Identification and structural characterization of further DNA elements in the potato and pepper genomes homologous to the transposable element-like insertion **TstI**

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Received May 21, 1991 / July 25, 1991

**Summary.** The molecular cloning and nucleotide sequence of elements from potato and pepper that are related to the recently identified **TstI** element are described. Sequence analysis reveals considerable conservation of sequences internal to both the **TstI** element and two of the related elements identified here. In six potato clones analysed, the 11 bp inverted repeat first identified in the **TstI** element is conserved. Several of the elements are flanked by an 8 bp direct repeat. DNA fragments which were amplified from several pepper genomes by polymerase chain reaction (PCR) amplification using the inverted repeat as sequence primers also display considerable conservation of sequences internal to the **TstI** element. These data further support the possibility that **TstI** is a non-autonomous transposable element and that **TstI** might be the first example of a transposable element which occurs in several genera of solanaceous plants.

**Key words:** Transposable element – Potato – Solanaceae – **TstI**

**Introduction**

Transposable elements, although they are probably ubiquitous in nature, have been functionally identified in few plants, such as maize and snapdragon (cf. e.g. Wienand and Saedler 1987; Döring 1989). DNA elements structurally related to transposable elements have, however, been identified in many more plants e.g., the **Tgm** element in the coding region of the **lel** locus of soybean (Vodkin et al. 1983) the **Tpcl** element in the promoter region of the chalcone synthase gene of parsley (Herrmann et al. 1988), the **PstI** element in the 5' flanking region of the **legC** gene (Shirsat 1988) and the **lps-r** element in the **rugosus** locus of pea (Bhattacharyya et al. 1990) and a 316 bp DNA fragment with 15 bp inverted repeats in the 5' flanking region of **wx-w23** of maize (Spell et al. 1988).

A transposable element-like insertion has recently been identified in the promoter region of a class II patatin gene (PGT3) from potato (**Solanum tuberosum**) (Köster-Töpfer et al. 1990). The insertion, called **TstI**, which is located in front of the transcription start site, is composed of 736 nucleotides, terminates by an 11 bp (one base mismatch) inverted repeat and is flanked by an 8 bp direct repeat that is present only once in all other patatin genes characterized. With respect to its inverted repeats, this element shows considerable homology to other transposable elements from higher plants, such as the **Ac/Ds** family from maize (7 out of 11 nucleotides are identical), the **Tpcl** family from parsley (6 out of 11 positions identical) and **Tam3** (4 out of 11 positions conserved) (Köster-Töpfer et al. 1990).

One characteristic feature of most transposable elements is that they are present in multiple copies in the host genome. Southern analysis has shown that sequences homologous to the **TstI** element are present in multiple copies in the potato and tomato genome (Köster-Töpfer et al. 1990). In order to characterize these sequences, six genomic clones hybridizing to the **TstI** element were isolated from the potato genome. In addition related sequences were amplified from three pepper genomes by PCR and analysed further. All of them display structural features related to the **TstI** element.

**Materials and methods**

**Plant material and genomic library.** A genomic library of the monohaploid **S. tuberosum** line AM80/5793 was
Table 1. Sequence of the primers used for the sequence analysis and PCR.

<table>
<thead>
<tr>
<th>Name</th>
<th>5'-Sequence-3'</th>
<th>Hybridizing region</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRM-1</td>
<td>CGTGGATTCCACACATC</td>
<td>530-515</td>
</tr>
<tr>
<td>PRM-2</td>
<td>GAACCTAAAAGTCAAATTGATC</td>
<td>1053-1074</td>
</tr>
<tr>
<td>PRM-3</td>
<td>CAGGGGCGTATCTGAGGGG</td>
<td>411-431</td>
</tr>
<tr>
<td>PRM-4</td>
<td>CAGGGGCGTATCTGAGGGG</td>
<td>1146-1123</td>
</tr>
</tbody>
</table>

Numbers refer to the nucleotide numbers of Tstl DNA indicated in Fig. 2.

screened using the XbaI—BclI internal fragment of the Tstl element as a probe. Genomic DNAs were prepared from seedlings of potato cv. Danshaku, fruits of eggplants, and seedlings of various pepper varieties (Shishitougarashi, Nikko-tougarashi, Kimuchi-tougarashi, Goshiki-tougarashi) and tomato. This plant material was purchased from local markets.

DNA sequencing. Sequence analysis of clones 465, 513, 574, D1, N1, K1 and G1 was performed by the dideoxy method (Sanger et al. /977) using commercial sequencing primers (Pharmacia) and synthetic oligonucleotides (Applied Biosystems DNA Synthesizer 380A). Sequences of the border regions were determined using internally hybridizing primers listed in Table 1.

PCR amplification. Genomic DNAs from potato, eggplant, peppers and tomato were used as templates for PCR reaction. The sequences of the primers used are listed in Table 1. The reaction mixture contains 50 mM KCl, 1.5 mM MgCl2, 10 mM TRIS-HCl pH 8.3, 10 pmol of each primer, 50 μmol of each dNTP, 2 units of Taq polymerase (Promega) and 1 μg of template DNA. Reaction cycles of 92 °C for 1 min (denaturation), 55 °C for 2 min (annealing) and 72 °C for 4 min (reaction) were repeated 35 times. An aliquot of the amplified DNA was separated on agarose gels and bands in the 700–500 bp size range were extracted and cloned into pUC18.

Southern hybridization. DNA blotting from agarose gels to Hybond N filters (Amersham) was done according to the standard method of Southern (1975). Hybridization was performed at 42°C in 50% formamide, 6X SSC, Denhardt's and 100 μg/ml salmon sperm DNA with a probe DNA which was labelled by 32P solution using a Multiprime labelling kit (Amersham). Washing was carried out in 0.5X SSC at 60°C for 100 min. Filters were autoradiographed at -80°C using X-ray film.

Results and discussion

Genomic cloning and sequence analysis

Southern hybridization analysis of genomic DNA of potato using the XbaI—BclI fragment of Tstl DNA as a probe revealed that the potato genome contains repetitive sequences hybridizing to internal Tstl sequences (Köster-Töpfer et al. 1990). In order to analyze whether these sequences also contain features characteristic of the whole Tstl element (i.e., terminal inverted repeats flanked by direct repeats), a genomic library was screened and eight independent lambda clones were identified. The restriction maps of these clones and the sequences homologous to Tstl DNA, determined by hybridizing with three different probes, are shown in Fig. 1. Relative to the Tstl element itself, no conserved restriction sites were observed, except for the XbaI sites present in the homologous regions of clone 465, 513, 748, 824, 929 and 1186. Southern hybridization of fragments of the patatin gene with these clones showed no significant signal (data not shown). This indicates that the clones were derived from chromosomal locations other than the site of the original Tstl element. The conserved XbaI sites were used as starting points for the sequence analysis of the homologous regions. The complete sequence

Fig. 1. Restriction maps of Tstl DNA and of the related genomic clones isolated from potato. Restriction sites are as B BamII; Bc, BclI; Hc, HincII; Hd, HindIII; K, KpnI; P, PstI; S, SalI; Sc, SacI; Sh, SphI; V, EcoRV; X, XbaI; Xh, XhoI. The boxed regions indicate the sequences homologous to Tstl DNA.