Transposon Display identifies individual transposable elements in high copy number lines

Dirk Van den Broeck, Tamara Maes, Matt Sauer, Jan Zethof, Peter De Keuleleire, Mariëlla D'Hauw, Marc Van Montagu and Tom Gerats
Laboratorium voor Genetica, Universiteit Gent, K.L.Ledeganckstraat 37, B-9000 Gent, Belgium

Summary
The dTph1 transposable element family of Petunia hybrida line W138 consists of between 100 and 200 members. A strategy that allows simultaneous detection of individual elements is described. Sequences flanking dTph1 elements are amplified by means of a ligation-mediated PCR. The resulting fragments are locus-specific and can be analysed by polyacrylamide gel electrophoresis. One of the applications of Transposon Display is the isolation of dTph1-tagged genes. Fragments that co-segregate with a mutant phenotype can be extracted from the gel and reamplified, providing access to tagged genes, as demonstrated in a reconstruction experiment. Data on the molecular identification of a phenotypic mutant, isolated in a random tagging experiment, is also presented. Upon sequencing, the obtained candidate fragment was found to be identical to part of the previously identified Fbp1 gene.

Introduction
Variegation in plants can in many cases be attributed to the action of transposable elements. In Antirrhinum majus and Zea mays, transposon tagging has been used successfully to isolate a range of genes (e.g. Coen et al., 1990; Goodrich et al., 1992; Walbot, 1992). In Petunia hybrida, instability has been described at several loci (Gerats et al., 1989; Kroon et al., 1994) and both transposon trapping and -tagging experiments have been carried out (Koes et al., 1995; Renckens et al., 1996; Souer et al., 1996). The first transposable element cloned from Petunia hybrida, dTph1, has been isolated from the dfrC gene of the line W138 (Beld et al., 1989; Gerats et al., 1990). It is 284 nucleotides long and contains perfect terminal inverted repeats (IR) of 12 nucleotides. Other, related insertion elements have since been isolated and sequenced. Based upon the IR and the size of the target site duplication, dTph1 belongs to the Ac/Ds group of transposable elements.

Most cultivated lines and the species from which P. hybrida is thought to be derived, contain between 5 and 25 hybridizing fragments that are highly homologous to the dTph1 element (Huits et al., 1995). The genome of the line R27 and of derivative lines like W138, contain between 100 and 200 dTph1 hybridizing fragments (Gerats et al., 1989; Huits et al., 1995; De Keuleleire et al., unpublished). Probably as a consequence of this high copy number, progenies of selfed plants of the line W138 display mutant phenotypes at a high frequency (Bianchi et al., 1978; Doedeman et al., 1984; Gerats et al., 1989). W138-derived mutable alleles that have been cloned so far contain elements that are highly homologous to the dfrC::dTph1 element (Huits et al., 1995; Renckens et al., 1996; Souer et al., 1996). Because of the high copy number, individual elements can not be analysed satisfactorily by conventional Southern blot analysis.

Here we present a strategy, derived from the AFLP© technique (Amplified Fragment Length Polymorphism; Vos et al., 1995), that solves this problem. The strategy is called Transposon Display and allows the simultaneous detection of individual dTph1 elements in high copy number lines. Individual transposons are identified by a ligation-mediated PCR that starts from within the transposon, and amplifies part of the flanking sequence up to a specific restriction site. Resulting PCR products can be analysed in a high resolution polyacrylamide gel system. Transposon Display provides detailed information on the copy number of the dTph1 transposon family and reveals insertion events (De Keuleleire et al., in preparation). It also allows detection of an insertion that is correlated with a particular phenotype. Such a co-segregating PCR product can be extracted from the identifying gel, reamplified, cloned, sequenced and used as a probe to further isolate the tagged gene. The feasibility of the strategy is demonstrated by means of a reconstruction experiment. We furthermore identified a recently isolated mutant as being caused by a dTph1 insertion into the Fbp1 gene (Angenent et al., 1993).

Results
General outline of the technique
Transposon Display combines the AFLP technique as developed by Keygene (Vos et al., 1995) with a nested PCR
Figure 1. Schematic representation of the dTph1 element of Petunia hybrida, the (imperfect) palindromic region around the MunI site is shown, as is the position of the tetracutter MseI. IR: Terminal Inverted Repeat.
to specifically amplify and display sequences that are flanking a dTph1 insertion. As in AFLP, genomic DNA is digested with a hexacutter and a tetracutter to generate fragments in a size range favourable for subsequent PCR amplification and gel analysis. Amplification is achieved by using a pair of primers, complementary to a pair of adaptors ligated to the hexacutter and tetracutter site. The hexacutter-specific adaptor is biotinylated so that after ligation, fragments that contain the hexacutter site can be specifically retrieved with streptavidin beads.

For Transposon Display, the restriction enzyme MunI (CAATTG) was chosen as a hexacutter. A MunI site is located in the center of a near palindromic region (see Figure 1) that appears to be conserved within the elements of the dTph1 family. The MunI-specific primer, complementary to the MunI-specific adaptor, is extended with four nucleotides complementary to the sequence of the dTph1 element. This represents a first enrichment step that eventually leads to the selection of element- plus border sequences. Since the palindrom is imperfect in the immediate vicinity of the MunI site (Figure 1), we can choose the direction of the first amplification. Extending the MunI-specific primer with ACAC leads to the amplification of the short fragment of the element and its flanking sequence, while an overhang of AACC will lead to the amplification through almost the entire element plus its flanking sequence (Figure 2). Further data will be presented mainly on the analysis and use of the short part of the element.

As a tetracutter, BflI (CTAG) can be chosen. The enzyme does not cut in the element, thus enabling amplification in both directions. The restriction enzyme MseI (TTAA), in contrast, is only useful in combination with the MunI-ACAC primer, amplifying the short fragment of the transposon, since the enzyme cuts the long fragment between MunI and the inverted repeat of the element. This procedure results in the amplification of fragments that are bordered by a BflI site (or MseI) and a MunI site flanked by ACAC or AACC. A further enrichment for transposon-specific fragments is achieved by using the obtained PCR products as a template for a second amplification with a nested primer that specifically recognizes a conserved region of the transposon. Two regions have been taken into account for the design of such primers. The conserved subterminal region (recognized by the Internal or Int primer) and the terminal inverted repeat (recognized by the IR primer). This nested amplification is further referred to as hot PCR since one primer is labelled with 3P. The radioactive fragments obtained are resolved on a 5% sequencing (PAA) gel. The fragments detected after autoradiography (see Figure 3), are expected to represent sequences that border dTph1 insertions.

Amplified fragments originate specifically from dTph1-like elements

To verify whether the detected fragments indeed result primarily from amplification of transposon flanking sequences, Transposon Display was performed by using either the IR primer (Figure 3, lanes 1–4) or the Int primer (Figure 3, lanes 5–8) in the hot PCR. Based upon the sequence of the dTph1-like element cloned from the DfrC gene (Gerats et al., 1990), it is possible to predict the exact migration shift that occurs if the Int primer is used instead of the IR primer (see Figure 4). Preamplification with the MunI-AACC primer followed by a hot PCR with the Int primer should result in fragments 233 bp longer than with the IR primer. When preamplification is done with the MunI-ACAC primer, the PCR products resulting from a hot PCR with the Int primer will be 24 bp longer than with the IR primer. Figure 3 shows the results of the amplifications for one individual of a high copy number line and one of a low copy number line. The obtained patterns for each individual are similar, except for the predicted migration shift. For the high copy number individual, 106 major fragments amplified with the IR primer (Figure 3, lane 3) reappear as fragments in the amplification with the Int primer (Figure 3, lane 7). Seven fragments are only present in the reaction with the Int primer and five fragments are only present in the reaction with the IR primer. This identifies about 95% of the detectable fragments as resulting from elements with sequences similar to the

Figure 2. Schematic representation of the Transposon Display strategy, using BflI as a tetracutter.

Step 1. Double digestion of genomic DNA with MunI BflI. MunI cuts the dTph1 element into two unequal parts (not drawn to scale). Since there is no BflI restriction site in dTph1, two dTph1 flanking fragments are generated per transposon insertion.

Step 2. BflI and biotinylated MunI adaptors are ligated to the DNA fragments. MunI fragments are selected with magnetic streptavidin beads (for clarity of the diagram, only the fragments containing the short end of the dTph1 element are represented).

Step 3. A first PCR amplification is performed with a BflI primer and the MunI-ACAC (short direction) primer. Amplification is also possible using the BflI primer and the MunI-AACC primer in the long direction (not shown).

Step 4. A nested hot PCR amplification is done with a BflI primer and a 3P labelled primer based on the terminal inverted repeat of the transposon (IR, step 4a) or with a BflI primer and a 3P labelled internal dTph1 primer (Int, step 4b).

Step 5. The obtained flanking fragments are separated by polyacrylamide gel electrophoresis. If the resulting patterns are too complex to be analysed, they may be subdivided into different reactions by adding extra nucleotides to the 3′ end of the BflI or the IR primer in the hot PCR, as in standard AFLP (Vos et al., 1995).

Other tetracutters may be used for Transposon Display of dTph1 elements (not shown). When MseI is used as a tetracutter instead of BflI, only the MunI-ACAC primer (short direction) can be used in the first amplification reaction.

The copy number of the dTph1 element in the W138 line

The presence of the MunI site within the element is a prerequisite to obtain a Transposon Display amplified fragment. Therefore an experiment was performed to analyze the degree of conservation of the MunI site in dTph1-like elements. Total genomic DNA was amplified with primers that recognize the terminal inverted repeats of the dTph1 element in an inward direction. The resulting products migrate predominantly as a 284 bp fragment (the entire element). Moreover, digestion of genomic DNA with MunI before amplification, does not result in detectable PCR products (results not shown). This indicates that most, if not all elements that contain conserved inverted repeats, also contain a MunI site. dTph1-like elements can then be defined operationally as those elements that result in the amplification of their flanking sequences by means of Transposon Display. The majority of flanking sequences amplified with the standard procedure (MseI or BfaI as tetacutter and IR in the hot PCR) falls within a window detectable with a standard 5% sequencing gel (up to around 700 nucleotides). In individuals of the line W138, about 180 fragments can be distinguished.

Because of the high number of fragments to be resolved, some fragments, resulting from independent insertions, might co-migrate. The AFLP approach itself offers an elegant solution to this problem. This is achieved by performing four hot PCRs instead of one, each with a primer that has a further 3′ extension of one nucleotide, such that only sequences fitting the overhang will be amplified (Figure 2). The total pattern then breaks up in four subpatterns, each representing about one quarter of the fragments. Instead of 180 fragments, an average of 45 different fragments has to be resolved, thereby decreasing the probability for co-migration of separate fragments. Indeed, the number of fragments then rises to about 50 for each 3′ extension (or 200 in total per individual), supporting the notion that up to 20 fragments might be co-migrating in the original set-up. Alternatively some of these fragments might represent somatic insertions, present in only a (very) limited part of the sampled tissue.

Figure 3. Transposon Display patterns of the high copy number line W138 and the low copy number line W80, resulting from a hot PCR with the IR (left) or Int (right) primer after preamplification with the MunI-ACAC primer (short direction). The IR and the Int patterns are almost identical apart from the expected 24 bp shift.
**Isolation of a tagged gene**

To test whether the Transposon Display approach would facilitate the isolation of tagged genes, we decided to perform a reconstruction experiment. We obtained a *dTph1* insertion in the *Ap2A* gene (*Ap2A-V2025-6*) by insertional mutagenesis (Koes et al., 1995; Maes et al., 1995). The *Ap2A* gene has been cloned and sequenced (Maes et al., unpublished results). Since the tetracutter sites within the sequences flanking the insertion are known, the length of the fragment to be obtained with Transposon Display can be predicted. Upon selfing of the originally isolated *Ap2A* insertion mutant, the *Ap2A-V2025-6* insertion allele segregated in the progeny, providing ideal starting material to conduct a reconstruction experiment. Individuals homozygous or heterozygous for the insertion, as well as individuals lacking the insertion (from the same and from related families) could be distinguished by means of a bridged PCR (see Figure 5, upper panel). Genomic DNA was subjected to Transposon Display in the ACAC direction (see Figure 5, lower panel). To simplify the pattern, the reactions were split in four by extending the *BfaI* primer with one nucleotide. Only one Transposon Display amplified fragment co-segregated with the *Ap2A*-*V2025-6* insertion (it should be mentioned that on the original gel, the uniqueness of the candidate fragment is much clearer than in the greatly reduced Figure 5). As predicted, this fragment migrates as a 231 bp fragment and is only amplified when the reactions are performed with the *BfaI*+T primer. The co-segregating fragment was isolated from the gel, reamplified and cloned. Ten clones were sequenced. All contained inserts identical to the part of the *Ap2A* gene that flanks the insertion. This demonstrates that co-segregating insertions can be identified within a background of over 200 insertions, and that the detected fragment is derived from the *Ap2A-V2025-6* insertion.

We subsequently undertook to molecularly identify a newly isolated mutant, originally called *greenback* because of its phenotype: enlarged green veins on the back of otherwise normal petals. Seven mutants and nine wild-type plants (7 hetero- and 2 homozygous) from the same family, as well as four homozygous wild-type siblings were analysed by Transposon Display. One candidate fragment was identified in a series of experiments, comprising digests with two different fourcutter enzymes, *MseI* and *BfaI*. The only perfect candidate fragment identified, was present in all mutant plants and in the seven heterozygotes, while it was absent in all homozygous wild-type plants tested. This candidate fragment was isolated from the PAA gel, cloned and used as a probe in a Southern blot experiment on progenies of an F2 cross between a mutant and the tester line W162 (Figure 6). The candidate fragment again co-segregated with the mutant allele and was sequenced. The sequence obtained was identical to part of the *Fbp1* gene. Our mutant is much weaker in phenotype than the one described by Angenent et al. (1993). A further analysis of the mutant allele will be presented elsewhere (Sauer et al., in preparation).

**Discussion**

The W138 line of *Petunia hybrida* exhibits several interesting features and has been presented as a genetic tool before (Gerats et al., 1989). Up to 20% of progenies of selfed W138 plants may have altered phenotypes. Unfortunately, this high mutation frequency goes hand in hand with a high copy number of the *dTph1*-like element. Classical Southern analysis turned out to be unsatisfactory for the detection of insertion events (Gerats et al., 1990; Huits et al., 1995). With the presented Transposon Display technique a powerful alternative becomes available.

The molecular tags used to follow insertion events by Transposon Display are amplified transposon flanking sequences that are small enough to be separated on a sequencing gel. The resolution of such gels is high compared to Southern analysis and allows the separation of around 100 fragments. Since W138 plants typically harbour between 100 and 200 elements, some of the amplified fragments might co-migrate.

As a consequence, insertion elements that co-segregate with a particular phenotype might not always be detectable. This problem can be solved by splitting up the Transposon Display pattern into four (or more) subpatterns by adding an extra (or more) selective nucleotide(s) at the 3’ end of the adaptor primer used in the hot PCR amplifications. Apart from increased resolution, Transposon Display also simplifies the cloning of a specific transposon flanking

![Figure 4](image-url)
sequence from a high copy number line. Amplified sequences can be extracted from the PAA gel, reamplified and cloned. Cloned fragments can be used to probe a blot of the original PAA gel, in order to confirm that the right fragment was cloned (results not shown). The feasibility of the Transposon Display was demonstrated by re-cloning the Ap2A gene in a reconstruction experiment. We were furthermore able to molecularly identify an unknown phenotypic mutant as an insertion allele of the Fbp1 gene (Sauer et al., in preparation).

Sauer and co-workers (1995) developed another solution for the isolation of dTph1-tagged genes from high copy number lines. Their method is based on differential screening of cloned iPCR products originating from the mutant plant. Such a strategy might be preferred when only a few individuals have to be compared to yield the tagged gene. To decrease the number of co-segregating insertions, one probes the cloned iPCR material of one mutant with iPCR material derived from other mutant plants, before performing a differential screening between wild-type and mutant. Such a comparison is much less laborious with the Transposon Display technique because different individuals can be screened by simply performing the PCR reaction on each DNA sample and running the products side by side on one gel.

The applicability of the Transposon Display technique is in principle universal and will be useful for the detection of other elements such as T-DNAs, and retroviral or retrotransposable elements, when the copy number per haploid genome becomes too high for analysis with more classical techniques. Hence, it might be hoped for that the technique discussed not only speeds up the study of the dTph1 element and its use for gene tagging in Petunia, but also of other systems such as the different subfamilies of the Mu transposable elements in Mutator stock lines of Zea mays (Lisch et al., 1995), Tc1 in Caenorhabditis elegans (Anderson et al., 1992), Tam1 in Antirrhinum majus (Loennig and Saedler, 1994), and the various transposable elements in Drosophila melanogaster (Domínguez and Alborno, 1996).

Aside from the use of Transposon Display to isolate tagged genes, several other applications can be envisaged. We are currently using the technique to assess the frequency of transposition events of dTph1 elements and to follow somatic transposition events throughout development and within the distinct tunica layers (De Keukeleire et al., in preparation).

### Experimental procedures

#### Preparation of DNA for PCR amplification

Pools of three to four leaves are harvested from one individual adult plant, frozen in liquid nitrogen and can be kept at −70°C. DNA is isolated basically according to Dellaporta et al. (1983), but extended with a CTAB cleaning step.

Each DNA sample (500 ng) is restricted with MunI and Msel or BflI by incubation for 3 h at 37°C in 35 μl restriction mix (5 units MunI, 5 units Msel or BflI, 1 X OPA-plus buffer of Pharmacia and 5 mM DTT). Adaptors are ligated by adding 10 μl of ligation mix (5 pmoI biotinylated MunI adaptor, 50 pmol Msel or BflI adaptor, 1.2 mM ATP, 1 X OPA buffer and 5 mM DTT) and a further 3 h incubation at 37°C.

#### Used adaptors and PCR primers

The adaptors used are: MunI adaptor: biotine-5'-CTCGTAG-ACTGCGTAGG-3' and 3'-CTGACGTCATTCAA-5'; Msel adaptor: 5'-GAGGATATGACTGAGTAA-3' and 3'-TACTCGAGTCTCAT-5'; BflI adaptor: identical to Msel adaptor. The primers used are: Munk-ACAC primer: 5'-AGACTCGCTAGAATTGACGAC-3'; Munk-AACC primer: 5'-AGACTCGTACGATTTAGGAC-3'; IR primer: 5'-GAATT-CGCTCAGCCCTTG-3'; IR + 1 primer: as IR primer, but with 3' extension of one nucleotide; Intr primer: 5'-AATTGGANCCCTTTG-3'; Msel primer: 5'-GACGATGAGCTGAGTAA-3'; Msel+1 primer: as Msel primer, but with 3' extension of one nucleotide; BflI primer: 5'-GACGATGAGCTGAGTAA-3'; BflI + 1 primer: as BflI primer but with 3' extension of one nucleotide.

Figure 5. Isolation of tagged genes by Transposon Display: a reconstruction experiment.

A W138 family segregating for the apa2a-V2025-6 insertion allele and nine more distantly related W138 plants were analysed. Top: PCR fragments were produced with two Ap2a specific primers, separated by agarose gel electrophoresis and visualized by ETBr staining. Presence of the dTph1 element and a target site duplication between the two primers leads to the amplification of a fragment that is about 300 bp longer (INS) than the wild-type product (WT).

Bottom: Transposon Display pattern of the same individuals. Preamplification reactions of the flanking sequences were performed with the MunI-ACAC primer and the BflI adaptor primer. For the hot PCR, four sets of reactions were done for each individual, in which the labelled IR primer was used in combination with a BflI adaptor primer extended with one of the four nucleotides. The fragments were separated on a PAA gel and visualized by autoradiography. The arrowhead depicts the only fragment co-segregating with the presence of the dTph1 element in the Ap2a gene in the V2025-6 descendants, which was amplified in the BflI + TIR = 0 subset of reactions (detail of the whole autoradiogram). This fragment is absent in the more distantly related W138 plants and could be resolved from an only slightly bigger band detected for some of these individuals.

Selection of biotinylated DNA fragments

Ten µl of streptavidine beads (Dynal®Dyneabeads© M.280, 6×10⁶ beads µ⁻¹) are washed once in 100 µl STEX buffer (10 mM Tris-HCl pH 8.0, 1 M NaCl, 1 mM EDTA, 0.1% Triton X-100), resuspended in 140 µl STEX buffer and mixed with a 45 µl DNA sample. After incubation for 30 min on a rotator at room temperature the beads are collected with a magnet and the supernatant is removed. The beads are washed once with 200 µl STEX buffer and transferred to another tube to avoid contamination due to specific binding of DNA fragments to the tube walls. The beads are collected again, washed another three times with 200 µl STEX buffer and resuspended in 200 µl T2E buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA). The 200 µl DNA-beads sample is then transferred to another tube, and can be kept for months at 4°C.

Selective pre-amplification

DNA-beads samples are PCR amplified with a MseI or BfaI primer in combination with a Muni primer with a 3’ ACAC or AACC overhang. For this, 5 µl of the above 200 µl DNA-beads samples are mixed with 75 ng of each primer, and 1 Unit Taq polymerase, in a PCR buffer with 10 mM Tris-HCl pH 8.3, 2.5 mM MgCl₂, 50 mM KCl and 0.2 mM dNTPs in a final 50 µl volume. The PCR cycler is programmed to run 24 cycles with a 30 sec step at 94°C, a 30 sec step at 60°C and a 60 sec step at 72°C. The resulting PCR products are checked by electrophoresing 20 µl on a 1% agarose gel. The PCR products should give a low intensity and low molecular weight smear (50–700 bp). The remaining 30 µl is diluted 20 times with T2E buffer and resuspended in 200 µl T2E buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA). The 200 µl DNA-beads sample is then transferred to another tube and can be kept at –20°C until used.

Labelling primers

For 100 PCR reactions the 5’ ends of 500 ng of IR primer is labelled with 1 Unit T4 polynucleotide kinase and 5 µl [γ-³²P]ATP (50Ci) in a final volume of 50 µl by incubation at 37°C for 30 min. The kinase is inactivated by heating at 80°C for 10 min.

Specific, hot PCR of the dTph1-flanking sequences

Five µl of the 20 times diluted pre-amplified material is taken as template for further amplification, by mixing with 5 ng of a labelled IR primer, 30 ng of the MseI or BfaI primer, and 0.4 Units Taq polymerase in a PCR buffer with 10 mM Tris-HCl pH 8.3, 2.5 mM MgCl₂, 50 mM KCl and 0.2 mM dNTPs in a final 20 µl volume. The PCR cycler is programmed to run 40 cycles with a 30 sec step at 94°C, a 30 sec step at 60°C and a 60 sec step at 72°C. The resulting PCR products are checked by electrophoresing 20 µl on a 1% agarose gel. The PCR products should give a low intensity and low molecular weight smear (50–700 bp). The remaining 30 µl is diluted 20 times with T2E and kept at –20°C until used.

Gel analysis

The reaction products are analysed on a 5% denaturing polyacrylamide gel. The gel is cast one day before use, by mixing 100 ml of a 5% (19:1) acrylamide:bisacrylamide, 7.5 M Urea, 1× TBE solution (TBE: 100 mM Tris, 100 mM boric acid, 2 mM EDTA) with 500 µl of 10% ammonium persulfate, and 100 µl of TEMED. The gel is pre-run, using 1× TBE as running buffer at a constant power to give constant heat development (about 40–50 V cm⁻¹). The samples are heated for 5 min at 95°C and quickly cooled on ice. About 3 µl of the 40 µl samples are loaded. After electrophoresis, the gel is dried on a slab gel drier. The gels are exposed to standard X-ray films (usually overnight) at room temperature without the use of Intensifying screens.

Reamplification

The material that contains a co-segregating fragment is sliced out of the gel, taken up 200 µl water and incubated for 1 h on ice; samples should be vortexed three or four times. The gel material is pelleted by centrifugation and 2 µl of the supernatant is used as template in a standard PCR amplification (see Pre-amplification); the amplification products are analysed by agarose gel electrophoresis. Reamplified material was sliced out and cloned in a pGEM-T vector (Promega). Clones were sequenced with a T7 sequencing kit (Pharmacia).

Hybridization

Southern blots were performed using Hybond N+ membranes (Amersham) according to the manufactures instructions. Fragments to be used as hybridization probes were labelled using transposon display primers and [α-³²P]d CTP. Hybridization was performed in a buffer containing 5× SSC, 0.1% SDS, 5× Denhardt’s solution and non-specific DNA at a concentration of 200 µg ml⁻¹ at 60°C for 16 h and washed with 0.1× SSC/0.1% SDS at 60°C. Blots were exposed to Molecular Dynamics phosphorimage screens for 16 h and visualized using a Molecular Dynamics phosphorimage analysis system 445 SI (Molecular Dynamics Inc.).

Acknowledgements

This work was supported by the Belgian Program on Inter-university Poles of Attraction (Prime Minister’s Office, Science Policy Programming #38; DVDB), IWT grant 943051 (TMI) and IWT grant 941391 (PdK). We thank Karel Spruyt for photography, Linda Jacobs for taking care of the plants and Wilson Ardiles Díaz for sequencing.

References


Beld, M., Martin, C., Huits, H., Stuitje, A.R. and Gerats, A.G.M. (1998) Partial transposon display primers and [γ-³²P] ATP. Hybridization was performed in a buffer containing 5× SSC, 0.1% SDS, 5× Denhardt’s solution and non-specific DNA at a concentration of 200 µg ml⁻¹ at 60°C for 16 h and washed with 0.1× SSC/0.1% SDS at 60°C. Blots were exposed to Molecular Dynamics phosphorimage screens for 16 h and visualized using a Molecular Dynamics phosphorimage analysis system 445 SI (Molecular Dynamics Inc.).


