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Genetic characterisation of *Act 1*, the activator of a non-autonomous transposable element from *Petunia hybrida*

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Abstract The line W138 of *Petunia hybrida* has variegated flowers because it is homozygous for the mutable *an1-W138* allele. Excision of the element, causing instability, depends on the presence of the activator *Act1*. The previously characterised non-autonomous element *dTph1* excises from the *dfrC* gene in response to *Act1*. This implies that both non-autonomous elements belong to the same transposable element family. In a range of distantly related cultivars we could detect a single functional *Act1* element. Linkage analysis for 11 of these lines showed that *Act1* was located on chromosome I in all cases, indicating that the element might be fixed in the genome. A group of cultivars that did not exhibit *Act1* activity could be traced back to a recent common origin ('Rose of Heaven'). Cultivars within this group presumably harbour the same inactivated *Act1* element. Among the lines tested were 7 lines representing the two species (*P. axillaris* and *P. integrifolia*) from which *P. hybrida* originated. None of these exhibited *Act1* activity. We assume that *Act1* is present in an inactive state in these lines and that it was activated upon interspecific crossing. In general, lines representing the two parental species and *P. hybrida* cultivars contain between 5 and 25 *dTph1* elements. The lines R27 and W138, however, contain significantly more *dTph1* elements (> 50) than all other lines.

Key words Transposition · *dTph1* · Two-element system · *Petunia*

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Introduction

Variegation in plants is in many cases associated with the presence of a transposable element that disturbs the expression of a specific gene. Mutable alleles in *Petunia hybrida* have been identified, for example for the flower colour genes *an1*, *an2*, *an3*, *an6*, *an11* and *rt* (Gerats et al. 1989). The genes *an3*, *an6* and *rt* encode the enzymes flavanone 3-hydroxylase, dihydroflavonol 4-reductase and anthocyanin-rhamnosyltransferase, respectively (Britsch et al. 1991; Huits et al. 1994; Kroon et al. 1993), whereas the genes *an1*, *an2* and *an11* are involved in the regulation of the expression of a number of structural flavonoid genes (Beld et al. 1989; Gerats et al. 1985; Huits et al. 1994; Jonsson et al. 1984; Quattrocchio et al. 1993).

Several mutable *An1* alleles have been isolated (Bianchi et al. 1978; Doodeman et al. 1984a; Gerats et al. 1982), of which the one described by Doodeman et al. (1984a) is genetically the best characterised. This allele was previously called $an1^{s/p-+}$, but it is referred to here as *an1-W138*. Plants homozygous for the *an1-W138* allele have white flowers with pink and red spots or sectors and give rise to progeny plants with spotted or white, pink or red flowers (Doodeman et al. 1984a). In general, transposable elements occur as families of structurally and functionally related elements that in some cases can be divided into two distinct types: autonomous elements that have the ability to excise and transpose on themselves and non-autonomous elements, the activity of which depends on the presence of an autonomous element. Salient examples of such two-element systems are the *Ac-Ds* and the *Spm-dSpm* (or *I/En*) system in maize (Federoff 1989). Wijsman (1986) demonstrated that the *an1-W138* allele contains a non-autonomous element that can be activated by an activator element, *Act1* (previously called *Bi*), located elsewhere in the genome.

A transposable element, *dTph1*, has been isolated from the *dfrC* gene of the line W138. It has 12-bp perfect terminal inverted repeats homologous to those of *Ac*

and other related elements, and it is flanked by an 8-bp target site duplication (Gerats et al. 1990). Molecular analysis of a mutable *rt* allele confirmed that *dTph1* is an active transposable element that can generate a variegated phenotype (Kroon et al. 1993). From the progeny of the line W138, plants were selected that were *an3*-mutable due to the insertion of *dTph1* elements (van Houwelingen et al. unpublished). Excision of the element leads to restoration of the wildtype sequence or the induction of a typical footprint (Gerats et al. 1990; van Houwelingen et al. unpublished data). DNA gel-blot analysis showed that *dTph1*-related sequences are repetitive in the genome of two *P. hybrida* lines (Gerats et al. 1990).

Here we present a genetic analysis of the *Act1* element that transactivates a non-autonomous transposable element in the *an1* gene of the line W138. A single functional *Act1* element was found in the majority of the petunia lines tested. The *dTph1* element in the *dfrC* gene of the line W138 is activated by the *Act1* element as well. In all cases analysed so far, the functional *Act1* element is located on chromosome I, implying that *Act1* itself does not transpose. None of the species from which *P. hybrida* originated had the capacity to activate the *an1-W138* element, even though *dTph1*-related sequences are present in these species.

Materials and methods

Plant material

Most of the *P. hybrida* lines used have been maintained as inbred stocks for at least four generations (usually much longer). Plants were grown under standard greenhouse conditions. A description of the different ancestral (S) lines and their origin is given in Wijsman (1983). We have noticed that the genetic nomenclature in reports about petunia is inconsistent. We therefore adopted the following nomenclature for petunia genetics: capital letters when the gene product is addressed (e.g. DFR); the first letter a capital plus italics when the locus is addressed (e.g. *An6*); lowercase plus italics if the gene is addressed (*an6*); for a dominant allele, the first letter a capital, italics plus the affix + ($An6^+$); for a recessive allele, lowercase, italics plus the affix - ($an6^-$); and for a mutable allele, lowercase, italics plus the affix mut ($an6^{mut}$). Genes of a gene family will be given the assignment A, B, etc. in capitals (*dfrA*, *dfrB* etc.).

Linkage analysis and genetic markers

Only those markers relevant for the genetic and molecular analysis presented in this paper are given. The lines S6, S11, S12, S14, V2, V13, V14, V23, V26, V28, V35 and Vu6 are *An1* dominant, *Hf1* dominant. The lines S1, S2, S7 and W115 are *An1* dominant and carry the recessive *hf1-1* allele. The lines M1, M3, M72, R4, R51, W22, W29, W80, and W138 are *An1* dominant, *hf1* recessive. The lines W144, W152 are *an1* recessive, *Hf1* dominant. The lines W20, W46, W55, W78, W83, W107, W110, W121, W126, W127, W147 and W148 are *an1* recessive, *hf1* recessive. The responsive lines W162 (*an1-W138*, *an1-W138*; *hf1-*, *hf1*) and W168 (*an1-W138*, *an1-W138*; *Hf1*, *Hf1*) were used to test for the presence of a functional activator *Act1*. The locus of the *Act1* element was determined by linkage of *Act1* with the *Hf1* locus. This gene is involved in the hydroxylation of the 5′ position of the anthocyanin molecule and affects the flower colour. In the B1 progeny of *Hf1*-dominant parental plants the status of the *hf1* gene could be directly scored in the variegated flowering plants. White flowering plants (*an1-W138*, *an1-W138*) were crossed to a tester line (R27) to determine the status of the *hf1* gene in the latter case. The *Hf1*-dominant responsive line W168 was developed to determine the linkage of *Act1* to the *hf1* gene in *hf1*-recessive parental lines. From the backcross (W162 × V23) × W162 a B1 progeny plant was selected, with white flowers (*an1-W138*, *an1-W138*), that proved to be *hf1* heterozygous in a subsequent testcross with the line R27. This plant was selfed, and from the progeny, plants were selected that were homozygous dominant for the *Hf1* locus to form the line W168. Lines to be tested for the presence of *Act1* and recessive for the *Hf1* locus were first crossed to the line W168 to give *hf1* heterozygous F_1 plants that were subsequently crossed to line W162.

The standard deviation was calculated with the formula: $SD = \sqrt{(f^{co} \times (1 - f^{co})/(n)} \times 100$ where f^{co} is the fraction of crossovers, n is the total number of plants and SD is the standard deviation.

DNA methods

Isolation of genomic plant DNA and DNA blot analysis were performed according to Gerats et al. (1990). Plant DNA for the polymerase chain reaction (PCR) amplification was isolated from leaves according to Dellaporta et al. (1983). To screen for somatic excision of *dTph1* from the *dfrC* gene, part of the *dfrC* gene was amplified via PCR using the primers cp1 (5′-CCACCCACTGTAATGCTGCAGTATT-3′) and cp2 (5′-AGCTAACGGATCCAAGCCACGCCCGT-3′). Primer cp1 anneals 107 bp upstream of the integration site of *dTph1*, whereas cp2 anneals 298 bp downstream of that site. PCR conditions were as described by Gerats et al. (1990) except that DNA was incubated at 70 °C for 20 min before adding DNA samples to the PCR reaction and SUPER TAQ was used (HT Biotechnology LTD). PCR-amplified DNA was electrophoresed through a 2% agarose gel and blotted onto Hybond N^+ membranes. [^{32}P]-labeled probes were prepared using standard random primer labeling procedures. All DNA blots were first washed at low (2 × SSC, 0.1% SDS; 60 °C) and then at high stringency (0.1 × SSC, 0.1% SDS; 60 °C).

Results

Test for the presence of a functional *Act1* element in a selection of *Petunia hybrida*, *P. axillaris* and *P. integrifolia* lines

Plants of the line W162 are homozygous for the *an1-W138* mutable allele and have white flowers because no functional *Act1* element is present. Introduction of a functional activator in line W162 restores variegation in the flowers (Wijsman 1986). To further characterize the *an1-W138* transposable element family, we tested whether specific petunia lines had the capacity to activate the *an1-W138* element present in the line W162.

In crosses with *an1*-recessive lines, activation of the *an1-W138* element was directly visible in the F_1 plants. The F_1 progeny plants of 10 lines tested (W20, W46, W55, W78, W83, W107, W121, W126, W127 and W147) all exhibited variegated flowers, demonstrating the presence of a functional activator in these lines (not shown). When F_1 plants from 4 of these crosses were backcrossed to the W162 parent, a 1:1 segregation for variegated versus white flowers was obtained, indicating that activation is caused by a single factor (Table 1). The F_1 progeny plants of 4 other *an1*-recessive lines (W110, W144, W148 and W152) exhibited only white flowers, indicating that these lines lack a functional *Act1*

Table 1 Test of *an1* recessive petunia lines for the presence of a functional *Act1* activator element

Backcrosses	Number of plants with phenotype	
	Variegated	White
(W20 × W162) × W162	38	47
(W46 × W162) × W162	43	35
(W121 × W162) × W162	39	31
(W147 × W162) × W162	58	44
(W110 × W162) × W162	0	60
(W144 × W162) × W162	0	60
(W148 × W162) × W162	0	60
(W152 × W162) × W162	0	60

element. As expected the W162 backcrosses of these F_1 plants produced only progeny plants with white flowers (Table 1).

In crosses with *An1*-dominant lines, activation of the *an1-W138* element could not be scored in the F_1 plants, as these plants have evenly coloured flowers (*An1*, *an1-W138*). Backcrossing of F_1 plants with W162 resulted in a 2:1:1 segregation for plants with coloured flowers (*An1*, *an1-W138*), plants with spotted flowers (*Act1*, *act1*; *an1-W138*, *an1-W138*) and plants with white flowers (*act1*, *act1*; *an1-W138*, *an1-W138*). Table 2 shows that out of 17 *P. hybrida* lines tested, 12 gave B1 progeny plants with variegated flowers, indicating that these lines harbour a functional *Act1* element. As with the *an1*-recessive lines, different *An1*-dominant lines typically gave rise to differences in frequency and timing of reversion events (results not shown). The backcross progenies of 5 other *P. hybrida* lines (R4, M1, M72, W80 and W115) segregated 1:1 for coloured versus white flowers, whereas no variegated flowers were observed. Apparently these lines lack a functional *Act1* element, although formally we cannot exclude that a functional *Act1* element tighlty linked to the *An1* locus is present in these lines. All *P. hybrida* lines lacking *Act1* activity appeared to be derived from the old cultivar M1 ('Rose of Heaven').

The first interspecific crosses, which eventually gave rise to *P. hybrida* cultivars, between the white flowering *P. axillaris* (ssp. *parodii* and *axillaris*) and the coloured flowering *P. integrifolia* (ssp. *inflata*, *occidentalis* and *integrifolia*) occurred around 1835 (Sink 1984; Wijsman 1982, 1983). Several of these species lines (S1, S2, S6, S7, S12, S13 and S14) of different geographical origin were tested for their ability to activate the *an1-W138* element. Surprisingly, none of these lines exhibited *Act1* activity (Table 2).

In summary, the results in Table 1 and 2 show that a single functional activator for the *an1-W138* element is present in a wide range of *P. hybrida* cultivars, whereas the element seems to be inactivated in (a progenitor of) line M1. None of the species' lines contains a functional *Act1* element.

Localisation of the activator element *Act1*

We determined the genomic position of the *Act1* element in 11 *P. hybrida* lines. Table 3 shows that in all cases *Act1* appeared to be linked to the *Hf1* locus on chromosome I. The crossing-over frequencies varied between 0 and 15%. However, this cannot be taken as

Table 2 Test of *An1* dominant petunia lines for the presence of a functional *Act* activator element

Backcrosses	Number of plants with phenotype		
	Fully coloured	Variegated	White
(V2 × W162) × W162	100	35	35
(V13 × W162) × W162	70	26	12
(V14 × W162) × W162	110	33	50
(V26 × W162) × W162	91	54	46
(V28 × W162) × W162	114	37	33
(Vu6 × W162) × W162	182	77	60
(W22 × W162) × W162	55	10	19
(W29 × W162) × W162	50	14	20
(W138 × W162) × W162	42	19	14
(R27 × W162) × W162	55	30	24
(R51 × W162) × W162	56	26	31
(M3 × W162) × W162	59	14	14
(R4 × W162) × W162	10	0	9
(M1 × W162) × W162	75	0	33
(M72 × W162) × W162	65	0	16
(W80 × W162) × W162	46	0	54
(W115 × W162) × W162	55	0	37
(*P. axillaris* (S1) × W162) × W162	32	0	10
(*P. axillaris* (S2) × W162) × W162	40	0	33
(*P. integrifolia* (S6) × W162) × W162	73	0	33
(*P. axillaris* ssp. *parodii* (S7) × W162) × W162	22	0	38
(*P. integrifolia* (S12) × W162) × W162	24	0	32
(*P. integrifolia* (S13) × W162) × W162	37	0	26
(*P. integrifolia* ssp. *inflata* (S14) × W162) × W162	32	0	43

Table 3 Linkage of the W138-*an1* activator *Act1* to the *Hf1* locus[a]

Backcrosses	Number of plants with phenotype				
	Variegated (*Act1*)		White (*act1*)		
	Hf1 hf1	*hf1 hf1*	*Hf1 hf1*	*hf1 hf1*	cM[b]
(V2 × W162) × W162	31	3	2	31	7.5 ± 3.2
(V13 × W162) × W162	6	0	0	9	0
(V14 × W162) × W162	37	0	1	31	1.4 ± 1.4
(V23 × W162) × W162	19	2	0	25	4.3 ± 3.0
(V26 × W162) × W162	23	2	1	24	6.0 ± 3.4
(V28 × W162) × W162	16	0	0	12	0
(V35 × W162) × W162	13	3	0	8	12.5 ± 6.8
(Vu6 × W162) × W162	35	2	2	22	6.6 ± 3.2
(R27[c] × W168) × W162	4	23	18	3	14.6 ± 5.1
(W22[c] × W168) × W162	1	12	7	1	9.5 ± 6.4
(W138[c] × W168) × W162	1	11	8	0	5.0 ± 4.9

[a] Segregation data for unlinked markers are not presented
[b] Linkage was assumed, if χ-square values for independent segregation of *Act1* and *Hf1* were > 7.8 ($P < 0.05$)
[c] All activator lines were *Hf1* homozygous dominant except R27, W22 and W138, which were *hf1* recessive

evidence that *Act1* is located at different positions in the different lines since recombination frequencies can vary significantly when linked markers are analysed in different genetic backgrounds (Cornu et al. 1989; de Vlaming et al. 1984). We conclude that *Act1* is located on chromosome I, presumably at a fixed position.

Act1 activates the *dTph1* element present in the *dfrC* gene

It was shown previously that the *dfrC* gene of the *an1* mutable line W138 contains an insertion, *dTph1*, that has the sequence characteristics of a transposable element. The tight linkage of the *dfrC* gene with the *An1* locus, in conjunction with the correlation between reversion of the *an1* mutable allele and excision of *dTph1* from the *dfrC* gene strongly suggested that the *An1* locus contained the *dfrC* gene and that *dfrC::dTph1* was responsible for the variegated phenotype of the W138 flowers (Gerats et al. 1990).

In order to confirm and extend these initial data, we PCR-amplified part of the *dfrC* gene from a number of W138 plants, all with a defined genotype, randomly chosen from several independent families. We used two *dfrC*-specific oligonucleotides that amplify a 700-bp fragment (fragment A) from plants homozygous for the *dfrC::dTph1* allele, whereas from plants homozygous for wildtype or excision alleles a 400-bp fragment (fragment B) is amplified (Fig. 1A). In heterozygous plants (*dfrC::dTph1*, *dfrC*), both fragments A and B are amplified in more or less equal amounts. Due to somatic excision of *dTph1* from the *dfrC* gene the 400-bp fragment can also be detected in plants homozygous for the mutable *dfrC* allele, but at a much lower intensity than in the heterozygous plants.

Figure 1B shows that out of 16 W138 plants tested, 9 gave a PCR pattern that did not match the *an1* genotype. For example, in lanes 10, 13, 14 and 15 only a 400-bp *dfrC* fragment was detected, indicating that these plants are homozygous for the wildtype or revertant *dfrC* alleles. However, genetic analysis showed that these

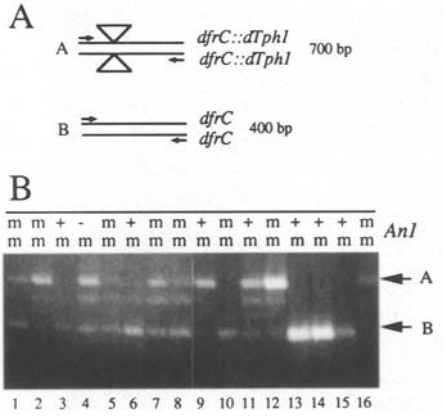


Fig. 1A,B Excision of transposable elements from the *dfrC* and *an1* gene is not correlated. **A** Diagram showing the expected *dfrC* PCR products as presented in *panel B*. **B** Comparison of the *dfrC* and the *an1* genotype in 16 W138 plants. The *an1* genotype was determined by selfing and is indicated *above* each lane (*m* mutable allele, + revertant allele, − recessive allele)

plants still contain at least one *an1-W138* allele. Lane 11 represents a plant homozygous for the *dfrC::dTph1* allele, whereas it is heterozygous for *an1*. In addition, PCR analysis of homozygous *An1*-revertant plants showed that in some of these plants *dTph1* was still present in the *dfrC* gene (data not shown). Thus, there is no correlation between mutability of the *an1* gene and the presence of *dTph1* in the *dfrC* gene. These data clearly prove that *an1* and *dfrC* are two distinct genes and that the *dTph1* element in the *dfrC* gene is not responsible for the variegation of W138 flowers.

The small size of the *dfrC::dTph1* element (284 bp; Gerats et al. 1989) suggested that it is a non-autonomous transposable element. We therefore examined whether somatic excision of the *dfrC::dTph1* element was dependent on the presence of *Act1*. The *dTph1*-containing part of the *dfrC* gene from W138 plants homozygous for the *dfrC::dTph1* allele was amplified using the same primers as before. Figure 2A, lane 3 shows that after blotting and hybridisation with a *dfrC* probe, four minor products could be detected (fragments

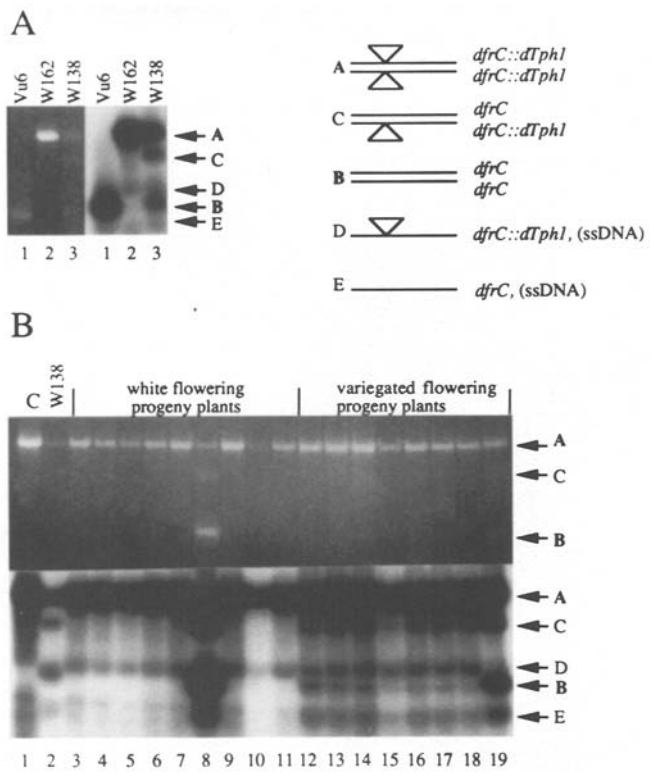


Fig. 2A,B Somatic excision of *dfrC::dTph1* depends on the presence of the *Act1* element. **A** Ethidium bromide-stained *dfrC* PCR products form the lines Vu6, W162 and W138 (*left*) were hybridised with a *dfrC* probe (*right*). The character of the hybridising fragments (*A–E*) is explained in the diagram on the *right* (see text for detailed explanation). **B** *dfrC* PCR products from a plasmid control (*lane 1*), a W138 plant (*lane 2*) and white- or variegated-flowering progeny plants of the cross (W162 × Vu6) × W162 (*lanes 3–19*). *Upper part* Ethidium bromides-stained gel; *lower part* same gel after blotting and hybridisation with a *dfrC* probe

B, C, D and E) in addition to the major 700-bp product that represents the *dfrC::dTph1* allele (fragment A). Based on its size (~ 400 bp), its hybridization to *dfrC* and the lack of hybridisation to a *dTph1* probe (not shown), we concluded that fragment B is a bona fide PCR product that originated from the *dfrC* gene after somatic excision of *dTph1*. Fragments C, D and E, however, are by-products of the polymerase chain reaction. Fragment C was not observed after separate amplification of Vu6 or W162 DNA (Fig. 2A, lanes 1 and 2, respectively). When Vu6 and W162 were mixed prior to amplification, fragment C was again detected (not shown). We concluded that fragment C most likely consists of one DNA strand of fragment A heteroduplexed to one strand of fragment B (Fig. 2A). The appearance of fragments D and E correlates to the presence of fragments A and B, respectively. We therefore assume that the two hybridizing bands D and E represent single-stranded DNA products corresponding to fragment A and B, respectively.

PCR analysis showed that plants of the line W162 are homozygous for the *dfrC::dTph1* allele. Somatic excision of *dfrC::dTph1* was detectable in W138 but not in W162 plants (Fig. 2A, lanes 2 and 3), indicating that W162 lacks a functional activator element for *dTph1*. The observation that both the *an1-W138* element and *dTph1* are activated in W138 but remain inactive in W162 was a first indication that both elements might respond to the same activator, *Act1*.

To further test whether activation of *dfrC::dTph1* was dependent on the presence of *Act1*, we analysed progeny plants from the W162 backcross of 5 different lines (V13, V26, Vu6, W115 and S14; see Table 2). Because the *dfrC* gene and the *An1* locus are tightly linked, most homozygous *an1-W138* plants with white or variegated flowers were expected to be homozygous for the *dfrC::dTph1* allele as well. Therefore, DNA was isolated from white and variegated flowering plants, and the *dfrC* region was amplified as before. In Fig. 2B we show the analysis of 9 white and 8 variegated flowering plants from the backcross (W162 × Vu6) × W162. In accordance to our expectations, most plants yielded a 700-bp fragment (fragment A) as the major product, indicating that they were indeed homozygous for the *dfrC::dTph1* allele. At a low frequency, however (2 plants out of 143), we found plants that yielded a 400-bp product (B) in amounts approximately equal to that of the 700-bp product (see e.g. Fig. 2B, lane 8). This indicates that these plants are heterozygous and contain a mutable plus a stable *dfrC* allele, either as a consequence of (1) crossing-over between the *dfrC* gene and the *An1* locus or (2) a sporogenic reversion of the *dfrC::dTph1* allele in the F_1 plant.

To detect somatic excision events in the *dfrC:dTph1* homozygous plants, PCR products were blotted and hybridised with a *dfrC* probe. The autoradiograph in Fig. 2 (panel C) shows that somatic excision events were detectable in plants with variegated flowers (*Act1*, *act1*) but not in those with white flowers (*act1*, *act1*). Table 4

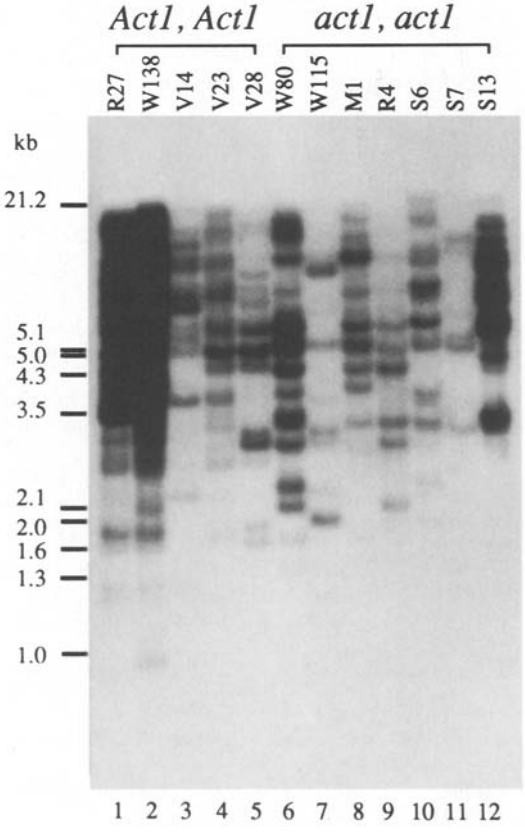
Table 4 Somatic excision of the *dTh1-dfrC* element is dependent on the presence of a functional *Act1* element

Backcross activity	Plants with variegated flowers		Plants with white flowers	
	Number of plants tested	Number of plants with detectable *dTph1* activity	Number of plants tested	Number of plants with detectable *dTph1*
(V13 × W162) × W162	14	10	5	0
(V26 × W162) × W162	29	20	18	0
(Vu6 × W162) × W162	25	18	18	0
(S14 × W162) × W162	–[a]	–	19	0
(W115 × W162) × W162	–	–	31	0

[a] –, Not applicable; see Table 2

summarises the results for all plants tested in the 5 different backcross progenies. Of the 41 white-flowered plants tested from the progenies that segregated for *Act1*, none showed somatic excision for *dTph1*, whereas all the 48 plants that did exhibit somatic excision for *dTph1* had variegated flowers. Examination of the variegated flowers of plants of these W162 backcrosses made it clear that the frequency of somatic excision of the *an1-W138* element can vary considerably. The differences in the intensity of the 400-bp hybridisation signal in Fig. 2B indicates that the frequency of somatic excision of *dTph1* from the *dfrC* gene may also vary (compare lanes 18 and 19). We assume, that *dfrC::dTph1* excision was not detected in the leaves of all plants with variegated flowers, since the frequency of somatic excision might be too low. The two lines that lack a functional *Act1* element (S14 and W115) also appear to lack a functional activator for *dTph1*, as we could not detect somatic excisions in any of the corresponding backcross progeny plants. Taken together, these data strongly suggest that *dTph1* is a non-autonomous transposable element that is activated by *Act1*.

Fig. 3 Copy number of *dTph1* elements in a selection of petunia lines. Genomic DNA (10 µg) of the different petunia lines was digested with *Eco*RI, fractionated in a 0.8% agarose gel, blotted and hybridised to a *dTph1* probe. After low stringency washing (2 × SSC, 0.1% SDS; 60 °C), the blot was autoradiographed



Distribution of *dTph1*-related elements in different petunia lines

We decided to study the copy number of *dTph1* elements in a selection of petunia lines for two reasons. First, we wanted to determine whether *dTph1* elements are present in all lines, especially in the species lines, and second, to determine whether *dTph1* copy number is affected by the presence of a functional *Act1* element. We therefore hybridised DNA gel blots of a selection of petunia lines with a *dTph1* probe. The analysis included 3 of the petunia species lines from which *P. hybrida* originated. Figure 3 shows that at low stringency, *dTph1*-related elements were detected in all these petunia lines, including the species lines. Washing the blot at higher stringency barely reduced the number of bands hybridising with *dTph1*, indicating that most of the fragments detected represented sequences highly homologous to *dTph1* (not shown). The differences in the hybridisation signals suggest that the strongly hybridising bands represent multiple *dTph1* copies. In most of the lines tested, between 5 to 25 *dTph1* copies were detected. However, 2 lines, R27 and W138, contain significantly more *dTph1* elements (> 50) than all other lines.

From these data, we conclude that there is no clear correlation between the presence or absence of a functional *Act1* element and the number of *dTph1*-related elements in a specific line (compare lanes 1–5 with lanes 6–12). Furthermore, we show that the *dTph1* family was already present in the species that gave rise to *P. hybrida*.

Discussion

In this paper, we describe the genetic characterisation of the *Act1* element that activates excision of a non-autonomous transposable element at two different loci in petunia: the element in the mutable *an1* allele of the line W138 and the *dTph1* element present in the *dfrC* allele of the same line.

A selection of petunia lines was tested for the capacity to activate the non-autonomous transposable element in the *an1-W138* allele. A large majority of the *P. hybrida* lines has this capacity, and in all of the cases tested *Act1* was linked with the marker *Hf1* on chromosome I (Table 1). Differences in cross-over percentages as presented in Table 3 are typical for linked genes in petunia (Cornu et al. 1989; de Vlaming et al. 1984). They most probably do not reflect actual differences in the position of *Act1* in the different lines studied. These lines are of diverse, but untraceable, origin, and most of them have been genetically separated for at least 50 years. We therefore conclude that *Act1* normally does not transpose. In maize, the elements that activate non-autonomous transposons like *Ac* and *Spm* are mobile themselves (Federoff 1989). It is conceivable that *Act1*, like *Ac* and *Spm*, encodes a transposase. Possibly, *Act1* has lost its mobility due to a mutation in the terminal inverted repeats or the subterminal regions. In *Ac* these regions

contain important *cis*-responsive sequences that act as a substrate for the transposase (Coupland et al. 1989; Kunze and Starlinger 1989).

In progenies of selfed W138 plants new mutable alleles occur at a high frequency (Bianchi et al. 1978; Doodeman et al. 1984b; Gerats et al. 1989). This may be due to the high copy number of *dTph1* in this line (Fig. 3). Indeed, all W138-derived mutable alleles that have been cloned so far contained elements that were virtually identical to the *dfrC::dTph1* element (van Houwelingen et al., unpublished results).

The *dTph1* element was found to be non-autonomous, as was already expected from its small size. The activator of *dTph1* transposition was localised in two lines on chromosome I, and we could not detect cross-overs between this element and *Act1*. Furthermore, the absence or presence of a *dTph1*-activating element correlated with that of a functional *Act1* element (Table 4). This indicates that the element in the *an1-W138* allele and *dTph1* are activated by the same activator (*Act1*) and thus belong to the same family of transposable elements.

P. hybrida lines derived from M1 (R4, M72, W80, W110, W144, W148, W152) failed to activate the element at the *an1-W138* allele in backcrosses with the W162 responsive line. This indicates that in these lines either (1) *Act1* is inactive or (2) the *Act1* element is active but tightly linked to the *An1* locus, in which case it would escape detection. For the lines W110, W144, W148 and W152 the latter possibility can be excluded since these (*an1* recessive) lines also failed to activate transposition in F_1 crosses. From this and the observation that the functional *Act1* element in the *P. hybrida* cultivars tested is always located on chromosome I, we assume that the line M1 and its derivatives contain an *Act1* element inactivated by a mutation.

No functional *Act1* element was detected in W162 backcrosses with species lines. This is surprising in view of the fact that all *P. hybrida* cultivars are derived from these species. We therefore assume that *Act1* is present in these species but in an inactive state. This is supported by the observation that members of the *dTph1* element family are present in the species lines tested (Fig. 3). Since most of the *P. hybrida* cultivars contain a functional *Act1* element, it was presumably activated during or soon after the interspecific crosses that gave rise to *P. hybrida*. Activation of transposable elements might be the result of any of a number of mechanisms or treatments defined as a "genomic shock", such as interspecific crosses, tissue culture, viral infection and mutagenic treatment (McClintock 1984; Nevers et al. 1986; Peterson 1987).

In summary, the *dTph1* transposable element family seems to consist of one immobilized activator element *Act1*, located on chromosome I, and a variable number of responsive *dTph1* elements. The isolation and characterisation of the *Act1* element could demonstrate whether *Act1* and *dTph1* are structurally related and may give an explanation for the immobility of *Act1* and its inactivity in the species.

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