35S SPT parent (two copies of *Ds* in plant), *lane 4* seed stock parent plant (1 copy of *Ds*), *lanes 5–25* regenerated plants. Bands marked with *P*● represent non-excised *Ds* elements that were amplified by inverse and nested PCR to use as probe to determine the 'launch' site of the *Ds* element. *Numbered bands* marked with an *asterisk* represent independent *Ds* insertion events that have been mapped and positions are shown in Fig. 6

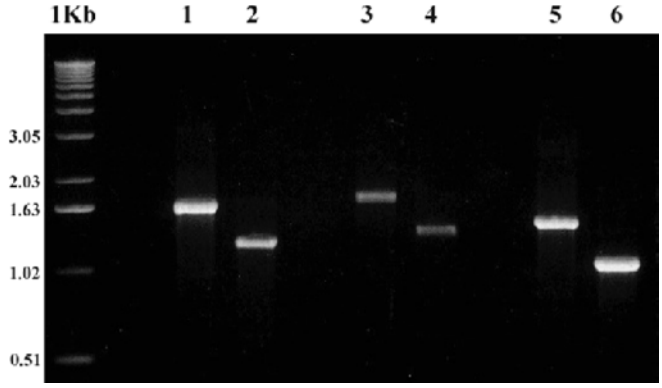

Fig. 3 Inverse and nested PCR for independent *Ds* insertion events. *Lanes 1*, *3* and *5* Inverse PCR using oligonucleotides HYG5′P1 and Ac3′P1; *lanes 2*, *4* and *6* nested PCR from previous inverse-PCR reaction using HYG5′P2 and Ac3′P2. Isolated amplified DNA from lane 4 was used as probe to map insert 9* on Fig. 2 and Fig. 6. DNA isolated from lanes 2 and 6 were used to map inserts 5* and 17*

were amplified from five different somatic plants including the F_3 805-2 parent. Figure 3 shows examples of probe production by inverse and nested PCR.

Mapping

Overview

The flanking genomic-DNA probes were radiolabelled and hybridised to filters from a mapping population, which consisted of 55 DH lines (out of 210 in the population) from a cross between two *B. oleracea* var. *alboglabra* (A12DHd) and *italica* (GDDH33).

Considering the proposed evolutionary background of *B. oleracea*, we expected to observe at least three sets of band signals on the autoradiographs. This is based on the theory that the *B. oleracea* genome is made up of three ancestral units which have undergone various rearrangements to finally produce the cultivated varieties of today (O'Neill and Bancroft 2000). With the autoradiography results, in most cases we found that we observed multiple bands specific to a probe: however, there was generally one band that had the strongest signal. The strongest signal, in theory, should be the true sequence or location due to highest sequence homology. To be able to map this strongest band must be polymorphic.

Launch site of Ds

The original T-DNA position, or launch site, of the *Ds* is the most important to be determined. In order to achieve this with a degree of conviction we used five different somatic plants. The five PCR probes all produced the correct inverse and nested PCR fragment sizes; all produced identical polymorphic patterns; and were all polymorphic in the strongest band. Figure 4 (top) shows

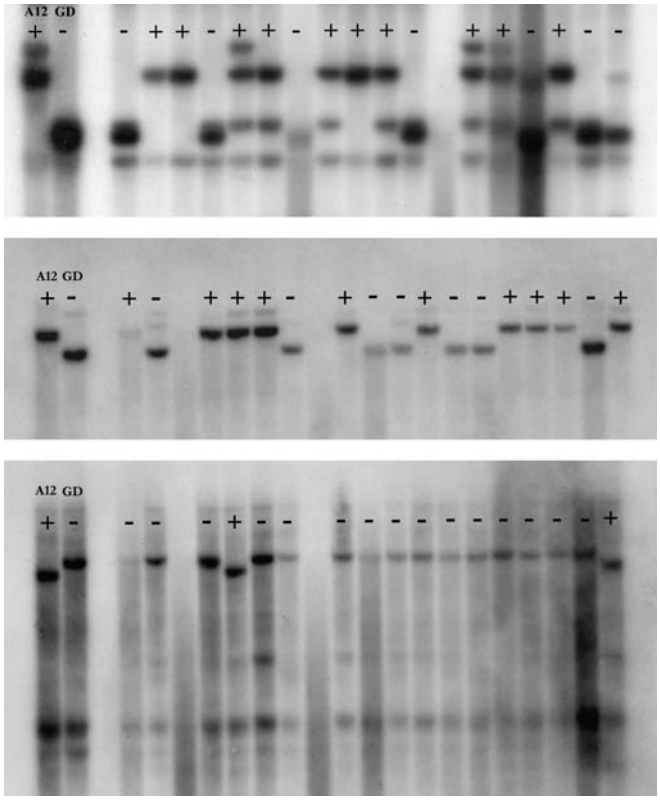

Fig. 4 Examples of autoradiographs of RFLP alleles detected using PCR probes specific for flanking sequences. Population parents labelled as *A12* for *B. oleracea* var. *alboglabra* and *GD* for *B. oleracea* var. *italica*. Alleles from A12 parent are marked with a *plus sign* and alleles for GD are marked with a *minus sign*. *Top* RFLPs for launch site of *Ds*, *middle* RFLPs for insert 13*, *bottom* RFLPs for insert 16*

an example of a mapping filter showing RFLPs for one of the probes.

Ds inserts

For the 37 probes that we used, we found 20 had polymorphic loci in the strongest, and in some cases, only, band (see Fig. 4, middle and bottom, for examples of autoradiographs showing RFLP alleles). The RFLP data from these results were scored and later analysed to give map locations. The remaining 17 probes in most cases produced a monomorphic strongest signal or had equally strong bands or were unreadable due to poor probe quality.

Map positions

The scored polymorphic loci were analysed through MAPMAKER version 3.0 (Lander et al. 1997) and compared with 124 RFLP markers which are spread over nine linkage groups; these nine linkage groups are thought to be comparable to the nine chromosomes of the *B.*

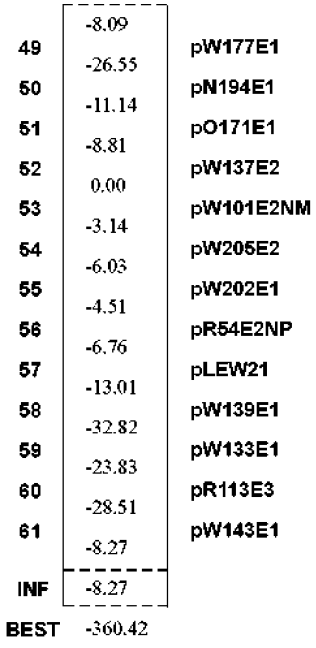

Fig. 5 Linkage analysis using MAPMAKER comparing data from an inverse-PCR probe representing the launch site of the *Ds* element with 13 RFLP markers for linkage group 4 (markers 49–61) showing the probable location of the *Ds* launch site being between markers 52 (pW137E2) and 53 (pW101E2NM)

oleracea genome. When comparisons between the RFLP markers and the probe data are made, map position is derived when a score of zero is calculated between two neighbouring markers (see Fig. 5 for the calculated scores for a flanking genomic DNA probe specific for the launch site of the *Ds* element). In this case the probe score was compared to 13 RFLP markers held in linkage group 4. The probable map position was found to be between markers 52 (pW137E2) and 53 (pW101E2NM) with a genetic distance between markers of 10 cM. The four other *Ds* launch-specific probes, isolated from separate plants, also mapped between the same two markers. The data from the 20 other probes were analysed in this way, and we were able to map 17 independent *Ds* inserts, which are represented in Fig. 6.

Discussion

Mapping T-DNA and transgenes in *Brassica* is difficult due to the complex genome. We have described here methods that can be applied to other *Brassica* species, as well as other chimeric plants and introduced practical solutions to some of the problems that can be expected with plant genomes with multiple copies and large numbers of repetitive DNA. We have also performed T-DNA mapping of transgenes in *B. napus*, which is a hybrid of *B. oleracea* and *B. rapa*, and has even more repeat DNA. At least six bands were visualised on the autoradiographs, but as found in this study there is generally one strong band (results not shown).

In this study we only used 55 individual progeny from a family of 210, so the mapping will not be very accurate, but is sufficient to give an indication of how far the elements move. Finer mapping techniques can be applied by using more individuals from the family and analysing through the more RFLP, AFLP or microsatellite markers that are available (Parkin et al. 1995; Sharpe et al. 1995; Bohuon et al. 1996; Szewc-McFadden et al. 1996).

The distribution of mapped inserts seems to show a degree of clumping of inserts, especially on linkage groups 3 and 7; although more inserts need to be mapped to make the distribution more statistically reliable. One possible theory for this is natural karyotyping of chromosomes. This is a hypothesis proposed by Bennett (1996) that in cell architecture, a chromosome will generally have a fixed location in relation to other chromosomes with two constant neighbour chromosomes within the cell. This is based on the mean spatial order of chromosomes, known as the Bennett model. In *B. oleracea*, a linkage group or chromosome 4 could have chromosomes 3 and 7 as constant neighbours based on Bennett's model. Therefore, if transposition took place during pre-meiotic interphase within the decondensed chromatids, then perhaps the nearest inter-chromosomal location to the launch site on chromosome 4 would be regions of chromosome 3 or 7, so the element might predominately move to these regions. Another possible theory for the distribution of inserts is pairing of cryptically homologous chromosomes where groups 3, 4 and 7 fall into the same homology segment. However, when this was tested for *B. napus* linkage groups N13, N14 and N17, which are equivalent to the *B. oleracea* groups, there was found to be no significant homology between them (M. Trick, JIC, personal communication). Yet another theory for the clustering is that this is due to a secondary transposition event. As the parent used was actively somatic, there is a possibility that when selection with streptomycin was made, the plants produced were showing a secondary linked transposition from a previous event. Perhaps there is a combination of the Bennett's model and secondary transposition; however, this is difficult to prove considering the limited number of mapped insertion events.

We have presented here ideas on the behaviour of *Ds* transposition in broccoli. However the question must be raised whether even though we selected 26 from a total of 73 plants and produced 40 probes from 24 of these plants, this is a true representation of the regenerant population as a whole. If not, then the distribution of insertion events might be biased and would undermine our conclusions. We must consider regions of the genome that might be highly monomorphic or devoid of restriction sites, and these areas might be under represented. For instance, the area around the launch site might not be very polymorphic and could contain several *Ds* elements that are unmappable. This is very difficult to prove and is a problem for any researcher trying to map positions of inserts or T-DNA. Further experiments could, in theory, be performed to confirm positions or reveal further

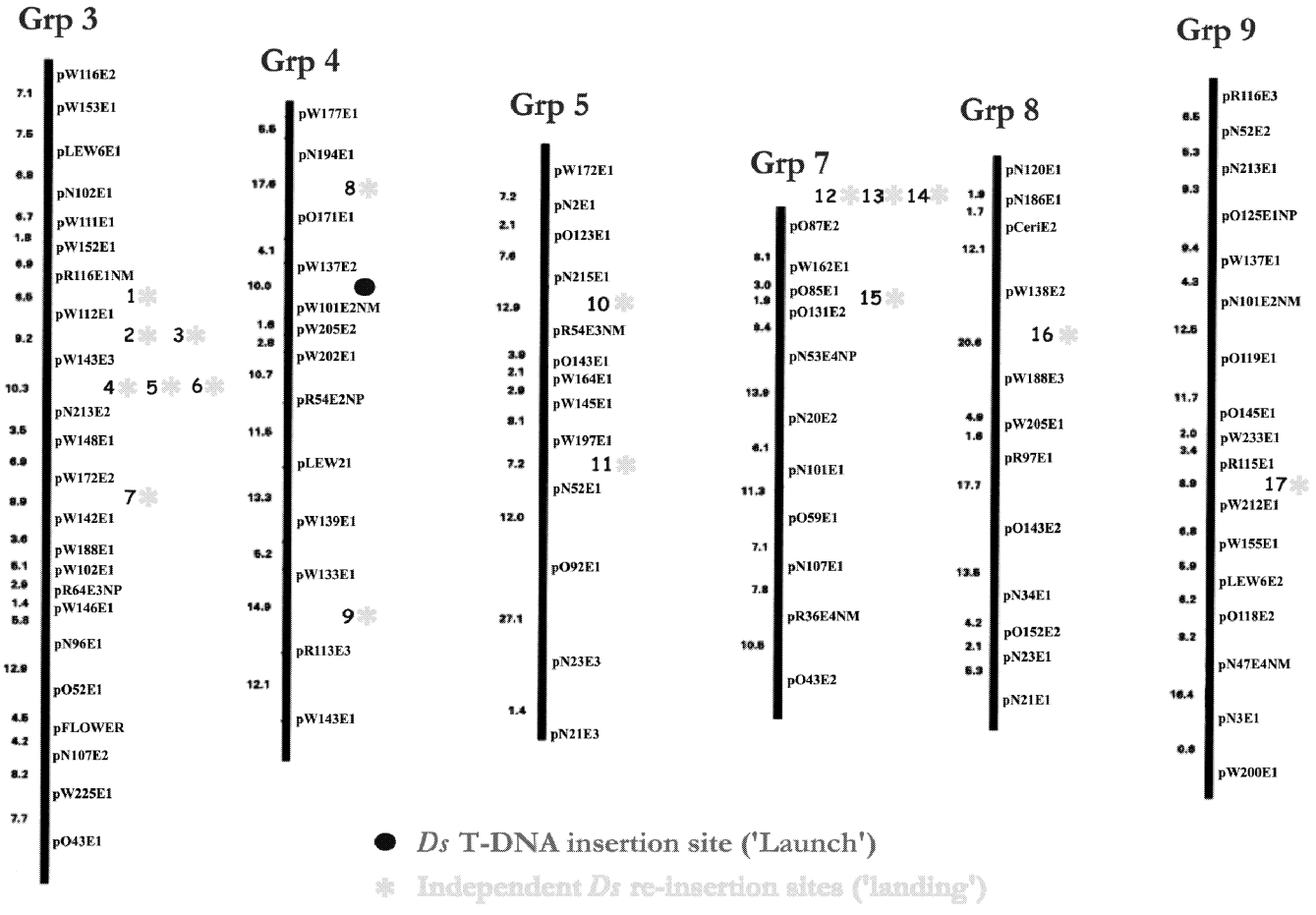

Fig. 6 RFLP map for six (out of nine) linkage groups of *B. oleracea* showing the position of 17 independent *Ds* reinsertion events and the original *Ds* T-DNA construct position. Figures on *left* of the linkage groups are genetic distances between RFLPs in centiMorgans

insertion events. For example, plant genomic DNA could be cut with different restriction enzymes and inverse PCR probes produced. Different mapping populations and enzymatic digestion could be used. Genomic sites could possibly be isolated by direct PCR, and the products compared to the inverse PCR products.

It is vitally important for tagging strategies to assess and determine how far the transposable elements move. If, as found for most heterologous species, the elements predominately move to linked locations, then a directed tagging strategy is preferred so that a *Ds* T-DNA insert can be selected in the region of a candidate gene, then encouraged to transpose in the hope that a transposed element will inactivate the gene in question (Bhatt et al. 1996). However in this study, contrary to other plant systems, there seems a tendency for the elements to move to unlinked chromosomal positions; so a directed tagging strategy is not feasible or potentially reliable to perform. We must consider the species of plant used; it is not ideal when growing large number of plants to try and 'knock out' a gene, as *Brassica* plants are large and have relatively long life cycles. Also with the repeat DNA, genes might be at least in triplicate, so knocking out one gene might not affect the plant phenotype. Considering these limitations, transposon mutagenesis is not really feasible for tagging of genes in these plant species. One possibility, however, would be the 'interesting' phenotype approach where plants are grown and any interesting visible mutation seen is analysed to see if it is transposon induced. However as mentioned above, this is hard to justify due to the economics of cost, space and potential reward.

So what potential use can this system have in *Brassica*? The ability to randomly insert transposable elements, then activate transposition to scatter the elements around the genome might have a use in *Brassica* for providing extra genetic markers that could be used in map-based cloning techniques. This is also possible if enough elements are available that might be used as an 'artificial' method of encouraging variation and diversity within the species. But a better transposon system for that would be the *Enhancer*/*Suppressor-Mutator* (*En*/*Spm*) or the *Mutator* (*Mu*) systems (Vodkin 1989; Walbot 1992) as these are generally at high-copy numbers, although it is difficult to track specific inserts with these systems. One potentially valuable application is for introduction of

isolated genes into the plant genome without the linked antibiotic markers required for plant transformation and selection. The current methodology for genetic modification of plants relies on selectable markers within the gene construct that is to be introduced. If a method can be applied that does not require selection markers or a method to rid of the markers from the desired gene would be a valuable method for plant researchers to overcome consumer concerns about antibiotic resistance markers in genetic modification. A 'clean gene' strategy may be applied for *Brassica* by modifying the *Ds* element just to contain the desired gene to be expressed in the genetically modified plant and the minimal transposon sequences required for transposition. This cassette could be introduced into a streptomycin excision marker system like in this study. This *Ds* construct could be introduced into desired *Brassica* species and crossed with a separate plant transformed with a *Tpase* construct. Streptomycin selection could then be performed to produce fully green plants, preferably with one gene locus for the desired *Ds* insert unlinked to the donor site and the *Tpase* source, followed by backcrossing and then selecting for progeny that contain only the desired *Ds* insert. Without the *Tpase* source, the *Ds* element would be stable. This strategy is feasible due mainly to the low excision rate, copy number and unlinked transposition of *Ds* in *B.oleracea*.

Acknowledgements The authors wish to thank Drs J. Jones, C. Dean and G. Coupland [John Innes Centre, Norwich, UK] for supplying the constructs, probe plasmids and also for providing details of the construction of plasmids. Special thanks to Penny Sparrow (JIC) for help with mapping data and analysis and also for maintaining plants used for construction of mapping lines. Thanks go also to Dr. Ian Bancroft, Dave Laurie and Eddie Arthur (JIC, Norwich) for critically reading the manuscript, as well as Martin Trick (JIC) for helpful advice throughout the project. This project was financially supported by the Biotechnology and Biological Sciences Research Council.

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