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Identification and mapping of a T-DNA induced flower mutation in *Arabidopsis thaliana*

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Abstract

Collection of the T-DNA tagged lines of *Arabidopsis thaliana* have been created by *Agrobacterium*-mediated root transformation. Transgenic lines produced by this method have been screened for morphogenic mutations. A flower mutation with increased number of stamens and carpels (*scaf1*) was identified. This mutation has similar but weaker phenotype than the known mutant *superman*. Two mapping procedures, with visible and molecular markers, were used to locate *scaf1* flower mutation. Genetic analysis showed that this mutation is located on chromosome 3 near *gl1* gene. It is probably one of the *SUPERMAN* epigenetic alleles.

Additional key words: linkage analysis, microsatellite markers, polymorphism, *SUPERMAN* gene.

Introduction

Insertional mutagenesis of *Arabidopsis thaliana* using the T-DNA of *Agrobacterium* is used as an effective technique to generate gene mutations, identify the corresponding genes, and characterize their function (Koncz *et al.* 1990, Forsthoefel *et al.* 1992). Mutations affecting a developmental or morphological trait can be easily identified, mapped to chromosomes and subjected to genetic analysis. Because the

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Abbreviations: hpt - hygromycinphosphotransferase; T-DNA - transferred DNA.

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T-DNA insertions carry dominant selectable markers and known DNA sequences, the T-DNA tagged genes can be cloned and characterized at the molecular level. Their chromosomal position can be mapped by linkage analysis. Historically genetic mapping utilized visible markers, but it is difficult to examine many such markers in a single cross. The recognition that distantly related individuals differ in DNA sequence throughout their genome (Botstein *et al.* 1980) led to the rapid incorporation of DNA markers into mapping strategies. Restriction fragment length polymorpism (RFLP) (Fabri and Schäffner 1994) and related codominant cleaved amplified polymorphic sequences (CAPS) (Konieczny and Ausubel 1993) or simple sequence length polymorphism (SSLP) markers (Bell and Ecker 1994) are more commonly used.

In a T-DNA transformed mutant population individual transformed lines may carry one, two or more inserts, the distribution of which is possibly dependent on the transformation method used (Ondřej *et al.* 1999).

Mutations affecting the initiation of flowering period and morphoregulation of inflorescence (Alvarez *et al.* 1992, Tsukuya *et al.* 1995) and flower development (Bowman *et al.* 1989) have been intensively studied during the last years using the methods of both classical mutagenesis and T-DNA mutagenesis. The floral meristem identity genes are responsible for the establishment of a floral meristem, while the floral homeotic genes are required for specifying particular organ identities. The mode of action of organ identity genes was explained in genetic terms by the ABC model (Coen and Meyerowitz 1991).

The genetic regulation of floral organ number has been actively studied in *Arabidopsis* (Crone and Lord 1993, Huang and Ma 1997) and rice (Nagasawa *et al.* 1996). *Clavata1* (*clv1*) plants have enlarged floral meristems, increased number of floral organs, inflorescence fasciation and also altered leaf morphology (Crone and Lord 1993). Two other *Arabidopsis* mutants, *clv2* and *clv3*, showing similar phenotypes have been identified (Clark *et al.* 1995). Mutations in *SUPERMAN* (*SUP*) gene result in additional stamens developing at the expense of the fourth whorl carpels. The number of extra stamens is variable, but some carpelloid tissue is usually present in the center of *sup* mutant flowers. A partial or complete loss of the gynoecium was also observed (Sakai *et al.* 1995). This mutation also causes specific alteration in ovule development. It mediates asymmetric growth of the outer integument of ovules (Gaiser *et al.* 1995). The interpretations of this phenotype is that *SUP* gene acts to restrict the number of stamens in the fourth whorl, to promote the formation of carpels and to limit floral meristem activity. The *SUP* gene encodes a protein containing regions with similarity to a zinc-finger and leucine zipper motif (Bowman *et al.* 1992, Takatsuji 1998). The *fon1* flowers have also increased stamen and carpel number and occasionally produces filamentous organs (Huang and Ma 1997). Although this mutation seemed to be different from *sup* it was found, that this is an epigenetic *SUP* allele associated with cytosine methylation within the *SUP* gene (Huang and Ma 1998). Seven heritable but unstable *SUP* epi-alleles (*clk*) were described. They are associated with nearly identical patterns of excess cytosine methylation within the *SUP* gene and a decreased level of *SUP* RNA (Jacobsen and Meyerowitz 1997).

In this paper we describe analysis of the other type of insertional mutant allele with similar phenotype to *sup* mutation This new allele was subjected to mapping using visible and molecular markers.

Materials and methods

Plants and transformation: *Arabidopsis thaliana* (L.) Heynh. plants (ecotype C24) were transformed according to Valvekens *et al.* (1988) modified by Koncz *et al.* (1994). *Arabidopsis thaliana* root culture was established in the liquid medium. Cut roots were co-cultivated with *Agrobacterium* for a short period and transferred to a solid callogenesis medium with cefotaxim 200 mg dm^{-3} and ticarcillin 500 mg dm^{-3} to suppress *Agrobacterium* growth. After two weeks, the roots with growing calli were transferred to shoot inducing medium with hygromycin 15 mg dm^{-3}. Auxin (2.0 mg dm^{-3} indole-3-acetic acid) conditioning in the previous medium followed by cytokinin [2.0 mg dm^{-3} N^{6}-(Δ^{2}-isopentenyl)adenosine] treatment leads to quick regeneration of shoots.

***Agrobacterium* strains and plasmids:** Vector plasmid pPCVR-tx has been used (Koncz *et al.* 1994). The T-DNA carries the hygromycin resistance gene (*hpt*) as a plant selectable marker. Closed to the right borderline sequence there is 35S promoter directed outwards to be able to activate silent genes if it inserts behind the plant gene promoter. The vector plasmid was harboured in *A. tumefaciens* strain GV3101 (pMP90RK) (Koncz *et al.* 1989). Bacteria were grown overnight in liquid media according to Langley and Kado (1972) supplemented with 100 mg dm^{-3} of rifampicin and 50 mg dm^{-3} of kanamycin at 28 °C.

Growth conditions: Seeds were cold treated before sowing by keeping at 4 °C for 5 d. They were sown in a 1:1 mixture of peat moss and soil. Plants were grown at 22 - 25 °C in growth chambers or in a greenhouse. For the tests of antibiotics resistance seeds were surface-sterilized by soaking in 5 % sodium hypochlorite containing few droplets of detergent for 15 min and then rinsed three times with sterile water. Seeds were placed on the MSAR (Koncz *et al.* 1994) medium containing 10 mg dm^{-3} hygromycin. After 14 d hygromycin resistant plants were selected and transferred into the soil.

Mapping using visible markers: Mutant was assigned to linkage group by crossing tester lines with visible markers as male and scoring F$_2$ plants for the flower mutation and visible markers. Five tester lines from Nottingham *Arabidopsis* Stock Centre were used to facilitate mapping studies: NW4 for chromosome 1 (mutations in genes *ch-1, ap1-1, gl2-1*), NW6 for chromosome 2 (*cp2-1, as-1, cer8-1*), NW7 for chromosome 3 (*hy2-1, gl1-1, tt5-1*), NW8 for chromosome 4 (*bp-1, cer2-2, ap2-1*) and NW9 for chromosome 5 (*ttg-1, yi-1*) described by Koornneef and Stam (1988). All visible markers were obtained in the Landsberg *erecta* (Ler) background. Crossing with two mutant lines were performed independently.

We planted 320 F_2 seeds for each linkage group (32 seeds per pot). However, fewer plants were screened because not all seeds germinated and some plants did not survive to maturity. F_2 plants were scored for mutant phenotypes at different stages: *1*) seedling stage for hypocotyl elongation, *2*) rosette stage for trichome analyses, *3*) flowering stage for the pistil structure and stamen number evaluation, *4*) maturity for the rest traits, and *5*) seed maturing for seed coat pigmentation. F_2 plants were divided into four groups according to their phenotypes: A: M Scaf1, B: M scaf1, C: m Scaf1, D: m scaf1.

The test of homogeneity was aimed at determination whether two sets of data were homogeneous enough to justify pooling them. Independence of segregation of two genes was tested with the χ^2 test by means of contingency table analysis for F_2 generation. Monogenic inheritance and recessiveness of both variants (3:1 ratio) were confirmed by χ^2 tests. Estimates of recombination frequency and standard error were obtained by RECF2 program (Koornneef and Stam 1988).

Mapping with molecular markers: The two parents used in the mapping cross were C24 *scaf1* and Landsberg *erecta* wild type. F_1 progeny from this cross was allowed to self-fertilize to produce F_2 individuals. DNA was prepared from individual F_2 plants according to Roger and Bendich (1988). Amplification of simple sequence length polymorphism (SSLP) markers (Bell and Ecker 1994) or cleaved amplified polymorphic sequences (CAPS) (Konieczny and Ausubel 1993) or heteroduplex analysis (Hauser *et al.* 1998) were performed using polymerase chain reaction (PCR). A population of 36 plants was screened. Oligonucleotide sequences are available on the internet (http://genome.bio.upenn.edu/SSLP_info/). PCR reactions were performed according to Bell and Ecker (1994). Polyacrylamide (5 %) gels were used to resolve SSLP and CAPS products, MDE gels to resolve heteroduplexes.

Scanning electron microscopy: Fresh flowers, flower organs of wild type and mutant were examined using scanning electron microscope *JSM 6300* (*JEOL*, Peabody, USA). Samples were fixed in 5 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), then washed three times and dehydrated through a graded alcohol series of ethanol. Coating and photographing was performed as described by Bowman *et al.* (1989).

Chemicals: Antibiotics *Hygromycin B* were purchased from *Boehringer Mannheim Co.* (Mannheim, Germany), *Ticarpen* from *Beecham Pharmaceuticals* (Brentford, UK), and *Claphoran* from *Roussel UCLAF* (Paris, France).

Results

Transformation, mutant isolation and genetic analysis: A population of about 300 *Arabidopsis* transformants were generated by root transformation method. Among these lines, 38 % segregated for *HPT* gene in monohybrid and 32 % in dihybrid Mendelian fashion, 12 % of the lines segregated for more than two T-DNA inserts and 18 % showed non-Mendelian patterns of T-DNA inheritance. This insertional lines collection was used to search for a broad spectrum of mutations. 87 phenotypic alterations were found. from them a flower mutant line was identified in the T_2 population.

The wild type *Arabidopsis* flower consists of four sepals, four petals, six stamens and two fused carpels which are organized into concentric whorls (Fig. 1*a*). The mutant plants exhibited normal vegetative development, but developed abnormal flowers with an increased number of stamens and carpels (Table 1, Fig. 1*b*). Most of the carpels failed to fuse on one or both sides (Fig. 2*b*,*d*,*e*). Some of the flowers produced chimeric stamen and carpel like mosaic organs (Fig. 2*c*). This mutation was named *scaf1* (stamen-carpel abnormal flowers). Some of the *scaf1* flowers contained completely or nearly completely fused two or three stamens (Fig. 1*c*). This mutant phenotype was visible mostly in the early appearing flowers. A correlation between the numbers of stamens produced and the amount or type of carpelloid mosaic organs was not observed.

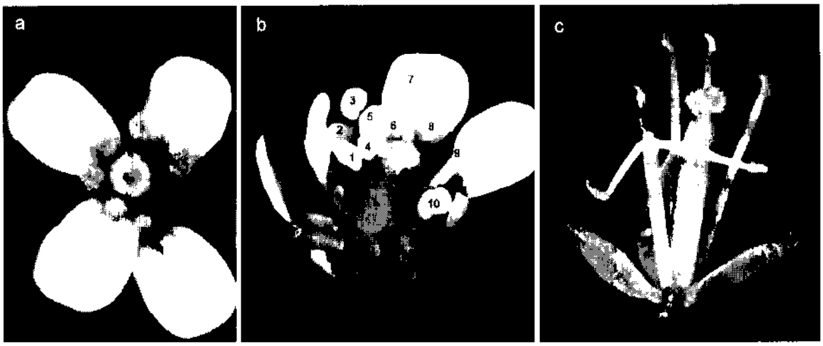

Fig. 1. *Arabidopsis thaliana* flowers photographed on *Leica MZ 12* stereomicroscope: mature wild type flower of ecotype C24 with six stamens (*a*), *scaf1* flower with ten stamens (*b*), *scaf1* flower with two stamens partially fused together (*c*).

Table 1. Number of plants studied and flower organs in *A. thaliana* wild type *C24* and *scaf1* mutant and known *sup1* mutant. The first 10 flowers on each given plant were analyzed. C.S. - chimeric stamens that grow in association with carpels (stamen-carpel mosaic), mean ± SD

Genotype	Plants	Sepal	Petal	Stamen	Carpel	C.S.
Wild type C24	40	4.0 ± 0	4.0 ± 0	5.8 ± 0.4	2.0 ± 0	0.0 ± 0
sup1	37	4.0 ± 0	4.0 ± 0	8.6 ± 1.6	2.3 ± 0.9	0.4 ± 0.6
scaf1	77	4.0 ± 0	4.0 ± 0.1	7.2 ± 1.8	2.9 ± 0.7	0.6 ± 0.8

Genetic and phenotypic analyses: Segregation ratios of the *scaf1* line in T_2 generation were 15:1 (Hyg^R:Hyg^S) and 3:1 (mutant.wild type). The mutant plant was backcrossed with wild type plants C24 and Landsberg *erecta* to separate both T-DNA inserts. Segregation data Hyg^R:Hyg^S for the F_2 progeny of F_1 self-crosses were as follows: for *scaf1* outcrossed to C24 466:172 and *scaf1* outcrossed to Landsberg *erecta* 259:94. Segregation and PCR analyses of F_2 plants showed that this mutation is linked to a T-DNA insertion with hygromycin resistance. The F_2 generation segregated for Hyg^R:Hyg^S in 3:1 or 15:1 ratio depending on the number of T-DNA inserts, and for wt:mutant plants in 3:1 ratio.

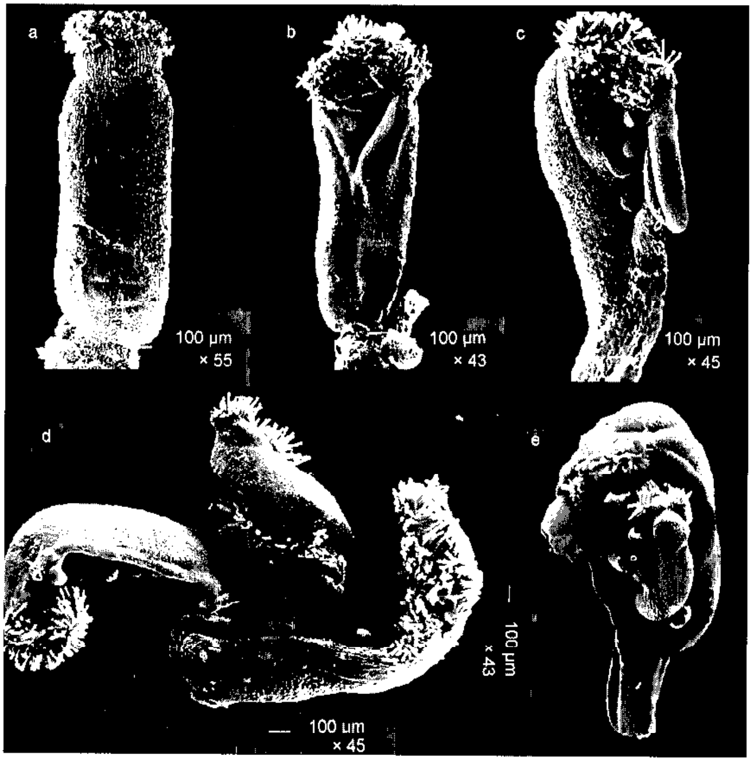

Fig. 2. Scanning electron microscopy of the *Arabidopsis* carpels: *a* - wild type gynoecium consisting of two fused carpels, *b* - *scaf1* gynoecium of two carpels, partially unfused on one side, *c* - *scaf1* stamen-carpel like mosaic organ, *d* - *scaf1* gynoecium of completely unfused three carpels with stigmatic papillae on the sides, *e* - *scaf1* gynoecium of four carpels failed to fuse on one side with a smaller additional carpel.

Linkage mapping procedures: To determine the position of the locus, mutant plants were crossed with lines carrying visible markers (Table 2). The χ^2 values of homogeneity corresponded to $P > 0.05$ in crosses with all signal lines. The only exception is crossing *ch1* × *scaf1* ($P > 0.02$). Therefore the data from the two independent experiments were comparable and could be summarized. Program

RECF2 confirmed linkage of *scaf1* to visible marker *gl1* on chromosome 3 (NW7). Recombination frequency is 0.244 ± 0.044, and its value after certation is 0.315 ± 0.046. Contingency table revealed linkage not only to marker *gl1* (chromosome 3) but even to markers *ap1*, *gl2* (chromosome 1) and *ap2* (chromosome 4).

Molecular markers: F_2 from the cross with Landsberg *erecta* ecotype was used for the linkage mapping of the mutant gene. Polymorphism between C24 and Ler ecotypes had to be proved. Among 25 SSLP. and two CAPS markers tested, 15 showed polymorphism between C24 and Landsberg *erecta* ecotypes (Table 3). The polymorphism of *gl1* marker was detected using heteroduplex analysis on MDE gel. The results showed linkage of the *SCAF1* gene to markers nga162, nga172 and *gl1* on chromosome 3, at position between nga162 and *gl1* (Table 4).

Table 2. Mapping using *Arabidopsis* lines with multiple visible markers. The tested ratio was 9:3:3:1. * - $P < 0.05$, ** - $P < 0.01$.

Cross	Linkage group	Marker genes	F_2 segregation ratio	χ^2 test
NW4 × *scaf1*	I	*ch-1*	354:28:35:2	0.18
		ap1-1	382:18:8:12	89.86**
		gl 2-1	363:24:30:5	4.28*
NW6 × *scaf1*	II	*cer8-1*	322:23:9:0	0.20
		cp2-1	310:20:28:2	0.09
NW7 × *scaf1*	III	*hy2-1*	336:25:75:2	1.88
		gl1-1	275:25:135:2	4.12*
		tt5-1	358:22:23:2	1.64
NW8 × *scaf1*	IV	*bp-1*	429:17:41:4	2.57
		cer2-2	439:19:36:2	0.50
		ap2-1	457:15:19:5	18.40**
NW9 × *scaf1*	V	*ttg -1*	284:10:23:2	1.64
		yi-1	409:15:25:2	1.05

Discussion

A flower mutation of *Arabidopsis* was induced by T-DNA mutagenesis. Although the used vector plasmid pPCVR-tx has been developed for activation insertion mutagenesis, recessive mutation was induced. It depends on the site of T-DNA integration.

Two different linkage mapping strategies were used. In the case of mapping visible markers, five crosses were required, while a single cross to another ecotype introduces a nearly unlimited supply of DNA polymorphic markers. Some visible mutations (*ap1-1*, *ap2-1*) also interacted genetically with the mapped mutation *scaf1*. This appears less problematic when crossing two ecotypes, although some mutant phenotypes become more or less extreme in different backgrounds.

Using visible markers, mutant gene *scaf1* has been assigned to the third linkage group. This method allowed only approximate location linked to *gl1* marker. The results obtained in F_2 generation revealed deviations from 3:1 ratios of the signal and also mutant genes. This indicates reduced viability of certain genotypes (the homozygous mutant). Another factor that may lead to a deficit of a class of genotypes is a reduced transmission of certain (mostly mutant) alleles through the gametophyte. This phenomenon is called certation (Dellaert 1980). It is the main cause for recessive deficits in induced mutants. Therefore recombination frequencies (r) were calculated by RECF2 computer program, which allows correction for certation (Koornneef and Stam 1988). According to our experience, the phenotypic segregation ratios in F_2 with insertional mutation can be unexpectable distorted by instability of the transgene. This can make the standard recombination analysis with T-DNA mutants very problematic (f.i. using the contingency table analysis in our experiments).

Table 3. Polymorphism of molecular markers between ecotypes C24 and Landsberg *erecta*. The sizes of the DNA fragments were estimated from 5 % native polyacrylamide gels.

Chromosome	Type	Name	DNA fragment size C24 (bp)	 Ler (bp)
I	SSLP	Atheat1	150	150
	SSLP	nga 248	120	120
	SSLP	nga 280	80	80
	SSLP	nga 111	180	160
	SSLP	nga 63	110	80
	SSLP	nga 59	115	115
	SSLP	AthATPase	70	70
II	SSLP	nga 1126	200	190
	SSLP	nga 361	100	110
	SSLP	AthBIO2	130	140
	SSLP	nga 168	130	130
III	SSLP	nga 172	150	130
	SSLP	nga 162	130	80
	SSLP	AthCDC2BG	120	120
	SSLP	nga 6	150	120
	MDE	*gl 1*	519	515
IV	SSLP	nga 8	200	200
	CAPS	ag 1 - HinfI digest	590:250:130:120	590:270:160:70
V	SSLP	nga 225	140	140
	SSLP	nga 151	150	100
	SSLP	nga 139	150	150
	SSLP	nga 76	280	200
	SSLP	nga 129	90	90
	SSLP	AthSO191	150	160
	SSLP	nga 249	110	110
	SSLP	nga 106	130	125
	SSLP	AthCTR1A	140	145

Table 4. Mapping using molecular markers

Linkage group	Markers type	name	Recombination frequency [cM]
I	SSLP	nga111	34.5
	SSLP	nga63	29.9
II	SSLP	nga1126	36.9
	SSLP	AthBIO2	33.3
III	SSLP	nga172	17.2
	SSLP	nga162	10.3
	MDE	gl1	14.0
IV	CAPS	ag1	44.8
V	SSLP	nga151	36.2
	SSLP	AthSO191	36.2

Molecular markers were used for more precise location. Except chromosome 4, from five to nine markers were tested for each linkage group. Location of the *SCAF1* gene on chromosome 3 was confirmed.

In F_1 complementation tests (data not shown), in some cases *scaf1* mutants failed to complement *sup* mutants. The reason is probably that this mutation is weakly semidominant. *scaf1* mutation seems to be an epigenetic allele of *SUP*. Jacobsen and Meyerowitz (1997) discovered that many of such mutations are caused by overmethylation of the *SUPERMAN* gene. Similar epigenetic alleles at other loci have not been recognized as such.

References

Alvarez, J., Guli, C.L., Yu, X.-H., Smyth, D.R.: Terminal flower. A gene affecting inflorescence development in *Arabidopsis thaliana*. - Plant J. **2**: 103-116, 1992.

Bell, C.J., Ecker, J.R.: Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. - Genomics **19**: 137-144, 1994.

Botstein, D., White, R.L., Skolnick, M., Davis, R.W.: Construction of a genetic linkage map in man using restriction fragment length polymorphisms. - Amer. J. human Genet. **32**: 314-331, 1980.

Bowman, J.L., Sakai, H., Jack, T., Weigel, D., Mayer, U., Meyerowitz, E.M.: *SUPERMAN*, a regulator of floral homeotic genes in *Arabidopsis*. - Development **114** : 599-615, 1992.

Bowman, J.L., Smyth, D.R., Meyerowitz, E.M.: Genes directing flower development in *Arabidopsis*. - Plant Cell **1**: 37-52, 1989.

Castle, L.A., Errampali, D., Atherton, T.L., Franzmann, L., Yoon, E.S., Meinke, D.W.: Genetic and molecular characterization of embryonic mutants identified following seed transformation in *Arabidopsis*. - Mol. gen. Genet. **241**: 504-514, 1993.

Clark, S.E., Running, M.P., Meyerowitz, E.M.: *CLAVATA3* is a specific regulator of shoot and floral meristem development affecting the same processes as *CLAVATA1*. - Development **121**: 2057-2067, 1995.

Clark, S.E., Running, M.P., Meyerowitz, E.M.: *CLAVATA1*, a regulator of meristem and floral development in *Arabidopsis*. - Development **119**: 397-418, 1993.

Coen, E.S., Meyerowitz, E.M.: The war of the whorls: genetic interactions controlling flower development. - Nature **353**: 31-37, 1991.

Crone, W., Lord, E.M.: Flower development in the organ number mutant *clavata1-1* of *Arabidopsis thaliana* (*Brassicaceae*). - Amer. J. Bot. **80**: 1419-1426, 1993.

Dellaert, L.M.V.: Segregation frequencies of radiation-induced viable mutants in *Arabidopsis thaliana* (L.) Heynh. - Theor. appl. Genet. **57**: 137 - 143, 1980.

Fabri, C.O., Schäffner, A.R.: An *Arabidopsis thaliana* RFLP mapping set to localize mutations to chromosomal regions. - Plant J. **5**: 149-156, 1994.

Forsthoefel, N.R., Wu, Y., Bennet, M.J., Feldmann, K.A.: T-DNA insertion mutagenesis in *Arabidopsis*: prospects and perspectives. - Aust. J. Plant Physiol. **19**: 353-366, 1992.

Franzmann, L.H., Yoon, E.S., Meinke, D.W.: Saturating the genetic map of *Arabidopsis thaliana* with embryonic mutations. - Plant J. **7**: 341-350, 1995.

Gaiser, J.C., Robinson-Beers, K., Gasser, C.: The *Arabidopsis SUPERMAN* gene mediates asymmetric growth of the outer integument of ovules. - Plant Cell **7**: 333-345, 1995.

Hauser, M.-T., Adhami F., Dorner, M., Fuchs, E., Glössl, J.: Generation of co-dominant PCR-based markers by duplex analysis on high resolution gels. - Plant J. **16**: 117-125, 1998.

Huang, H., Ma, H.: *FON1*, an *Arabidopsis* gene, that terminates floral meristem activity and controls flower organ number. - Plant Cell **9**: 115-134, 1997.

Huang, H., Ma, H.: The nature of the *Arabidopsis fon1* mutations.- Plant Cell **10**: 3-4, 1998.

Irish, V.F., Sussex, I.M.: Function of the *apetala-1* gene during *Arabidopsis* floral development. - Plant Cell **2**: 741-753, 1990.

Jacobsen, S.E., Meyerowitz, E.M.: Hypermethylated *SUPERMAN* epigenetic alleles in *Arabidopsis*. - Science **277**: 1100-1103, 1997.

Koncz, C., Martini, N., Mayerhofer, R., Koncz-Kalman, Z., Koerber, H., Redei, G., Schell, J.: High-frequency T-DNA-mediated gene tagging in plants. - Proc. nat. Acad. Sci. USA **86**: 8467-8471, 1989.

Koncz, C., Martini, N., Szabados, L., Hrouda, M., Bachmair, A., Schell, J.: Specialized vectors for gene tagging and expression studies. - In: Gelvin, S.B. (ed.). Plant Molecular Biology Manual B2. Pp. 1-22. Kluwer Academic Publishers, Dordrecht 1994.

Koncz, C., Mayerhofer, R, Koncz-Kalman, Z., Nawrath, C., Reiss, B., Rédei, G.P, Schell, J.: Isolation of a gene encoding a novel chloroplast protein by T-DNA tagging in *Arabidopsis thaliana*. - EMBO J. **9**: 1337-1346, 1990.

Konieczny, A., Ausubel, F.M.: A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype specific PCR-based markers. - Plant J. **4**: 403-410, 1993.

Koornneef, M., Stam, P.: Procedures for mapping by using F_2 and F_3 populations. - *Arabidopsis* Inf. Serv. **25**: 35-40, 1988.

Langley, R.A., Kado, C.I.: Studies on *Agrobacterium tumefaciens*: conditions for mutagenesis by N-methyl-N-nitrosoguanidine and relationship of *A. tumefaciens* mutants to crown gall induction. - Mutat. Res. **14**: 277-286, 1972.

Nagasawa, N., Miyoshi, M., Kitano, H., Satoh, H., Nagato, Y.: Mutations associated with floral organ number in rice. - Planta **198**: 627-633, 1996.

Ondřej, M., Kocábek, T., Rakouský, S., Wiesnerová, D.: Segregation of the T-DNA inserts in the offspring of *Arabidopsis thaliana* after *Agrobacterium* transformation. - Biol. Plant. **42**: 185-195, 1999.

Roger, S.O., Bendich, A.J.: Extraction of DNA from plant tissue. - In: Gelvin, S.B. (ed.). Plant Molecular Biology Manual A6, Pp. 1-10. Kluwer Academic Publishers, Dordrecht 1988.

Sakai, H., Medrano, L.J., Meyerowitz, E.M.: Role of *SUPERMAN* in maintaining *Arabidopsis* floral whorl boundaries. - Nature **378**: 199-203, 1995.

Takatsuji, H.: Zinc-finger transcription factors in plants. - Cell mol. Life Sci. **54**: 582-596, 1998.

Tsukuya, H., Inaba-Higano, K., Komeda, Y.: Phenotypic characterization and molecular mapping of an *acaulis*2 mutant of *Arabidopsis thaliana* with flower stalks of much reduced length. - Plant Cell Physiol. **36**: 239-246, 1995.

Valvekens, D., Van Montagu, M., Van Lijsebettens, M.: *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana* root explants by using kanamycin selection. - Proc. nat. Acad. Sci. USA **85**: 5536-5540, 1988.