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Germinal excision and reinsertion frequencies of the mobile element *Ds* transposed from two unlinked T-DNA loci in tomato

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Abstract

Acceptor sites of unlinked transposed *Ds* element from two T-DNA loci in tomato were mapped. Experimental data obtained from TC_1 progeny testing were employed for estimation of germinal excision frequency (GEF) of *Ds* element and frequency of its reinsertion (FR). The donor T-DNAs 1481J and 1601D, containing a 35S:*NPT* transformation marker, a 35S:*BAR* or nos:*BAR* excision marker conferring phosphinothricine resistance and a *Ds* element in the 5' untranslated leader of the nos (or 35S):*BAR* gene, were located on chromosome 7 and 8, respectively. *Ds* transposition was induced by 10512I T-DNA carrying stabilized *Ac* (s*Ac*) which provides a source of transposase and 2':*GUS* marker conferring β-glucuronidase activity. Tomato plants harbouring the *Ds* in 1481J or 1601D locus and s*Ac* were crossed and F_1 seedlings heterozygous for both *Ds* and s*Ac* were identified. These plants, 72 with *Ds* in 1481J and 65 with *Ds* in 1601D, were crossed individually as seed parents to wild-type plants to generate TC_1 progenies. TC_1 seed was germinated on phosphinothricine (Basta)-containing medium, and individual seedlings carrying a transposed *Ds* and lacking s*Ac* were identified by PCR (to detect the *Ds*) on phosphinothricine resistant individuals that lacked β-glucuronidase activity. From segregation ratio in TC_1 the germinal excision and reinsertion frequencies of the *Ds* element were estimated for individual F_1 plants. A total of 14560 TC_1 seedlings of 1481J and 16195 TC_1 seedlings of 1601D was analyzed. We observed high variation between individual plants as regards both GEF and FR despite of donor locus (1481J or 1601D), however, the average germinal excision frequencies as well as average frequencies of reinsertion were very similar for both donor loci: $GEF_{1481J} = 24$ %, $GEF_{1601D} = 25$ %, $FR_{1481J} = 42$ %, $FR_{1601D} = 46$ %.

Additional key words: *Lycopersicon esculentum*, phosphinothricine (Basta) resistance, β-glucuronidase, stabilized *Ac*.

Introduction

When studying chromosome breakage in the 1940s, McClintock (1947, 1948) discovered transposable elements *Activator* (*Ac*) and *Dissociation* (*Ds*) in maize. Since then intensive genetic and molecular studies have revealed that transposons are present in prokaryotic and eukaryotic genomes and how mobile elements behave (Shapiro 1995). *Ac/Ds* elements, (Baker *at al.* 1986) have been shown to be active in a heterologous plant species (tobacco). Soon afterwards, *Ac* was shown to be able to transpose in many plants (see review Kunze 1996) and was used for isolation of genes by strategy called transposon tagging (for review see Balcells *at al.* 1991). In these studies, and in numerous others, the characteristic features of transposon behaviour were revealed - among others the frequency of transposition was estimated for variety of different species. There are plant species where *Ac/Ds* elements were shown to be very active (tomato - Yoder 1990, Carroll *et al.* 1995; tobacco - Jones *et al.* 1989, 1991). On the contrary, *Arabidopsis* (Schmidt and Willmitzer 1989), lettuce (Yang *et al.* 1993), flax (Ellis *et al.* 1992), *Petunia* (Robbins *et al.* 1994) and *Nicotiana plumbaginifolia* (Marion-Poll *et al.* 1993) revealed very

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low transposition frequency of *Ac*. In tomato, Carroll *et al.* (1995) found that germinal excision frequencies of *Ds* averaged 15 - 40 % but large variation between and within plants were observed. The authors found that reinsertion frequency of excised *Ds* was 27 - 61 %, this means lower when compared with 50 - 70 % of maize (McClintock 1956, Dooner and Belachew 1989).

In this paper, the data obtained by testing of TC_1 progeny generated within the framework of experimental strategy designed for the generation, recovery and mapping of unlinked transposed *Ds* elements from two T-DNA loci are used for estimation of germinal excision and reinsertion frequencies. High variation between individual plants despite of donor locus was observed both for germinal excision and reinsertion frequency. However, the average values of both frequencies were very similar for both donor loci.

Materials and methods

Plants harbouring T-DNAs with *Ds* or s*Ac* and crossing strategy: The DNA constructs used in this project as well as transgenic tomato lines of variety Moneymaker were designed and described by Carroll *et al.* (1995). Briefly, the T-DNAs harbouring *Ds* element and lying on chromosome 7 and 8, respectively, contained a marker of transformation (35S:*NPT*), a marker of *Ds* excision (35S or *nos:BAR* conferring resistance to Basta) and a *Ds* element in the 5' untranslated leader sequence of the *BAR* gene. To mobilise the *Ds* element, the *Ds* lines were crossed to transgenic line carrying stabilised *Ac* (s*Ac*) as a source of transposase and 2':*GUS* gene coding for β-glucuronidase. Number of plants of F_1 generation, heterozygous for both *Ds*- and s*Ac*-containing T-DNAs (verified by PCR, see Fig. 1), were pollinated with non-transformed plants of the same variety to form TC_1 generation. Each F_1 plant gave rise to one TC_1 population that consisted of about 5 sub-populations derived from individual fruits of the plant.

Selection of Basta^R plants and GUS assay: All TC_1 sub-populations were screened separately to identify the plants expressing *Ds* excision marker (*i.e.* resistant to herbicide Basta) and, at the same time, lacking GUS activity (*i.e.* without s*Ac*). The screening was performed essentially as described elsewhere (Bříza *et al.* 2000). In this way, four classes of the TC_1 seedlings could be identified: Basta^R/GUS$^+$, Basta^R/GUS$^-$, Basta^S/GUS$^+$, and Basta^S/GUS$^-$. Individual seedlings of choice (*i.e.* Basta^R/GUS$^-$) were assayed by PCR for *Ds* (Fig. 2) and the plants harbouring *Ds* (*i.e.* with reinserted tr*Ds*) were recovered for next greenhouse propagation and self-pollination (Bříza *et al.* 2000).

Germinal excision and reinsertion frequency estimations: The estimations were calculated essentially as described by Carroll *et al.* (1995) in individual progenies of F_1 plants. Since *BAR* gene codes for phosphinothricin acetyltransferase that shows noncell autonomous phenotype in tomato (Jones *et al.* 1993), somatic and germinal transpositions cannot be distinguished in plants carrying the transposase gene. Therefore, only progeny lacking the s*Ac* were employed for the germinal excision frequency estimation.

Isolation of genomic DNA and Southern analysis: The method of genomic DNA isolation was essentially as described by Tai and Tanksley (1991). Following that Southern analyses were performed as described by Bříza *et al.* (2000) to determine number of tr*Ds* and uniqueness of transposition events.

Results

After PCR verification of *Ds* and s*Ac* heterozygous constitution of F_1 plants (Fig. 1) all plants (*i.e.* 72 with *Ds* in 1481J locus and 65 with *Ds* in 1601D locus) were pollinated with non-transformed tomato. Seeds from 5 individual fruits harvested from individual F_1 plants were sown for screening separately and number of seedlings in Basta^R/GUS$^+$, Basta^R/GUS$^-$, Basta^S/GUS$^+$, and Basta^S/GUS$^+$ phenotype classes were scored (Table 1 and 2). Since mapping of unlinked transposed *Ds* elements was a final aim of the study only Basta^R seedlings lacking s*Ac*, *i.e.* lacking GUS activity that was used as a histochemical marker for the presence of s*Ac*, were recovered and subjected PCR assay for *Ds*, *i.e.* for reinsertion of excised *Ds* (Fig. 2).

From a total of 16195 TC_1 individual seedlings the average germinal excision frequency (GEF) over the 65 double heterozygotes with *Ds* in 1601D locud was 27 % and reinsertion frequency (FR) was 47 % (Table 1). The average GEF and FR for 1481J locus from 72 heterozygotes was 25 % and 42 %, respectively and these values were derived from 14560 TC_1 individuals (Table 2). Thus, both *Ds*-containing lines revealed very similar average values of both frequencies. However, large variation was detected between individual F_1 plants (Table 1 and 2) as well as between individual fruits on the same plant (data not shown). Differences between F_1 individuals in the GEF ranged from 0 to 89 % and reinsertion frequency varied from 0 to100 %. These data

confirm that transposition activity in tomato is high: only one F_1 plant (No. 104) showed no Basta^R TC_1 progeny even among GUS$^+$ seedlings, and two F_1 plants (Nos. 5 and 71) revealed no Basta^R progeny among GUS$^+$ seedlings. On the contrary, a few F_1 individuals (Nos. 132, 83, 84, 69) gave rise progeny consisting of several tens Basta^R/GUS$^+$ seedlings indicating either high transposition activity during F_1 plant development or excision event in early developmental stage of the F_1 plants. Analysis of one out of such progeny derived from F_1 plant No. 132 is given in Table 3. From data in the table is apparent that even plants originated in the same fruit harboured different transposition events. On the contrary, there were plants derived from different fruits of different fruit bunches that harboured same transposition events. However, F_1 plants were found too that gave rise progeny largely consisting of seedlings harbouring identical transposition - 9 out of 15 Basta^R/GUS$^-$ seedlings derived from F_1 plant No. 23 harbouring unlinked tr*Ds* carried a clonal transposed *Ds* element defined by 3.5 kb *Eco*R I band (data not shown). Totally, we hybridised DNA from 240 TC_2 families and revealed that 12 % out of that harbouring single copy of *Ds* carried the same transposition events.

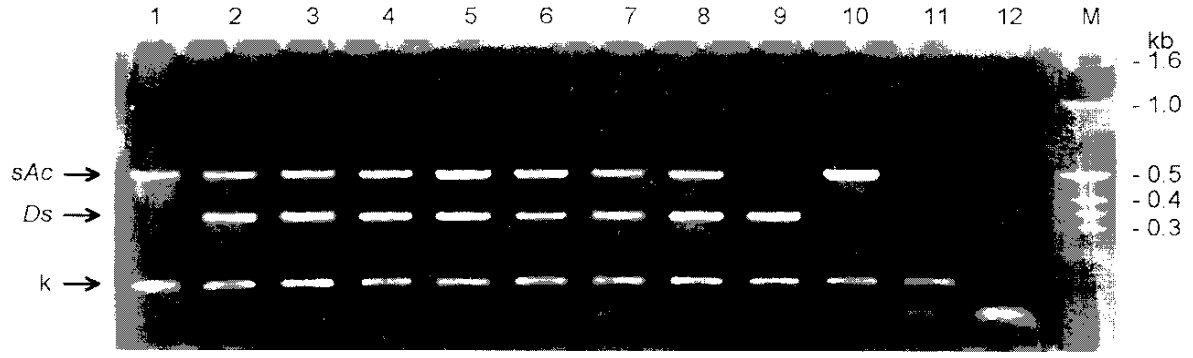

Fig. 1. Example of ethidium bromide stained products of PCR analysis for detection of T-DNAs carrying *Ds* and *sAc* elements in plants of F_1 generation. PCR analysis was carried out using intact leaf or cotyledon tissue as a template as described by Klimyuk *et al.* (1993) and three pairs of oligonucleotide primers were added to each reaction: D60 (5'-GTG ATC CAG ATG TGA AGC AAG-3') and D75 (5'-ACG AAC GGG ATA AAT ACG GTA ATC-3') amplified a 334-bp fragment of the 3' end of *Ds*, JB1 (5'-GCG ACA GCA AAC AGC CCA TGC ATC-3') and JB2 (5'-ACC CCT TTT GAA GCA TAG TGG TCA-3') amplified a 512-bp fragment of the *sAc* element, 2995AL (5'-CGA GAG AGA TTC AAG AAT AGA CCC-3') and 2995AR (5'-TAT AAC CAA ATG CAA CTC CGT CTT-3') amplified a 141-bp fragment from tomato chromosome 11 as a positive control. *Lane 1 - 8*, F_1 individuals; *lane 9*, line 1481J harbouring T-DNA with *Ds*; *lane 10*, line 10512I harbouring T-DNA with *sAc*; *lane 11*, nontransformed tomato; *lane 12*, negative control; *lane M*, marker DNA (1kb ladder, BRL). *sAc*, 512-bp fragment of *sAc*; *Ds*, 334-bp fragment composed of the 3' end of *Ds*; k, 141-bp fragment of chromosome 11 (positive control). It is obvious that F_1 plant in *lane 1* does not contain *Ds* element; therefore, it was discarded from next experiments.

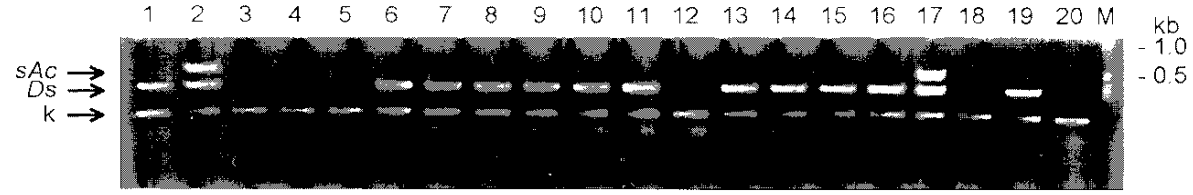

Fig. 2. Example of ethidium bromide stained products of PCR analysis for detection of reinserted *Ds* element and for lacking of *sAc* element in plants of TC_1 generation. For details of PCR and primers used see Figure 1. *Lane 1 - 20*, individual TC_1 plants; *lane M*, marker DNA (1-kb ladder, BRL). *sAc*, 512-bp fragment of *sAc*; *Ds*, 334-bp fragment composed of the 3' end of *Ds*; k, 141-bp fragment of chromosome 11 (positive control). Plants in *lanes 2* and *17* harboured *sAc*; therefore, they were omitted from next experiments.

Discussion

Using *sAc/Ds* system of maize mobile element we estimated the average frequency of germinal excision of *Ds* element from two loci on tomato chromosomes 7 and 8, and we also calculated *Ds* reinsertion frequency. Our findings, mentioned in Tables 1 and 2 and derived from about 31000 TC_1 seedlings, show that average values of

Table 1. Germinal excision (GEF) and reinsertion (FR) frequencies of the *Ds* element in progeny derived from individual F_1 plants harbouring the *Ds* in 1601D locus on chromosome 8.

Plant number of F_1	Segregation in TC_1 GUS$^+$/Basta^R	GUS$^+$/Basta^S	GUS$^-$/Basta^R	GUS /Basta^S	GUS /Basta^R/$_+$ Ds$^+$ plants	GEF [%]	FR [%]
1	29	68	7	86	3	15	43
2	1	65	1	85	1	2	100
3	76	164	39	228	16	29	41
4	21	41	9	53	6	29	67
5	5	147	0	157	0	0	0
6	16	75	4	98	3	8	75
7	36	69	22	88	0	0	0
8	39	92	19	113	9	29	47
9	50	107	19	131	11	25	58
10	45	100	9	142	4	12	44
11	14	69	22	94	11	38	50
12	23	49	13	98	12	23	92
13	15	33	5	47	4	19	80
14	50	77	13	105	5	22	38
15	60	102	27	147	7	31	26
16	31	70	2	102	1	4	50
17	32	71	13	71	6	31	46
18	16	34	2	54	2	7	100
19	31	67	6	93	2	12	33
20	19	41	18	48	12	55	67
21	25	41	13	78	8	29	62
22	43	109	20	137	5	25	25
23	27	84	21	105	16	33	76
24	30	70	16	93	10	29	63
25	21	54	11	101	5	20	45
26	27	87	39	118	3	50	8
27	22	64	8	67	4	21	50
28	52	103	19	135	9	25	47
29	40	95	10	128	3	14	30
30	36	75	8	105	2	14	29
31	25	75	11	105	4	19	36
32	19	53	6	65	6	17	100
33	24	57	4	73	2	10	50
34	49	137	16	157	6	18	38
35	4	40	19	41	2	63	11
36	36	84	16	104	5	27	31
37	22	46	20	56	8	53	40
38	51	107	26	174	19	26	73
111	30	70	17	87	16	33	94
112	20	80	13	86	9	26	69
113	24	71	13	82	3	27	23
114	31	111	11	153	5	13	45
115	52	84	29	155	9	32	31
116	15	67	6	66	2	17	33
117	44	75	15	115	9	23	60
118	57	104	34	142	16	39	47
119	31	83	8	116	4	13	50
120	35	76	6	112	1	10	17
121	40	93	17	152	10	20	59
122	43	24	6	48	6	22	100
123	8	58	4	64	0	12	0
124	70	64	12	119	12	18	100
125	45	52	38	76	30	67	79
126	176	130	33	250	19	23	58
127	83	164	24	216	15	20	63
128	28	66	22	86	16	41	73
129	49	61	25	105	14	38	56
130	52	95	30	148	12	34	40
131	70	128	28	197	9	25	32
132	127	165	93	216	22	60	24
133	53	60	11	105	5	19	45
134	69	89	16	125	9	23	56
135	56	71	37	96	17	56	46
136	31	41	14	80	4	30	29
137	68	135	27	186	16	25	59
1601D	2569	5239	1122	7265	522	27	47

Table 2. Germinal excision (GEF) and reinsertion (FR) frequencies of the *Ds* element in progeny derived from individual F_1 plants harbouring the *Ds* in 1481J locus on chromosome 7.

Plant number of F_1	Segregation in TC_1 $GUS^-/Basta^R$	$GUS^+/Basta^S$	$GUS^+/Basta^R$	$GUS^-/Basta^S$	$GUS^-/Basta^R$/ Ds^+ plants	GEF [%]	FR [%]
39	34	103	8	124	5	12	63
40	33	49	36	66	33	71	92
41	42	65	3	116	2	5	67
42	26	42	5	72	4	13	80
43	11	30	5	33	1	26	20
44	22	67	11	82	5	24	45
45	28	63	13	88	4	26	31
46	33	50	11	82	7	24	64
47	34	63	2	88	2	4	100
48	30	56	8	89	6	16	75
49	60	77	21	102	3	34	14
50	9	23	6	28	5	35	83
51	37	78	10	131	7	14	70
52	31	58	8	65	2	22	25
53	43	75	7	90	7	14	100
54	22	50	16	46	1	52	6
55	35	79	11	94	7	21	64
56	22	79	14	71	4	33	29
57	35	68	14	65	14	35	100
58	44	80	8	115	5	13	63
59	35	58	11	79	7	24	64
60	12	51	3	69	1	8	33
61	29	52	30	80	2	55	7
62	24	75	9	97	4	17	44
63	47	68	11	126	8	16	73
64	42	93	8	153	2	10	25
65	31	74	6	97	3	12	50
66	35	73	11	101	4	20	36
67	49	77	7	117	6	11	86
68	46	74	16	113	9	25	56
69	61	69	63	79	52	89	83
70	27	65	7	90	1	14	14
71	32	72	0	91	0	0	0
72	52	121	22	175	12	22	55
73	37	84	10	121	3	15	30
74	20	48	4	59	1	13	25
75	44	56	6	111	2	10	33
76	18	31	3	55	3	10	100
77	26	43	8	74	2	20	25
78	37	73	13	129	8	18	62
79	23	75	6	110	2	10	33
80	25	45	12	65	5	31	42
81	83	49	26	177	14	26	54
82	46	39	8	105	4	14	50
83	82	30	77	174	1	61	1
84	105	50	68	130	0	69	0
85	58	32	21	172	13	22	62
86	15	17	5	54	3	17	60
87	27	28	11	100	6	20	55
88	42	64	17	123	3	24	18
89	32	56	13	83	5	27	38
90	50	84	35	81	1	60	3
91	70	87	22	135	4	28	18
92	33	105	10	115	4	16	40
93	15	37	2	40	2	10	100
94	7	28	1	17	0	11	0
95	11	24	2	29	1	13	50
96	67	122	18	165	10	20	56
97	17	46	3	55	2	10	67
98	18	48	6	62	3	18	50
99	14	32	7	39	4	30	57
100	3	13	4	14	1	44	25
101	21	74	6	90	2	13	33
102	31	59	3	83	2	7	67
103	32	66	12	93	8	23	67
104	0	50	0	47	0	0	0
105	17	47	8	63	5	23	62
106	15	25	2	44	0	9	0
107	24	43	8	59	6	24	75
108	88	150	40	243	18	28	45
109	49	96	30	94	14	48	47
110	75	90	9	126	6	13	67
1481J	2530	4423	957	6650	403	25	42

both frequencies are, irrespective of donor loci of *Ds* element, almost the same and that both frequencies reveal very high variance among individual plants or even among fruits of the same plant. Since all F_1 plants used in the study harboured s*Ac* in the identical chromosome position and T-DNA with *Ds* were localised in two different loci only (1601D or 1481J) we cannot use the position effect when explaining variability among different plants. In addition, we mentioned high variation among fruits of the same plant. High variability of GEF in tomato was also observed by Carroll *et al.* (1995) and Peterson and Yoder (1995). The latter authors reported high or low activity state of the *Ac* element even the *Ac* lied in the identical chromosomal position and they connected this activity state with amplification of the element - they revealed that amplification of the element is the consequence of its transposition. According to Schwartz and Dennis (1986) methylation of the *Ac* seems to be responsible for this different status of *Ac* element activity.

Among progeny of 137 F_1 plants several progenies (No. 69, 83, 84, 125, 132) were revealed which exhibited high excision activity of *Ds* element. Since estimation of germinal excision and reinsertion frequencies of the *Ds* element was not main aim of the whole project only F_1 progenies harbouring unlinked tr*Ds* were assayed for *Ds* copy number and uniqueness of transposition by Southern analysis. Therefore, results for only one out of high activity *Ds* F_1 progeny could be presented (Table 3). In this progeny, 9 out of 15 families (*i.e.* 60 %) were shown to carry two or more copies of tr*Ds*. From these 9 families 3 and 2 ones harboured same transposition events while remaining 4 families carried independent transpositions. Out of 6 families with single *Ds* copy, 2 families had the same transposition event and 4 harboured tr*Ds*s in unique locations. Conversely to the F_1 plants exhibiting very high transposition activity of *Ds*, a few plants of F_1 generation were shown to exhibit no or very low activity of *Ds* transposition (F_1 plants No. 104, 71 and 5). It is probable that differences observed among GEF of individual F_1 progenies harbouring both *Ds* and transposase source (s*Ac*) in identical chromosomal locations are caused by different degree and specificity of s*Ac* methylation like in *Arabidopsis* (VanSluys *et al.* 1993). However, which factor(s) influence(s) such different methylation pattern in the plants with same genetic background is not clear. According to McClintock (1951) the developmental timing of transposition is controlled during transposition of mobile elements. Levy and Walbot (1990) found that the excision frequency of the *Ds*2 element reached 0.2 - 1 % during proliferation of the aleurone layer in maize. This reflects either temporal fluctuations in transposase expression or the influence of a transposition-modulating host factor. In maize, Heinlein (1995) did not prove the presence of a modifier gene. It suggests that mild changes in transposase expression may result in spatially and temporally characteristic transposition patterns.

Table 3. Analysis of progeny derived from F_1 plant No. 132 ([a] - the same Romanic figure means the same fruit bunch, [b] - Bříza *et al.* (2000). [c,d,e] - the same transposition events).

Family number	Number of Basta^R/GUS plants	Number of Basta^R/GUS *Ds* plants	Number of plants derived from same fruit [a]	Number of families with linked/unlinked tr*Ds* [b]	Number of families with different copy number of unlinked tr*Ds* and size of the *Ds* containing band [kb]			Map position of tr*Ds* in family with 1 copy of *Ds* [b]
					1 copy	2 copies	>2 copies	
132	93	22	9 (I 1)	1/8	3 (2.8, 3.7[c], 3.7[c])	3 (7.5+13, 2.1+4[d], 2.1+4[d])	2 (2.2+3.4+6.5, 2.25+4.8+8)	Ch 12 (family with 3.7-kb band)
			2 (I 2)	0/2	1 (6)	1 (1.9+4)[e]	0	Ch 11
			2 (I 3)	0/2	0	1 (1.9+4)[e]	1 (8.5+10.5+13)	-
			2 (II 2)	0/2	1 (2.5)	1 (2.1+4)[d]	0	no RFLP polymorphism
			2 (III 1)	2/0	0	0	0	-
			3 (III 2)	2/1	1 (4.2)	0	0	Ch 1
			2 (died)	-	-	-	-	-

Average reinsertion frequency of excised *Ds* almost reached identical value for both donor T-DNAs - 46 and 42 %. The findings are consistent with data presented by Carroll *et al.* (1995) for tomato as well as by Dooner and Belachew (1989) for maize. Frequency of reinsertion for progeny of individual F_1 plants also revealed high variation when ranged from 0 to 100 %. It is probable (but not verified by Southern analysis) that progeny of F_1 plants exhibiting very high FR (80 - 100 %) consisted of plants harbouring the same transposition events (for example 30, *i.e.* 91 %, out of 33 TC_1 plants derived from F_1 plant No. 40 or 48, *i.e.* 92 %, out of 52 TC_1 plants

derived from F_1 plant No. 69 showed very closed linkage between donor T-DNA and tr*Ds*). Similarly when FR is 0 we can suppose that all such Basta^R/GUS plants originated from single transposition events without following *Ds* reinsertion (for example see progeny of F_1 plant No. 84 where none out of 68 Basta^R/GUS TC_1 plants carried *Ds*).

The data shown in this study suggest that, in some cases, high values of germinal excision frequency resulted from early transposition events (F_1 plant No. 23 for example) but in other cases, the high GEF is a consequence of high transposition activity *Ds* during F_1 plant development when many independent transposition events generate many new independent *Ds* locations in genome. In addition, in some cases transposition gives rise to multiple transposed *Ds* element in individual plants which is in agreement with findings that transposition of *Ac/Ds* from replicated to unreplicated DNA makes for increasing of copy number of DNA mobile elements (Greenblatt 1984, Chen *et al.* 1992).

In conclusion, for the use and prospects of transposon tagging of genes of interest in tomato is important that transposition activity of maize *Ac/Ds* system is very high in this species, and at the same time that sibling progeny frequently harbour unique transposition events.

References

Baker, B., Schell, J., Loerz, H., Fedoroff N.: Transposition of the maize controlling element "*Activator*" in tobacco. - Proc. nat. Acad. Sci. USA **83**: 4844-4848, 1986.

Balcells, L., Swinburne, J., Coupland, G.: Transposons as tools for the isolation of plant genes. - TIBTECH **9**: 31-31, 1991.

Bříza, J., Niedermeierová, H., Pavingerová, D., Thomas, C.M., Klimyuk, V.I., Jones, J.D.G.: Transposition patterns of unlinked transposed *Ds* elements from two T-DNA loci on tomato chromosomes 7 and 8. - Plant J., in press, 2000.

Carroll, B.J., Klimyuk, V.I., Thomas, C.M., Bishop, G.J., Harrison, K., Scofield, S.R., Jones, J.D.G.: Germinal transpositions of the maize element *Dissociation* from T-DNA loci in tomato. - Genetics **139**: 407-420, 1995.

Chen, J., Greenblatt, I.M., Dellaporta, S.L.: Molecular analysis of *Ac* transposition and DNA replication. - Genetics **130**: 665-676, 1992.

Dooner, H.K., Belachew, A.: Transposition pattern of the maize element *Ac* from the *bz-m2(Ac)* allele. - Genetics **122**: 447-457, 1989.

Ellis, J.G., Finnegan, E.J., Lawrence, G.J.: Developing a transposon tagging system to isolate rust-resistance genes from flax. - Theor. appl. Genet. **85**: 46-54, 1992.

Greenblatt, I.M.: A chromosome replication pattern deduced from pericarp phenotypes resulting from movements of the transposable element, *Modulator*, in maize. - Genetics **108**: 471-485, 1984.

Heinlein, M.: Variegation patterns caused by excision of the maize transposable element *Dissociation* (*Ds*) are autonomously regulated by allele-specific *Activator* (*Ac*) elements and are not due to *trans*-acting modifier genes. - Mol. gen. Genet. **246**: 1-9, 1995.

Jones, J.D.G., Carland, F.M., Maliga, P. Dooner, H.K.: Visual detection of transposition of the maize element *Activator* (*Ac*) in tobacco seedlings. - Science **244**: 204-207, 1989.

Jones J.D.G., Harper, L., Carland, F.M., Ralston E.J., Dooner, H.K.: Reversion and altered variegation of an SPT:*Ac* allele in tobacco. - Maydica **36**: 329-335, 1991.

Jones, J.D.G., Jones, D.A., Bishop, G.J., Harrison, K., Carroll, B.J., Scofield, S.R.: Use of the maize transposons *Activator* and *Dissociation* to show that phosphinothricin and spectinomycin resistance genes act non-cell-autonomously in tobacco and tomato seedlings. - Transgen. Res. **2**: 63-78, 1993.

Klimyuk, V.I., Carroll, B.J., Thomas, C.M., Jones, J.D.G.: Alkali treatment for rapid preparation of plant tissue for reliable PCR analysis. - Plant J. **3**: 493-494, 1993.

Kunze, R.: The maize transposable element *Activator* (*Ac*). - In: Saedler, H., Gierl, A. (ed.): Transposable Elements. Pp. 161-194. Springer, Heidelberg 1996.

Levy, A.A., Walbot, V.: Regulation of the timing of transposable element excision during maize development. - Science **248**: 1534-1537, 1990.

Marion-Poll, A., Martin, E., Bonnefoy, N., Pautot, V.: Transposition of the maize autonomous element *Activator* in transgenic *Nicotiana plumbaginifolia* plants. - Mol. gen. Genet. **238**: 209-217, 1993.

McClintock, B.: Cytogenetic studies of maize and *Neurospora*. - Carnegie Inst. Wash. Year Book **46**: 146-152, 1947.

McClintock, B.: Mutable loci in maize. - Carnegie Inst. Wash. Year Book **47**: 155-169, 1948.

McClintock, B.: Chromosome organization and genic expression. - Cold Spring Harbor Symp. Quant. Biol. **16**: 13-47, 1951.

McClintock, B.:Controlling elements and the gene. - Cold Spring Harbor Symp. Quant. Biol. **21**: 197-216, 1956.

Peterson, P.W., Yoder J.I.: Amplification of *Ac* in tomato is correlated with high *Ac* transposition activity. - Genome **38**: 265-276, 1995.

Robbins, T.P., Jenkin, M., Courtney-Gutterson, N.: Enhanced frequency of transposition of the maize transposable element *Activator* following excision from T-DNA in *Petunia hybrida*. - Mol. gen. Genet. **244**: 491-500, 1994.

Schwartz, D., Dennis, E.: Transposase activity of the *Ac* controlling element in maize is regulated by its degree of methylation. - Mol. gen. Genet. **205**: 476-482, 1986.

Shapiro, J.A.: The discovery and significance of mobile genetic elements. - In: Sherratt, D.J. (ed.): Mobile Genetic Elements. Pp. 1-17. IRL Press and Oxford University Press, New York 1995.

Schmidt, R. Willmitzer, L.: The maize autonomous element *Activator* (*Ac*) shows a minimal germinal excision frequency of 0.2 % - 0.5 % in transgenic *Arabidopsis thaliana* plants. - Mol. gen. Genet. **220**: 17-24, 1989.

Tai, T., Tanksley, S.: A rapid and inexpensive method for isolation of total DNA from dehydrated plant tissue. - Plant mol. Biol. Rep. **8**: 297-303, 1991.

VanSluys, M.A., Scortecci, K.C., Tempe, J.: DNA methylation associated to *Ac* element in *Arabidopsis*. - Plant Physiol. Biochem. **31**: 805-813, 1993.

Yang, C.H., Ellis, J.G., Michelmore, R.W.: Infrequent transposition of *Ac* in lettuce, *Lactuca sativa*. - Plant mol. Biol. **22**: 793-805, 1993.

Yoder, J.I.: Rapid proliferation of the maize transposable element *Activator* in transgenic tomato. - Plant Cell **2**: 723-730, 1990.