Theor Appl Genet (1995) 90: 957–968

T. P. Robbins · A. G. M. Gerats · H. Fiske
R. A. Jorgensen

Suppression of recombination in wide hybrids of *Petunia hybrida* as revealed by genetic mapping of marker transgenes

Received: 1 May 1994 / Accepted: 8 December 1994

Abstract In the course of a heterologous transposon tagging experiment in *Petunia hybrida* (n=7), 135 independent T-DNA loci were tested for linkage to the target genes *Hf1* and *Fl*, which are located on the two largest chromosomes . Approximately one-third (47) of these T-DNA loci were linked to one of these two markers. Of these 47 linked-loci, 19 mapped within 1 cM of its marker, indicating a highly non-random genetic distribution of introduced loci. However, rather than non-random integration within both of the marked chromosomes, this probably reflects a suppression of recombination around these marker loci in the particular wide hybrids used for mapping. This hypothesis was tested by measuring recombination between linked T-DNAs in an inbred background. Inbred recombination levels were found to be at least 3-fold higher around the *Hf1* locus and 12-fold higher around *Fl* compared to the wide hybrids. These findings may reflect the origin of *P. hybrida* by hybridization of wild species, and while relevant to genetic mapping in petunia in particular they may also have more general significance for any mapping strategies involving the use of wide hybrids in other species.

Key words Petunia · Transformation · T-DNA · Recombination · Wide hybrids

Communicated by H. K. Dooner

T. P. Robbins (✉)[1] · H. Fiske · R. A. Jorgensen[3]
DNA Plant Technology Corporation, 6701 San Pablo Avenue, Oakland CA 94608, USA

A. G. M. Gerats[2]
Department of Genetics, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

Present addresses:
[1] Department of Molecular Genetics, John Innes Centre, Colney, Norwich, NR4 7UH, UK
[2] Laboratorium Genetika, Universiteit Gent, Ledeganckstraat 35, B-9000 Gent, Belgium
[3] Department of Environmental Horticulture, University of California, Davis CA 95616, USA

Introduction

The advent of routine plant transformation procedures using *Agrobacterium* has allowed a novel class of genetic marker to be developed. The markers used to select for transformation (e.g. antibiotic or herbicide resistance) can serve equally well as dominant traits for segregation analysis. Given an efficient transformation system a potentially limitless number of single-locus markers that segregate in Mendelian fashion can be generated (Deroles and Gardiner 1986). Despite the wide availability of introduced selectable markers, in a variety of genetically well-defined species (e.g. Feldmann and Marks 1987) there have been relatively few attempts to incorporate these into existing genetic maps.

However, in a study using *Agrobacterium*-mediated transformation of *Petunia hybrida*, nine T-DNA integration sites were localized to four out of the seven chromosomes (Wallroth et al. 1986). Of four integrations placed on chromosome I, all were found to map to genetically distinct sites. Similarly, ten T-DNA insertions in the tomato genome were found to map to distinct sites on at least 5 of the 12 chromosomes (Chyi et al. 1986). In no instance were two inserts found at an identical location. These studies provided the first evidence that selectable markers could be placed at a variety of loci scattered apparently at random throughout plant genomes. Additional support came from the direct physical mapping of a limited number of T-DNA insertions in *Crepis* (Ambros et al. 1986).

In the course of a transposon tagging experiment described herein we mapped a large number of independent transgene loci (135) with respect to several target genes. Our approach differed from those used previously in that the selectable marker was used to test for skewed segregation of linked phenotypic markers. This allowed us to screen a large number of transformants for linkage because it was not necessary to assay progeny individually for inheritance of the transgene as in previous studies (Wallroth et al. 1986, Chyi et al. 1986). The high density of T-DNA markers allowed us to address a number of problems re-

lated to recombination and the distribution of T-DNA insertion sites within petunia chromosomes. The results showed that the level of recombination between the T-DNA markers and linked phenotypic markers is generally much less than would be expected for a typical genetic map. We subsequently showed that the level of recombination between linked T-DNA markers in an inbred background is apparently greater than the hybrid mapping data would suggest. This presumably results from unexpectedly poor recombination between chromosomes of distinct but interfertile lines. The implications of the results presented here are discussed with respect to both genetic mapping in petunia in particular and also mapping strategies based on wide hybrids in general. In addition, we have extended and confirmed the previous suggestions that T-DNA insertions occur at random with respect to chromosomes.

Materials and methods

Petunia hybrida genetic stocks

The inbred lines of *Petunia hybrida* used in this study, V26, M1 and M59, were obtained from the stock centre of the Department of Genetics at the Free University of Amsterdam. These lines were originally derived from commercial stocks and have been inbred for at least ten generations. Each line has been characterized for a large number of genetic factors influencing anthocyanin pigmentation in petunia. The factors that are relevant to the mapping scheme used here are as follows:

V26: *Hf1, Fl, Ph1, ph2, po, an4*
M1: *hf1, fl, Ph1, Ph2, Po, An4*
M59: *hf1, fl, ph1, Ph2, po, An4*

The *Hf1* gene is required for 3′5′-hydroxylation of the B ring of flavanones and dihydroflavonols. The *Fl* gene affects the production of flavonol copigments, and *ph1* and *ph2* affect the pH of the corolla cell sap. Finally, *Po* and *An4* determine the pollen colour. All these factors can be scored independently by the visual inspection of corollas or anthers. For further details see Wiering and de Vlaming (1984).

Transformation of *P. hybrida*

Two constructs were introduced into the line V26 using a previously established transformation/regeneration protocol (Napoli et al. 1990) based on the general method of Horsch et al. (1985). The pJJ2853 construct contains a neomycin phosphotransferase (NPTII) coding region with a NOS promoter and an OCS 3′ end that confers resistance *in planta* to the antibiotic kanamycin (Jones et al. 1989). The pJJ4411 construct contains a hygromycin phosphotransferase (HPT) coding region with a p1′ promoter and a NOS 3′ end that conferred resistance to hygromycin (Keller et al. 1992). The transformations with pJJ2853 used the conditions described previously (Napoli et al. 1990) while those with pJJ4411 were performed using 15ug/ml hygromycin in the selection media.

Seedling selections

Transgenic seed was surface-sterilized for 20 min with 20% bleach, 1% Tween and rinsed at least five times with sterile water before drying in a laminar flow hood. Sterile seed were sprinkled onto petri plates containing Basal MS medium pH 5.8, 0.8% agar, 1% glucose or 3% sucrose and the appropriate antibiotic [100–200 ug/ml kanamycin sulphate (Sigma) or 15 ug/ml hygromycin (Calbiochem)]. Plates were incubated at 24°C under 3000–5000 lux for 2–4 weeks prior to scoring. Seedlings resistant to kanamycin (producing both true leaves and long branched roots) or hygromycin (fully expanded cotyledons and extended hypocotyls) were transferred to Magenta boxes containing Basal MS medium pH 5.8, 0.8% tissue culture agar (JRH Biosciences, Lenexa Kan.) and 3% sucrose. Once the roots were well-established, seedlings were transplanted into soil and placed in a greenhouse for scoring flower colour.

Statistical methods

Due to the mapping scheme employed, only kanamycin-(or hygromycin)-resistant plantlets were analysed for segregation of phenotypic markers (Fig. 1). Linkage between the T-DNA insert and a phenotypic marker was presumed if the marker exhibited a segregation deviating significantly from that of the controls. The segregations were not tested against a 1:1 ratio because of inherent segregation distortion, but instead against a pooled segregation ratio derived from a number of mapping populations in which the T-DNA was clearly linked (within 10 cM) to a distinct marker. Thus, the expected ratio for *Hf1:hf1* segregations was derived from the actual numbers found in crosses where the T-DNA insert appeared to be linked within 10 cM of *Fl*. In the first experiment presented in Table 1A, *Hf1:hf1* segregation in 10 *Fl*-linked progenies[1] (Ac9, Ac12, Ac21, Ac80, Ac83, Ac112, Ac118, Ac128, Ac145, Ac172) gave a pooled ratio of 1195:655. Conversely, *Fl:fl* segregation in 14 *Hf1*-linked progenies (Ac8, Ac11, Ac27, Ac32, Ac46, Ac48, Ac52, Ac73, Ac107, Ac114, Ac119, Ac163, Ac171, Ac208) was 1231:1060. In the second experiment (Table 1B), we used a ratio of 946:512 for *Hf1:hf1* derived from 8 *Fl*-linked populations (Ac352, Ac395, Ac424, Ac448, Ac522, Ac530, Ac563, Ac625) and a ratio of 789:607 for *Fl:fl* derived from 4 *Hf1*-linked populations (Ac332, Ac388, Ac399, Ac617). The control populations were shown to be homogeneous using a Chi-square test, and all gave a probability of at least 5%. The observed segregation ratios for particular lines were then tested with the relevant control segregation using a standard Chi $^2_{2\times2}$ contingency test:

$$\mathrm{Chi}^2 = \frac{((\mathrm{ad}-\mathrm{bc})-0.5\,\mathrm{N})^2\,\mathrm{N}}{(\mathrm{a}+\mathrm{b})(\mathrm{a}+\mathrm{b})(\mathrm{b}+\mathrm{d})(\mathrm{c}+\mathrm{d})}$$

where **a** represents the number of dominant plants observed, **b** the number of recessive plants observed, **c** the expected number of dominant plants, **d** the expected number of recessives and **N** the total number of plants.

Results

The results presented here were obtained from two distinct populations of transgenic petunias, both derived by *Agrobacterium*-mediated transformation of the same inbred line, V26, by the procedure of Napoli et al. (1990). The experiments differ in the T-DNA construct employed: pJJ2853 confers a kanamycin-resistant phenotype and pJJ4411, a hygromycin-resistant phenotype (see Materials and methods for details). Both constructs also contain an *Ac* element inserted into a gene conferring streptomycin-resistance (for further details see Jones et al. 1989; Keller et al. 1993).

[1] The line Ac129 was also tightly linked to Fl (0 cM) but the Hf1 ratio was significantly different from the other 10 lines when pooled (P< 5%) and so it was omitted from the control ratio

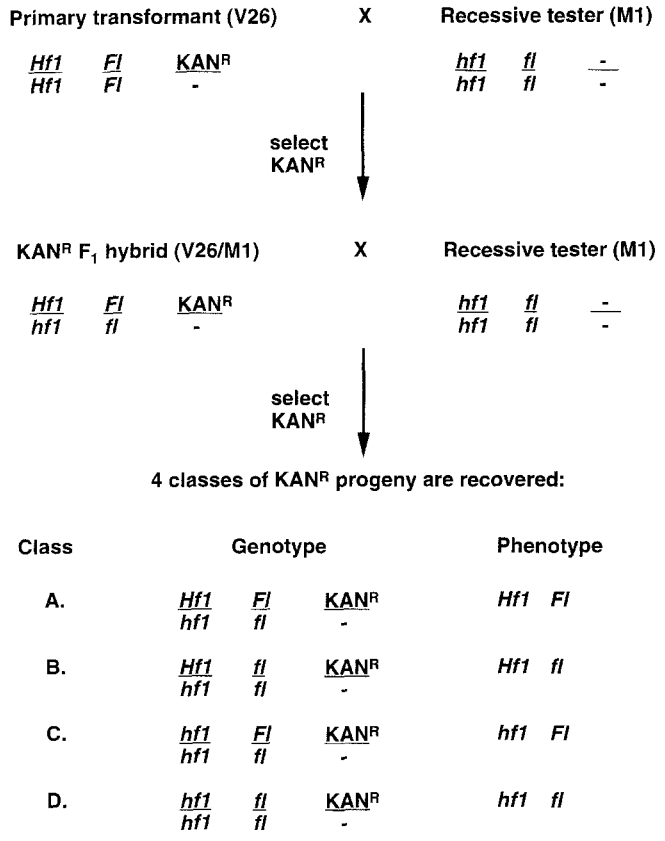

Fig. 1 Scheme for mapping T-DNAs with phenotypic markers. The genotypes for the two inbreds used in the initial study (V26 and M1) are indicated for the marker genes *Hf1* and *Fl* on chromosomes I and II, respectively. An unlinked T-DNA conferring kanamycin resistance in the transformation host V26 is shown. Kanamycin-resistant F_1 hybrids were selected and backcrossed with M1 as shown. The selection of kanamycin-resistant backcross seedlings will result in four possible phenotypic classes (*A–D*), all of which are readily distinguished by visual inspection of corollas (Wiering and de Vlaming 1984). If the T-DNA was unlinked to either *Hf1* or *Fl* then the four classes would be present in approximately a 1:1:1:1 ratio. If the T-DNA was linked to *Hf1* then classes A+B (*Hf1*)>C+D (*hf1*) or linked to *Fl* then classes A+C (*Fl*)>B+D (fl)

Screening kanamycin-resistant transformants for linkage to marker loci

Kanamycin-resistant transformants were produced by introducing the T-DNA of pJJ2853 into the inbred line, V26. These primary transformants were crossed to the inbred tester line M1 to produce V26/M1 hybrids that are heterozygous at a number of phenotypic marker loci (see Materials and methods). Hybrid seed was plated on kanamycin-containing medium, and lines with good penetrance for the kanamycin-resistance phenotype that were also apparently single locus (based on a 1:1 segregation) were selected for backcrossing as females to the M1 tester line. The selection of kanamycin-resistant backcross progeny allowed the detection of linkage between the T-DNA and marker loci on chromosomes I (*Hf1*) and II (*Fl*) as illustrated in Fig. 1. Linkage between the transgene and either marker gene would result in a distorted segregation in favour of the allele derived from the transformation parent (V26).

Table 1A presents the results of scoring kanamycin-selected backcross seed derived from 84 transformants for the two phenotypic markers *Hf1* and *Fl*. The allele ratios (*Hf1*: *hf1* and *Fl*: *fl*) were determined by pooling data from the four phenotypic classes as described in the legend to Fig. 1. A preliminary screen of 30–60 kanamycin-resistant selections was used as an initial linkage test (Table 1A, preliminary dataset). Several lines gave apparent skewing of phenotypic ratios towards the dominant allele of the transformed parent (*Hf1* or *Fl*), as would be expected in the case of linkage. To confirm a putative linkage the data were extended to larger confirmatory populations (typically more than 100 individuals) for selected lines (Table 1A, confirmatory dataset). The significance of any observed skewed ratios was calculated using a Chi-square test as described in the Materials and methods. Briefly, the observed ratio for a putative *Hf1*-linked line was compared to that expected based on a pooled sample of lines for which the T-DNA was tightly linked (within 10 cM) to *Fl*. Conversely, for a putative *Fl*-linked line, a control ratio based on a pooled sample of lines for which the T-DNA was tightly linked to *Hf1* was employed. In neither case was the control ratio exactly 1:1, indicating inherent segregation distortion. Segregation distortions are a common occurrence in petunia linkage analysis and can be more extreme than those reported here (e.g. Cornu et al. 1980). The basis for these segregation distortions are unknown, but in this case we can exclude pollen competition because the F_1 hybrid was used as the female in all cases.

The analysis of the preliminary linkage screen indicated that 17 lines showed linkage to *Hf1* and 18 lines showed linkage to *Fl* at the 5% significance level (Chi^2>3.84). In general, the significance of a linkage was improved when confirmatory populations were scored, with the exception of Ac29, which remained the same. Three families (Ac17, Ac37 and Ac222) gave results that were inconsistent with the preliminary screen when expanded populations were analysed. One line (Ac102) appeared to show significant linkage to both *Hf1* and *Fl*. The cause of this anomaly remains to be determined and could be a simple bookkeeping error. Alternatively, it could result from a translocation between chromosomes I and II. Such translocations have been reported previously following irradiation treatment (Maizonnier and Cornu 1971). It is conceivable that such an event might have been induced during the transformation-regeneration procedure. Tissue culture is a well-known source of chromosomal abnormalities.

Confirmatory or expanded datasets were also prepared for 20 lines for which no significant linkage was detected in the preliminary dataset. In 3 cases new significant linkages (Ac95, Ac124, Ac171) were revealed as might be expected for a larger sample. An indeterminate number of additional linkages may have been undetected for lines where the linkage populations were not expanded. Mapping data for lines that were both significant at the 1% level (appropriate for the number of lines studied) and free of anomalies are summarized in Table 2A. The estimates for link-

Table 1A Segregation data for kanamycin-selected backcross mapping populations for 84 independent single-locus transformants. A preliminary screen of 30–60 individuals was used to detect linkage by comparison with control ratios (see Materials and methods for details). Tentative linkages were confirmed with larger populations to improve the significance by the Chi^2 test

Ac number	Preliminary dataset			Confirmatory dataset		
	Hf1:hf1	*Fl:fl*	Chi^2 [a]	*Hf1:hf1*	*Fl:fl*	Chi^2
Ac004	39:15	27:27	NS	71:31	51:51	NS
Ac006	46:14	34:26	NS	88:27	61:54	NS
Ac007	26:13	23:16	NS; *ph2*			
Ac008	*53: 3*	27:29	14.0	*211: 4*	105:110	78.6
Ac009	17:11	*28:0*	14.4	123:72	*191:4*	101.8
Ac010	37:16	24:29	NS	122:73	103:92	NS
Ac011	*41: 2*	23:20	11.1	*192:9*	110:91	59.8
Ac012	37:18	*55:0*	30.6	132:67	*193:6*	97.8
Ac013	20:11	14:17	NS; *ph2*	56:47	55:48	NS
Ac014	30:11	*39:2*	16.4			
Ac015	*47:9*	26:30	4.6	106:28	70:64	6.5
Ac016	45:34	45:34	NS; *po*			
Ac017	30:11	*41:0*	22.2	*[b]	*	*
Ac021	33:20	*53:0*	29.3	124:51	*175:0*	102.8
Ac022	29:11	20:20	NS	139:70	112:97	NS
Ac023	24:17	23:16	NS			
Ac025	26:15	19:22	NS			
Ac027	*56:1*	20:37	19.3	*232:3*	120:115	90.1
Ac028	22:7	17:12	NS			
Ac029	*37:1*	20:18	11.4	*65:8*	39:34	11.2
Ac030	22:17	20:19	NS; *po*			
Ac032	*53:4*	26:31	12.2	*89:4*	42:51	26.7
Ac036	23:9	16:16	NS	169:83	134:18	NS
Ac037	16:13	21:9	NS	*	*	*
Ac038	33:27	32:28	NS; *po*	71:54	66:59	NS
Ac041	18:10	16:12	NS; *an4*			
Ac043	26:9	22:13	NS; *an4*			
Ac045	27:9	19:17	NS	104:46	75:75	NS
Ac046	*36:2*	26:12	9.1	*131:13*	80:64	28.0
Ac048	*23:0*	13:10	7.7	*141:2*	77:66	53.5
Ac051	27:13	26:14	NS; *ph2*	73:48	71:50	NS
Ac052	*25:1*	18:18	6.4	*129:3*	76:66	45.7
Ac057	34:6	21:19	NS			
Ac058	25:14	29:10	NS			
Ac065	25:8	20:13	NS	195:94	169:120	NS
Ac066	24:10	26:8	NS	57:23	52:28	NS
Ac068	27:15	19:23	NS			
Ac070	11:10	12:9	NS	40:17	35: 24	NS
Ac073	*28:1*	16:13	7.7	*301:5*	175:131	114.7
Ac078	13:12	14:11	NS; *po*			
Ac079	19:8	22:5	NS	133:77	126:83	NS
Ac080	28:11	*39:0*	21.0	128:61	*188:1*	107.8
Ac083	20:13	*33:0*	17.4	155:112	*256:11*	123.7
Ac085	*36:5*	26:15	5.0			
Ac090	25:11	25:11	NS			
Ac091	26:7	17:13	NS			
Ac093	19:12	15:16	NS			
Ac094	25:9	21:15	NS	59:30	52:39	NS
Ac095	30:7	27:10	NS	*217:29*	135:111	37.5
Ac101	29:13	26:16	NS			
Ac102	*44:3*	24:23	10.5	*217:25*	*174: 68*	42.5[c]
Ac107	*34:2*	24:12	8.3	*200:5*	122:83	71.4
Ac112	46:30	*69:7*	24.3			
Ac114	*39:0*	20:19	14.6	*251:1*		103.8
Ac118	40:22	*62:0*	34.8	230:135	*364:1*	213.8
Ac119	*40:0*	26:14	15.1	*279:1*	144:135	116.0
Ac124	31:5	19:17	NS	*142:29*	88:81	13.3
Ac125	23:11	21:13	NS			
Ac126	24:12	17:19	NS; *an4*	274:162	239:198	NS
Ac128	27:13	*40:0*	21.5	128:69	*200:0*	117.9
Ac129	33:9	*42:0*	22.7	111:34	*143:0*	83.6
Ac133	29:12	17:24	NS			
Ac135	31:5	*29:7*	4.8; *ph2*			
Ac138	21:13	20:14	NS; *ph2*			

Table 1A Continued

Ac number	Preliminary dataset			Confirmatory dataset		
	Hf1:hf1	*Fl:fl*	Chi^{2} [a]	*Hf1:hf1*	*Fl:fl*	Chi^{2}
Ac143	21:14	14:21	NS			
Ac145	19:11	*30:0*	15.6	88:39	*124:1*	69.4
Ac151	20:6	21:5	NS; *an4*			
Ac152	22:15	14:23	NS; *an4*			
Ac155	26:8	*27:7*	3.9			
Ac158	26:20	*41:5*	12.5			
Ac163	*27:2*	21:12	5.6	*127:10*	88:53	31.1
Ac165	38:10	25:23	NS; *ph2*			
Ac169	62:48	*86:24*	13.6			
Ac171	7:0	3:4	NS	*92:0*	45:47	37.5
Ac172	22:13	*35:0*	18.6	41:27	*68:0*	38.4
Ac175	13:8	7:14	NS	51:41	56:36	NS
Ac177	16:12	12:18	NS; *an4*			
Ac179	22:14	21:15	NS			
Ac180	20:11	14:17	NS	269:136	221:184	NS
Ac184	32:18	24:26	NS			
Ac189	17:16	19:14	NS; *an4*			
Ac208	*52:0*	26:26	20.3	*100:8*	27:29	24.0
Ac222	26:13	*36:3*	12.9	*	*	*
Ac234	20:16	17:19	NS			

[a] Chi $^{2}_{2\times2}$ values were calculated by comparison with control segregation ratios as described in the Materials and methods. Significantly skewed allele ratios ($P< 0.05$ or Chi$^{2}>3.84$) are italicized. Lines with no significant linkage to either marker are indicated NS. Lines with a significant linkage to others markers tested (*an4*, *po*, *ph2*, data not shown) are also indicated

[b] Some confirmatory datasets were not consistent with preliminary datasets (indicated *), and these lines have therefore been omitted from the summary in Table 2

[c] The confirmatory dataset showed a significant linkage to *Fl* with a Chi^{2} of 16.4, perhaps a result of a translocation between chromosomes I and II

age distance include a correction factor for segregation distortion as described in the footnote. A disproportionate number of very tight linkages is apparent and will be discussed in the following section. The minimum number of linked lines for *Hf1* (18) and *Fl* (14) represent 21.4% and 16.7% respectively of the total number of kanamycin-resistant loci mapped with respect to these markers as summarized in Table 3A.

Screening hygromycin-resistant transformants for linkage

A second population of 51 transformants was generated by introducing the pJJ4411 construct (Keller et al. 1992), which confers resistance to hygromycin into the same inbred line, V26. In this case, a different recessive tester line, M59, which is only distantly related to M1, was employed (for reasons relating to transposon tagging experiments). Again the backcross scheme shown in Fig. 1 (substituting M59 for M1) was used to test for linkage of the transgene loci to *Hf1* and *Fl*, but in a distinct hybrid genotype (V26/M59) and using a different dominant selectable marker (hygromycin-resistance).

The results of both preliminary and confirmatory scores for *Hf1* and *Fl* markers in this experiment are presented in Table 1B. The control segregation ratios used for determining the significance of linkage were calculated as before and were slightly different from those obtained with the V26/M1 hybrid (see Materials and methods for further details). The preliminary screen produced 16 lines (7 *Hf1*; 9 *Fl*) that were tentatively linked (5% level of significance), of which nearly all were highly significant when confirmatory populations were scored. Table 2B presents a summary of lines showing significant linkage (1% level of significance) to either *Hf1* or *Fl*. In a few cases a control for the normal transmission of alleles in the absence of hygromycin selection was performed (see footnote for details). If a borderline *Fl*-linked line was included in the analysis (Ac422) then 6 *Hf1* linkages and 9 *Fl* linkages were found, representing 11.8% and 17.6%, respectively, of the 51 lines tested. The distribution of T-DNA insertions between chromosomes 1 and 2 is summarized in Table 3B. The results are in broad agreement with the results of the kanamycin-resistance study, although apparently fewer *Hf1* linkages were detected in the V26/M59 hybrids.

As for the kanamycin-resistant (KAN^{R}) loci, the majority of linkages to hygromycin-resistant (HYG^{R}) loci indicated a very low level of recombination. The average linkage to *Hf1* was particularly tight for the HYG^{R} loci, with two-thirds of the linkages (4/6) being within 1 cM compared to apparently fewer *Hf1*-linked KAN^{R} loci (3/17). Both populations showed frequent tight linkages to *Fl* with approximately half of both HYG^{R} (5/9) and KAN^{R} (7/13) loci being mapped within 1 cM. This is unlikely to reflect an enhanced ability to detect tight linkages since highly significant linkages were detected at distances up to 30 cM (e.g. Ac616).

Table 1B Segregation data for hygromycin-selected backcross mapping populations for 51 independent single-locus transformants. A preliminary screen of 30–60 individuals was used to detect linkage by comparison with control ratios. Tentative linkages were confirmed with larger populations to improve significance by the Chi^2 test

Ac number	Preliminary dataset			Confirmatory dataset		
	Hf1:hf1	*Fl:fl*	Chi^2 [a]	*Hf1:hf1*	*Fl:fl*	Chi^2
Ac301	32:17	23:26	NS			
Ac331	79:38	69:48	NS			
Ac332	*38:0*	11:27	13.8	*273:0*	145:128	113.9
Ac352	25:14	*36:3*	11.3	101:55	*128:28*	22.7
Ac366	33:24	26:31	NS			
Ac371	36:22	33:25	NS			
Ac386	33:18	27:24	NS			
Ac388	*54:0*	37:17	20.6	*472:1*	275:198	195.7
Ac389	17:15	18:14	NS	59:40	54:45	NS
Ac393	26:8	27:7	NS			
Ac395	37:21	*56:2*	23.7			
Ac397	42:18	33:27	NS			
Ac398	34:18	30:22	NS	102:69	98:73	NS
Ac399	*57:0*	32:25	21.9	*326:2*	198:130	130.8
Ac400	40:28	36:32	NS			
Ac405	27:17	29:15	NS	67:34	60:41	NS
Ac406	*66:13*	49:30	6.24	249:133	232:150	NS
Ac420	32:29	42:19	NS			
Ac422	24:9	*29.4*	6.60			
Ac423	41:16	22:35	NS			
Ac424	36:24	*60.0*	30.8	122:88	*210:0*	114:1
Ac438	37:19	34:22	NS			
Ac441	29:22	31:20	NS			
Ac448	40:19	*56:3*	21.6	87:66	*148:5*	66.8
Ac456	33:26	38:21	NS			
Ac458	42:22	42:22	NS			
Ac461	27:18	21:24	NS			
Ac469	36:19	31:24	NS			
Ac472	38:19	35:22	NS			
Ac478	24:18	21:21	NS			
Ac488	26:19	25.20	NS			
Ac493	33:27	38:22	NS			
Ac494	26:29	20:35	NS			
Ac501	35:24	38:21	NS			
Ac507	18:12	17:13	NS			
Ac511	31:27	38:0	NS	59:42	62:39	NS
Ac522	37:22	*59:0*	30.3	207:92	*299:0*	163.6
Ac530	22:16	*37.1*	15.6	179:93	*271:1*	145.2
Ac540	22:20	22:20	NS			
Ac555	15:21	25:11	NS			
Ac562	42:17	44:15	NS			
Ac563	42:15	*57:0*	29.2	119:54	*173:0*	93.6
Ac568	33:26	33:26	NS			
Ac576	37:23	42:18	NS			
Ac584	25:6	17:14	NS			
Ac615	27:20	24:23	NS			
Ac616	*45:10*	29:26	NS	*184:55*	140:99	7.92
Ac617	*56:0*	33:23	21.4	*321:1*	171:151	131.4
Ac625	25:14	*37.2*	13.6	94:43	*135:2*	67.0
Ac630	31:31	36:26	NS			

[a] $Chi^2_{2\times2}$ values were calculated by comparison with control segregation ratios as described in the Materials and methods. Significantly skewed allele ratios ($P < 0.05$ or $Chi^2 > 3.84$) are italicized. Lines with no significant linkage to either marker are indicated NS. Lines with a significant linkage to others markers tested (*an4*, *po*, *ph2*, data not shown) are also indicated

Mapping T-DNA inserts to other chromosomes

The mapping scheme described in Fig. 1 in which either M1 or M59 is used as the recurrent parent allows mapping to markers on the two largest chromosomes of petunia; *Hf1* (I) and *Fl* (II). It was possible to test linkage of T-DNA loci to additional markers on distinct chromosomes using V26 as the recurrent parent as this line is recessive for *Ph2* (IV), *Po* (V) and *An4* (VII). A segregation analysis was carried out for these markers for 78 of the lines described in Table 1A. A Chi-square test was used to establish significant linkages by the same criteria as those used for *Hf1* and *Fl*,

Table 2A Summary of linkage data for KAN^R transformants that reveal a significant linkage[a] to either *Hfl* or *Fl* in Table 1A

Hfl-linked lines	*Hfl:hfl* total	Map distance[b] (cM)
Ac008	211:4	2.4
Ac011	192:9	5.8
Ac015	106:28	27.2
Ac027	232:3	1.7
Ac029	65:8	14.2
Ac032	89:4	5.6
Ac046	131:13	11.7
Ac048	141:2	1.8
Ac052	129:3	3.0
Ac073	301:5	2.1
Ac095	217:29	15.3
Ac107	200:5	3.2
Ac114	251:1	0.5
Ac119	279:1	0.5
Ac124	142:29	22.0
Ac163	127:10	9.5
Ac171	92:0	0.0
Ac208	100:8	9.6
Fl-linked lines	*Fl:fl* total	Map distance (cM)
Ac009	191:4	2.2
Ac012	193:6	3.2
Ac014	39:2	5.2
Ac021	175:0	0.0
Ac080	188:1	0.6
Ac083	256:11	4.4
Ac112	69:7	9.9
Ac118	364:1	0.3
Ac128	200:0	0.0
Ac129	143:0	0.0
Ac145	124:1	0.9
Ac158	41:5	11.6
Ac169	86:24	23.3
Ac172	68:0	0.0

Table 2B Summary of linkage data for HYG^R transformants that reveal a significant linkage[a] to either *Hfl* or *Fl* in Table 1B

Hfl-linked	*Hfl:hfl* total	Control[c]	Map distance[b] (cM)
Ac332	273:0	53:41	0.0
Ac388	472:1	85:56	0.3
Ac399	326:2	63:33	0.8
Ac451	134:34	N.D.	26.3
Ac616	184:55	N.D.	29.9
Ac517	321:1	60:50	0.4
Fl-linked	*Fl:fl* total	Control	Map distance (cM)
Ac352	128:28	57:42	20.3
Ac395	56:2	56:54	0.4
Ac422	29:4	N.D.	13.7
Ac424	210:0	58:52	0.0
Ac448	148:5	N.D.	3.7
Ac522	299:0	55:45	0.0
Ac530	272:1	55:42	0.4
Ac563	173:0	N.D.	0.0
Ac625	135:2	N.D.	1.6

[a] Significantly linked lines are those that gave a significantly skewed allele ratio in the mapping backcross described in Fig. 1. Only lines with that gave a significant Chi square of 6.63 or more ($P<0.01$) are summarized from Tables 1A (in 2A) and 1B (in 2B)

[b] The map distances were calculated from the recombination fraction and adjusted as follows to take account of the skewed transmission observed in controls (see Materials and methods section). Thus, distances in Table 2A were multiplied by factors of 1.30 for *Hfl* linkages or 1.07 for *Fl* linkages. Likewise, distances in Table 2B were multiplied by factors of 1.30 for *Hfl* linkages or 1.13 for *Fl* linkages

[c] For certain HYG^R lines a control sowing of unselected seed was scored to ensure normal segregation of linked alleles in the absence of selection (N.D.=not determined). However, these data were insufficient to act as a control for all linkage tests

and the significant linkages that resulted are indicated in Table 1A. When the significant linkages for all markers were summarized, it was possible to determine the proportion of inserts mapped to each chromosome (Table 3A). Since each marker identifies a unique linkage group in petunia it is possible to compare the observed number of linked lines with the maximum expected number based on the approximate physical size of the chromosome (Smith et al. 1973). None of the markers tested appear to have significantly more linkages than expected based on chromosome size, although several approach the theoretical maximum assuming all T-DNAs on a given chromosome show linkage to the marker gene.

The data collected with the *Hfl*, *Fl*, *Ph2*, *Po* and *An4* markers did not establish the chromosomal location of 37 (or possibly only 31) of the 84 KAN^R loci tested. The stringency with which the linkage tests can establish linkage to the markers employed at the 1% level of significance varied from one population to another because population sizes were variable. Also the severe skewing of *Hfl:hfl* (2:1 in controls) would tend to obscure linkage, especially in small populations. Thus, some of the 37 (or 31) unmapped loci could map to sites loosely linked to one of the markers. Further analysis would be required with larger populations, and probably additional markers, to determine whether any chromosome is more or less likely to carry T-DNA inserts than would be expected on the basis of physical or genetic size.

An inbred recombination assay

One possible explanation for the extremely tight linkages observed in the above mapping schemes is that recombination around the marker loci was suppressed in the F_1 hybrid. In an attempt to measure recombination in an inbred situation, a novel scheme for mapping T-DNAs with respect to each other was devised (Fig. 2A). The method requires that homozygous stocks are available for 2 (or more) lines that carry dominant introduced markers (e.g. KAN^R) located in the same linkage group. In the example shown, 2 homozygous lines are crossed to give a doubly hemizygous F_1 hybrid. Recombination between the transgenes was detected by the appearance of kanamycin-sensitive seedlings on outcrossing to an untransformed line.

Table 3A Summary of 49 KAN^R loci showing significant linkage to markers on five distinct petunia chromosomes

Marker gene	Significant[a]	Possible[b]	Proportion[c]
Hf1 (I)	18	2	21.4% (23.8%)
Fl (II)	14	4	16.7% (21.4%)
Ph2 (IV)	6	–	7.7%
Po (V)	4	–	5.1%
An4 (VII)	7	–	9.0%
Unlinked	35	29	40.1% (33.0%)

Table 3B Summary of 15 HYG^R loci linked to markers on two distinct petunia chromosomes

Marker gene	Significant[a]	Proportion
Hf1	6	11.8%
Fl	9	17.6%
Unlinked	36	70.6%

[a] Significant linkages are those at the 1% significance level (Chi^2>6.63) from Tables 1A (summarized in Table 3A) and Table 1B (summarized in Table 3B)

[b] Possible linkages are those borderline cases significant at the 1–5% level of significance (Ac85, Ac135 and Ac155) and those with problematical datasets (Ac17, Ac102 and Ac222)

[c] The proportion showing linkage to *Hf1* or *Fl* is based on mapping data for all 84 KAN^R loci using the M1 backcross (Fig. 1). The proportion showing linkage to *Ph2*, *Po* or *An4* is based on a subset of 78 KAN^R loci that were mapped using the V26 backcross (see text for details)

In order to test the suitability of our available KAN^R homozygous lines for this analysis, each was crossed to an untransformed V26 stock and plated on kanamycin to test the penetrance of the KAN^R marker (Table 4). Three *Hf1*-linked lines (Ac11, Ac46 and Ac163) were selected that were loosely linked to *Hf1* in the hybrid assay (5.8–11.7 cM). All 3 lines were shown to be highly penetrant (>99%) with only a single "sensitive" seedling scored. Similarly, 3 *Fl*-linked lines (Ac9, Ac80 and Ac118) that were rather more tightly linked (0.3–2.2 cM) were tested. All 3 lines again appeared to be highly penetrant (100%), although the population for Ac118 was small. Intercrosses were then made between the 3 *Hf1*-linked homozygous lines, as shown in Table 5. Similarly, intercrosses were made between the 3 *Fl*-linked lines. As a control for two unlinked T-DNAs, Ac9 (*Fl*-linked) was crossed to Ac11 (*Hf1*-linked). The F_1 hybrid seed was plated on kanamycin and, as anticipated, almost all progeny were resistant (Table 5; 2nd column). F_1 selections were grown up and crossed as females to a sensitive tester line (V26) to provide seed for the recombination assay; the results are also presented in Table 5 (3rd column). The control cross between an *Hf1*-linked line and an *Fl*-linked line (Ac9×Ac11) gave approximately the 3:1 ratio expected for independent segregation of two dominant markers. Surprisingly, several of the testcrosses also gave approximately 3:1 ratios. Only 2 marker pairs (Ac163 and Ac46 for the *Hf1* linkage group and Ac80 and Ac118 for the *Fl* linkage group) gave significantly fewer than 25% sensitive seedlings. Thus, for each linkage group one of the markers (Ac11 for *Hf1* and Ac9 for *Fl*) was behaving essentially as an unlinked marker.

The fraction of sensitive seedlings can be converted to a map distance (in cM) between T-DNAs by multiplying by a factor of 2 to account for the doubly resistant class of recombinants not detected in the assay (Fig. 2A). The observed map distance between the KAN^R markers (cM obs, Table 5; 3rd column) was consistently higher than the maximum map distance expected (cM exp, Table 5; 4th column) based on the hybrid mapping data. This map expansion appears to be less dramatic for the *Hf1*-linked lines (1.0–3.5 fold) than for the *Fl* linkage group (12.2–21.8 fold). This may reflect the more loosely linked lines that were used for *Hf1* than for *Fl*, and a comparable expansion for more tightly linked lines cannot be excluded. These map expansions are almost certainly underestimates since the map distance for markers that appear unlinked cannot be accurately determined. Moreover, the worst case scenario for hybrid distances was used, with T-DNAs assumed to flank either side of the phenotypic marker gene. The most reliable estimate of map expansion is probably between Ac80 and Ac118, which were 0.6 and 0.3 cM from *Fl* on the hybrid map. These T-DNA inserts showed a separation of 11 cM on the inbred map suggesting at least a 12-fold expansion of the map in this region.

One potential drawback of the above scheme is that one or both transgene loci could be silenced (for recent reviews see Jorgensen 1992; Matzke and Matzke 1993). The silencing of either transgene locus in the F_1 hybrid would result in kanamycin-sensitive progeny that could not be distinguished from recombinants. To circumvent this problem we adopted an alternative strategy of scoring doubly resistant recombinant progeny by utilizing loci with distinct marker phenotypes, i.e. KAN^R and HYG^R. To evaluate this approach we chose 3 KAN^R loci linked to *Fl* for crossing to a HYG^R locus also linked to *Fl*. An F_1 hybrid was made between homozygous KAN^R and HYG^R stocks as shown in Fig. 2B. The resulting double hemizygote was outcrossed to V26, and linkage was measured by the frequency of doubly resistant outcross progeny as shown. As a control, the F_1 seed populations were tested and shown to be doubly resistant, and so both loci were fully penetrant (Table 6; 2nd column). To calculate the recombination frequency, the proportion of doubly resistant seedlings in the outcross was multiplied by 2 to produce the linkage dis-

Table 4 Penetrance of KAN^R marker for lines selected for inbred mapping study based on an outcross of homozygous stocks to a sensitive tester line

Ac line	Linkage [a]	KAN^R	KAN^S	Penetrance
Ac11	*Hf1* (5.8 cM)	302	0	100%
Ac46	*Hf1* (11.7 cM)	422	0	100%
Ac163	*Hf1* (9.5 cM)	480	1	99.8%
Ac9	*Fl* (2.2 cM)	256	0	100%
Ac80	*Fl* (0.6 cM)	218	0	100%
Ac118	*Fl* (0.3 cM)	16	0	(100%)

[a] Linked marker and linkage distance based on data in Table 2A

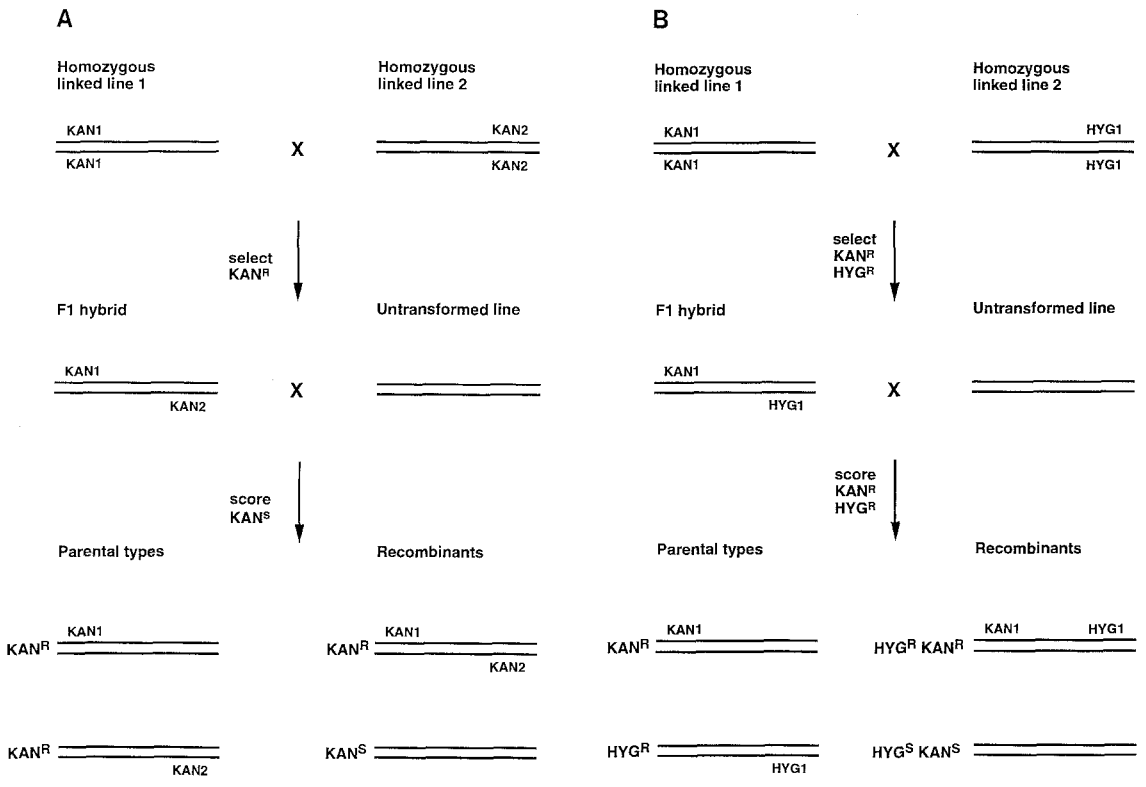

Fig. 2A, B Two alternative schemes for measuring recombination between transgene markers in an inbred line. Two lines homozygous for distinct T-DNAs that have previously been placed in the same linkage group are crossed as shown. All F_1 progeny will carry one copy of each T-DNA (denoted KAN1 and KAN2 for kanamycin-resistant loci and HYG1 for a hygromycin-resistant locus) at distinct sites on the respective homologues. By crossing the F_1 progeny to an untransformed line it is possible to assay recombination in the KAN1–KAN2 interval by assaying for KAN^S seedlings (**A**) or in the KAN1–HYG1 interval by scoring for doubly resistant (i.e. HYG^R+ KAN^R) seedlings (**B**). In scheme **A** sensitive seedlings can only result from crossovers in the KAN1–KAN2 interval. Since the reciprocal recombination event will be resistant and therefore indistinguishable from the parental chromosomes, the actual recombination frequency will be twice the frequency of sensitive seedlings. Similarly in scheme **B** the actual recombination frequency will be twice the observed frequency of doubly resistant seedlings

tance, presented as cM observed in Table 6. These distances can be compared with the maximum distance predicted by the hybrid mapping data (taken from Table 2A and B). In each case, the linkage distances derived from intercrosses between transformants were greater than the maximum expected distances from the hybrid-based mapping, and the expansion in map distances was of a similar magnitude (4–28 fold) to that found with the recombination assay based on kanamycin sensitivity.

Discussion

Among 135 *Agrobacterium* T-DNA loci introduced into petunia, at least 47 were shown to be significantly linked to either *Hf1* or *Fl* (markers for chromosomes I and II). Of these linked T-DNAs, a highly disproportionate number were found to be very tightly linked; 7 of the 23 loci on chromosome I mapped within 1 cM of *Hf1*, whilst 12 of the 22 loci on chromosome II mapped within 1 cM of *Fl* (Table 2). Thus, 19 of the linked loci (40.4%) are located in two distinct 2-cM genetic intervals. If random T-DNA integration with respect to the genetic map is assumed, these data might suggest a total genetic length for the petunia genome of only 28 cM. Obviously, the petunia genetic map must be much larger than this because a minimum of one genetic exchange per chromosome pair is required for normal disjunction (i.e. one would expect a minimum size of 350 cM). Therefore, we conclude that T-DNA integration must be substantially non-random with respect to the recombination map in petunia. Two possible explanations for this are: (1) there are hotspots for T-DNA integration near the marker loci or (2) the region surrounding the markers have suppressed recombination compared to the genome as a whole. The second possibility is favoured by two independent lines of evidence presented in this paper.

Firstly, the data presented in Table 3 indicates that there is no significant bias for integration into any one particular chromosome. These findings confirm and extend similar results in petunia (Wallroth et al. 1986) and tomato (Chyi et al. 1986) that argue for integration of T-DNA apparently at random throughout the genome. More recently, the application of inverse polymerase chain reaction (PCR) has enabled 37 T-DNA inserts to be placed on the tomato restriction fragment length polymorphism (RFLP) map, again apparently at random with respect to the chromo-

Table 5 Inbred recombination assay between KAN^R markers in the same linkage group based on an outcross of F_1 hybrids to a sensitive tester line (see Fig. 2A for details)

Cross	F_1 (R:S)[a]	$F_1 \times$ V26 (R:S)[b]	cM obs[c]	Max cM exp[d]
Hf1-linked × *Hf1*-linked intercrosses				
Ac11 × Ac46	210:0	132:42	48.3	17.5
Ac11 × Ac163	132:2	104:38	53.5	15.3
Ac163 × Ac46	249:0	588:69	21.0	21.2
Fl-linked × *Fl* -linked intercrosses				
Ac9 × Ac80	152:1	184:58	47.9	2.8
Ac9 × Ac118	189:1	115:43	54.4	2.5
Ac80 × Ac118	214:1	411:24	11.0	0.9
Fl-linked × *Hf1*-linked intercross (control)				
Ac9 × Ac11	112:0	316:87	43.2	"50.0"

[a] Homozygous stocks of KAN^R lines for which the T-DNA was linked to either *Hf1* or *Fl* were crossed as indicated, and the F_1 seed was plated on kanamycin and scored for resistance (R) versus sensitivity (S)

[b] KAN^R F_1 plants (doubly hemizygous) were grown, and crossed as females to a sensitive tester line (V26) and scored for kanamycin resistance. Sensitive seedlings can only result from recombination between the linked transgenes as shown in Fig. 2A

[c] Observed recombination between transgenes was calculated from the fraction of sensitive seedlings in the previous column. The recombination estimate is expressed as a map distance in cM taking account of the fact that only 50% of recombination events are detected (see Fig. 2A)

[d] For comparison the maximum expected distances based on the hybrid mapping data were calculated assuming that the KAN^R markers are on opposite sides of the phenotypic marker. The expected distances would be considerably less if both KAN^R markers are on the same side of the phenotypic marker

Table 6 Inbred recombination assay by scoring doubly resistant seedlings derived from F_1 hybrids between KAN^R and HYG^R markers in the same linkage group (II)

Cross (KAN^R/HYG^R)	F_1 (R:S)[a]	$F^1 \times$V26[b]	cM obs[c]	cM exp[d]
Ac9 × Ac424	90:0	7:145	9.2 cM	2.2 cM
Ac80 × Ac424	96:0	5:212	4.6 cM	0.6 cM
Ac118 × Ac424	101:0	9:197	8.6 cM	0.3 cM

[a] Crosses were made between homozygous lines for 3 different KAN^R loci and 1 HYG^R locus all linked to the same phenotypic marker, *Fl* on chromosome II. F_1 seed was plated on double-selection plates containing 15 ug/ml hygromycin and 100 ug/ml kanamycin and scored for double resistance by the emergence of true leaves. In control platings neither singly HYG^R or KAN^R lines were able to produce true leaves under double-selection conditions

[b] HYG^R KAN^R F_1 plants were crossed as females to a sensitive tester (V26) and scored for double resistance as above. Only recombinant seedlings should carry both resistance markers, as shown in Fig. 2B

[c] The observed distance is based on twice the frequency of doubly resistant seedlings since the doubly sensitive class of recombinants will not be scored (Fig. 2B)

[d] The expected distance is the maximum possible based on the sum of hybrid map distances in Table 2, (i.e. assuming the markers to be on opposite sides of the phenotypic marker)

somes (Thomas et al. 1994). Neither the data in Table 3 nor these other studies test adequately the possibility of non-uniform integration within a chromosome (i.e., hotspots within each of the chromosomes). Nonetheless, because of the large number of transformants studied here, the data constrains the magnitude of any chromosomal bias to a greater degree than do the previous studies.

Secondly, we present evidence for a suppression of recombination in the region surrounding each marker locus. This was provided by an approach based entirely on selectable markers in an inbred genotype. This method directly assesses recombination between T-DNA loci integrated independently into the same chromosome of an inbred line. Analysis of 3 T-DNA loci around *Hf1* demonstrated that two intervals measured to be 15.3 and 17.5 cM in crosses between two distinct inbred lines (the transformation host V26 and the tester line M1) were expanded to at least 50 cM (i.e. unlinked) in crosses within a single inbred line (V26). The situation for 3 *Fl*-linked T-DNA loci was even more extreme such that markers previously mapped to a 0.9-cM interval were found to span at least 11 cM in the inbred map. Thus, these data indicate that the level of recombination in the vicinity of *Hf1* may be at least 3-fold higher, and in the vicinity of *Fl* 12-fold higher, within V26 as compared to a V26/M1 hybrid. Though these data could be influenced by some degree of gene silencing, this problem does not pertain to the data obtained by the double resistance assay (Table 6), which indicate a similar degree of map expansion neighbouring the *Fl* locus.

The results presented here confirm some unusual aspects of recombination in petunia that were suggested by previous linkage studies. Clusters of loci that exhibit low levels of recombination are common in the petunia genetic map (Cornu et al. 1989). In many such clusters, recombination distances vary widely depending on the parents employed (Cornu et al. 1980). The causes of these variations could be either structural incompatibilities between homologues or unlinked genetic factors that regulate recombination frequency throughout the genome. A locus called *Rm1* has been proposed to enhance recombination in most petunia chromosomes (Cornu et al. 1989), including those studied here (I and II). It is suggested that *Rm1* is a semidominant enhancer of recombination that gives intermediate levels of recombination in a *Rm1/rm1* heterozygote

(Robert et al. 1991). In our results, recombination is higher in the inbred (V26) than in the hybrid (V26/M1) and so if V26 carries *Rm1*, and M1 does not, then the hybrid would be expected to show less recombination than the inbred, which was the situation observed. In the study of Robert et al. (1991) the *Rm1/rm1* heterozygote showed no less than half the recombination of the *Rm1/Rm1* homozygote, which seems inconsistent with the 12-fold suppression of recombination observed for the *Fl* interval in this study. Therefore, the presence or absence of the *Rm1* factor alone does not appear to fully explain the effects of genetic background on recombination observed here.

The origin of cultivated *P. hybrida* is believed to have involved hybridization between two or more wild species of the genus *Petunia* (Sink 1984). Molecular evolutionary studies of the chalcone synthase gene family demonstrate that much of the diversity of the genus *Petunia* at these loci has been retained in modern *P. hybrida* (Koes et al. 1987). Thus, a cross between any two inbred lines could bring together two homologues derived from two different wild species. If so, structural differences between the parental genomes could be sufficient to inhibit recombination. In the case of chromosomes I and II in the genotypes studied here, this interpretation would predict that the V26 chromosomes derive from a different species than their homologues in M1 and M59[2]. Recently, a collection of random amplified polymorphic DNA (RAPD) markers were used to determine the relative contribution of the parental wild species to the chromosomes of *P. hybrida* (Peltier et al. 1994). It appears that chromosome blocks have been derived intact from the wild species of *Petunia* that are believed to have been used by plant hybridizers in the mid-nineteenth century to create *P. hybrida*. In addition, there is evidence that recombinant chromosomes have appeared, and so modern genotypes are comprised of alternating segments derived from the parental species. The conservation of chromosomal blocks of linked polymorphic markers over the albeit brief history of *P. hybrida* is consistent with the idea that little or no recombination is occurring over large segments of the petunia genome when maintained as wide hybrids.

The results presented here have significant implications for the construction of both classical and molecular genetic maps in petunia. This could influence the design of either a directed transposon tagging scheme or, alternatively, any map-based cloning scheme. Both schemes require the identification of tight linkage between the target locus and either a transposable element or a flanking set of RFLP markers. As we have shown, linkage testing in a petunia hybrid may result in apparent tight linkages while the recombination distance in the inbred background could be very large. Clearly, schemes that involve mapping in an inbred background, either with induced mutants or selectable markers as described here, would have considerable advantages over schemes involving wide hybrids.

The extent to which this is also a problem with RFLP-based mapping schemes in general is uncertain. However, as RFLPs by their nature require divergent chromosomes, this should be considered a possibility. For example, the tomato RFLP map is based on a single interspecific hybrid (Bernatzky and Tanksley 1986), and yet it is clear that domains introgressed from wild species can have a suppressive effect on recombination (Rick 1969). Nonetheless, the total hybrid map length in tomato, 1276 cM (Tanksley et al. 1992), is significantly greater than that suggested for petunia here and in previous work. This suggests that the effects of chromosome divergence are probably less extreme in tomato than in petunia but may nevertheless be locally significant.

Acknowledgements The authors would like to express their gratitude to C. Lemieux and C. Napoli for generating the transformants discussed in this paper. In addition, we thank M. Jenkin, N. Rethmeier and S. Harding for help with the seedling selections and flower scoring. For greenhouse care of the plants we are indebted to T. Lemieux and C. Velton. We also thank H. Dooner and N. Courtney-Gutterson for comments on the manuscript. This research was supported by a contract from Florigene BV, Rijnsburg, The Netherlands.

[2] It is interesting to note that the recessive *fl* allele is found in the purple-flowered species (*P. violacea*, *P. inflata*) whereas the dominant *Fl* allele is found in one of the white-flowered species (*P. parodii*). This is consistent with the notion that in a V26/M1 or V26/M59 hybrid which is heterozygous for *Fl*, the chromosome II region around *Fl* may therefore differ in parental origin

References

Ambros PF, Matzke AJM, Matzke MA (1986) Localization of *Agrobacterium rhizogenes* T-DNA in plant chromosomes by *in situ* hybridization. EMBO J 5:2073–2077

Bernatzky R, Tanksley SD (1986) Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887–898

Chyi Y-S, Jorgensen RA, Goldstein D, Tanksley SD, Loaiza-Figueroa F (1986) Locations and stability of *Agrobacterium*-mediated T-DNA insertions in the *Lycopersicon* genome. Mol Gen Genet 204:64–69

Cornu A, Maizonnier D, Wiering H, de Vlaming P (1980) Petunia genetics. III. – The linkage group of chromosome V. Ann Amelior Plant 30(4):443–454

Cornu A, Farcy E, Mousset C (1989) A genetic basis for variations in meiotic recombination in *Petunia hybrida*. Genome 32:46–53

Deroles SC, Gardiner RC (1986) Expression and inheritance of kanamycin-resistance in a large number of transgenic petunias generated by *Agrobacterium* mediated transformation. Plant Mol Biol 11:355–364

Feldmann KA, Marks MD (1987) *Agrobacterium*-mediated transformation of germinating seeds of *Arabidopsis thaliana*: A non-tissue culture approach. Mol Gen Genet 208:1–9

Horsch RB, Fry JE, Hoffmann NL, Eichholtz D, Rogers SG, Fraley RT (1985) A simple and general method of transferring genes into plants. Science 227:1229–31

Jones JDG, Carland FM, Maliga P, Dooner HK (1989) Visual detection of the maize element *Activator* (*Ac*) in tobacco seedlings. Science 244:204–207

Jorgensen R (1992) Silencing of plant genes by homologous transgenes. Ag Biotechnol News Info 4:265–273

Keller J, Lim E, James DW, Dooner HK (1992) Germinal and somatic activity of the maize element *Activator* (*Ac*) in Arabidopsis. Genetics 131:449–459

Koes RE, Spelt CE, Mol JNM, Gerats AGM (1987) The chalcone synthase family of *Petunia hybrida* (V30): sequence homology, chromosomal localization and evolutionary aspects. Plant Mol Biol 10:159–169

Maizonnier D, Cornu A (1971) A telocentric translocation responsible for variegation in Petunia. Genetica 42:422–436

Matzke MA, Matzke AJM (1993) Genomic imprinting in plants: parental effects and *trans*-inactivation phenomena. Ann Rev Plant Physiology and Plant Mol Biol 44:53–76

Napoli C, Lemieux C, Jorgensen R (1990) Introduction of a chimeric chalcone synthase gene into petunia results in reversible cosuppression of homologous genes *in trans*. Plant Cell 2:279–289

Peltier D, Farcy E, Dulieu H, Berville A (1994) Origin, distribution and mapping of RAPD markers from wild *Petunia* species in *Petunia hybrida* Hort lines. Theor Appl Genet 88:637–645

Rick CM (1969) Controlled introgression of chromosomes of *Solanum pennellii* into *Lycopersicon esculentum*: segregation and recombination. Genetics 62:753–768

Robert N, Farcy E, Cornu A (1991) Genetic control of meiotic recombination in *Petunia hybrida*: dosage effect of gene *Rml* on segments *Hfl-Lgl* and *An2-Rt*; role of modifiers. Genome 34:515–523

Sink K (1984) Taxonomy. In: Sink KC (ed) Petunia. Springer, Berlin, Heidelberg, New York, pp 3–9

Smith FJ, Oud JL, Jong JH de (1973) A standard karyogram of *Petunia hybrida*. Hortic Genet 44:474–484

Tanksley SD, Ganal MW, Prince JP, de Vicente, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, Young ND (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Thomas CM, Jones DA, English JJ, Carroll BJ, Bennetzen JL, Harrison K, Burbridge A, Jones JDG (1994) Analysis of the chromosomal distribution of transposon-carrying T-DNAs in tomato using the inverse polymerase chain reaction. Mol Gen Genet 242:573–585

Wallroth M, Gerats AGM, Rogers SG, Fraley RT, Horsch RB (1986) Chromosomal localization of foreign genes in *Petunia hybrida*. Mol Gen Genet 202:6–15

Wiering H, de Vlaming P (1984) Genetics of flower and pollen colors. In: Sink KC (ed) Petunia. Springer, Berlin, Heidelberg, New York, pp 49–67