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Genome-wide linkage analysis of *Arabidopsis* genes required for leaf development

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Abstract In most crop species, primary productivity depends mainly on the leaf. However, the genes that contribute to the making of plant leaves remain largely unknown. With a view to identifying the genes involved in leaf development in *Arabidopsis thaliana*, we previously isolated EMS-induced mutants with abnormally shaped leaves and demonstrated that they fall into 94 complementation groups. We present here the map positions of 76 of these genes, which have been obtained using a high-throughput genetic mapping method, based on the simultaneous coamplification by PCR of 21 polymorphic microsatellites and the semiautomated fluorescent detection of the products. The map positions and F_2 mapping populations obtained in this work will be instrumental in the positional cloning of these genes, which are essential for leaf development.

Keywords Plant leaf morphogenesis · *Arabidopsis* · Genetic map

Introduction

The primary pathway for carbon and energy uptake by plants is the leaf, an organ that is of the utmost importance to agriculture. However, little is known about the genetic controls that underlie leaf development, in spite of the fact that its biotechnological manipulation offers great potential. At the present time, the nature, action and interactions of the genes which drive the sequence of developmental events that contribute to the making of a leaf remain to be unraveled (reviewed in Hake and Sinha 1991; Smith and Hake 1992; Sinha et al. 1993; Telfer and Poethig 1994; Tsukaya 1995; Hall and Langdale 1996; Poethig 1997; Brutnell and Langdale 1998; Tsiantis and Langdale 1998; Van Lijsebettens and Clarke 1998; Pozzi et al. 1999; Sinha 1999).

In an attempt to identify genes involved in leaf morphogenesis in *Arabidopsis thaliana*, we have screened for new mutants that form abnormal leaves (Berná et al. 1999) and conducted genetic analyses of mutants that were already available (Serrano-Cartagena et al. 1999; 2000). Our large-scale mutant search, which came close to, but did not quite reach, saturation of the genome, showed that the lines obtained fell into 94 complementation groups (Berná et al. 1999). We present here the results of our linkage analysis of most of these genes which were identified by mutation. We have taken advantage of the availability of a high-throughput mapping procedure previously developed for this purpose in our laboratory, based on the simultaneous coamplification by PCR of 21 polymorphic microsatellites and the fluorescent semiautomated detection and sizing of the products (Ponce et al. 1999). The molecular markers used are spaced evenly over the entire genome at intervals of 6.7 to 57.7 cM.

Materials and methods

Plant materials and growth

The *Arabidopsis thaliana* mutant lines studied in this work had already been isolated and subjected to complementation analysis as described in Berná et al. (1999). All the mutations studied here are recessive, with only two exceptions (*ven3-2* and *ven6*, both semidominant), and were obtained in the genetic background of the ecotype L*er* after ethyl methanesulfonate (EMS) treatment. Plants were grown at 20 ± 1°C and 60–70% relative humidity under continuous illumination of 7,000 lx, under both sterile (in 150 mm petri dishes containing agar medium, each plate sown with 100 regularly spaced seeds) and non-sterile (in pots containing a 2:2:1 mixture of perlite, vermiculite and sphagnum moss) conditions, as described in Ponce et al. (1998).

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Linkage analysis

To assess linkage to polymorphic microsatellite markers, also called Simple Sequence Length Polymorphisms (SSLP; Bell and Ecker 1994), at least one mutant line from each of the complementation groups described in Berná et al. (1999) was outcrossed to the Columbia-0 (Col-0) ecotype; mutant plants were always used as female parents, allowing us easily to recognize any self-pollination event. The seeds of two or three F_1 plants were harvested and stored. For each complementation group, about 300 seeds from a single F_1 plant were sown on petri dishes. Inheritance patterns, although already studied in F_2 populations obtained from backcrosses (Berná et al. 1999), were tested again in the F_2 families derived from the outcrosses.

F_2 plants displaying the recessive phenotype were harvested 3–4 weeks after sowing, and stored at –20°C. DNA isolation, multiplex PCR, electrophoresis and sizing of amplification products were performed as described in Ponce et al. (1999). In brief, DNA from each F_2 plant was amplified in a single-tube multiplex PCR mix containing 21 primer sets, each set including an oligonucleotide labeled with one of three different fluorescent dyes. The amplification products were electrophoresed in a single lane of a polyacrylamide gel, in an ABI PRISM 377 DNA sequencer, and sized with the help of the GeneScan 2.1 fragment analysis software (Applied Biosystems). Most map positions were determined from DNA samples extracted from 19 F_2 plants, the maximum number of samples per gel, since our attempts to use all the 36 wells available in our ABI 377 sequencing gels were unsuccessful: the intensity of the signal from some bands in a given lane was so high that it was also detected in the neighboring lanes (Ponce et al. 1999).

Estimation of map distances

The microsatellite markers used in this work were the following, their corresponding chromosome and map positions in cM are indicated in parentheses: AthACS (1; 3.31), AthZFPG (1; 37.36), T27k12Sp6 (1; 61.22), AthGENEA (1; 88.90), nga111 (1; 115.55), nga1145 (2; 9.60), nga1126 (2; 50.65), nga361 (2; 63.02), nga168 (2; 73.77), AthCHIB (3; 19.10), nga162 (3; 20.56), AthGAPab (3; 43.77), nga6 (3; 86.41), nga12 (4; 22.92), nga1111 (4; 29.64), nga1139 (4; 83.41), nga1107 (4; 104.73), AthCTR1 (5; 9.32), nga139 (5; 50.48), AthPHYC (5; 71.13), MBK5 (5; 119). These map positions were taken from the latest version of the recombinant inbred (RI) map of Lister and Dean (http://nasc.nott.ac.uk/new_ri_map.html), the only exception being MBK5, which is known to be 2 cM distal to *LFY3*, which in turn is at 116.88 cM in the RI map.

Recombination percentages (r) and their standard errors (S_r) were obtained as described in Koornneef and Stam (1992), and transformed into map distances in centimorgans (D), using the mapping function of Kosambi (1944), as follows:

$$r = \frac{number\ of\ recombinant\ chromosomes\ found}{total\ \ number\ of\ \ chromosomes\ studied} \cdot 100;$$

$$S_r = \sqrt{\frac{r(100-r)}{n}}$$

$$D = 25ln\left[\frac{100+2r}{100-2r}\right]; \quad S_D = \frac{2500S_r}{2500-r^2}$$

where n is the number of chromosomes studied. In those cases where linkage between a mutation and a marker was complete, the standard deviation of map distances (S_D) was calculated as:

$$S_D = \frac{1}{\sqrt{n}}$$

Map distances between a mutation and its neighboring markers, as well as their relative positions (their maximum-likelihood map), were determined using the "lod table" and "compare" commands, respectively, in the Mapmaker 3.0b program (Lander et al. 1987).

Results

In this work, we have mapped 76 of the 94 genes that we previously identified by complementation analysis of EMS-induced *Arabidopsis* mutants with aberrantly shaped leaves. Their phenotypes are named and described in Table 1 and illustrated in Fig. 1. All the mutant lines of four of the 94 loci were lost (*api2*, *den14*,

Table 1 Phenotypic classification of mutants studied in this work

Phenotypic class	Brief description of most representative phenotypic trait	Complementation groups
Asymmetric leaves	Rounded lamina, with some degree of bilateral asymmetry and margins slightly revolute (Fig. 1b)	*AS1*, *AS2*
Rotunda	Broad and rounded lamina (Fig. 1c)	*RON1–RON3*
Rugosa	Wrinkled lamina (Fig. 1d)	*RUG1*, *RUG2*
Ondulata	Undulated lamina (Fig. 1e)	*OND1–OND4*
Exigua	Small and dark leaves (Fig. 1f)	*EXI1–EXI8*
Orbiculata	Small, rounded and yellowish leaves (Fig. 1g)	*ORB1*, *ORB2*
Elongata	Narrow and elongated lamina and long petiole (Fig. 1h)	*ELO1–ELO4*
Angusta	Narrow lamina (Fig. 1i)	*ANG1–ANG4*
Apiculata	Pointed lamina, with slightly incised margins (Fig. 1j)	*API1–API7*
Denticulata	Pointed lamina, with dentate margins (Fig. 1k)	*DEN1–DEN17*
Angulata	Yellowish leaves with dentate margins (Fig. 1l)	*ANU1–ANU12*
Erosa	Rounded lamina, with dentate margins (Fig. 1m)	*ERO1–ERO3*
Scabra	Rounded and protruded lamina (Fig. 1n)	*SCA1–SCA5*
Venosa	Conspicuous venation. Some lines displaying incise margins (Fig. 1o)	*VEN1–VEN6*
Dentata	Serrated margins (Fig. 1p)	*DEA1*
Serrata	Small leaves with strongly serrated margins (Fig. 1q)	*SEA1–SEA4*
Transcurvata	Margin obliquely revolute (Fig. 1r)	*TCU1–TCU3*
Ultracurvata	Lamina spirally rolled downwards (Fig. 1s)	*UCU1*
Incurvata	Involute margins (Fig. 1t)	*ICU1*, *ICU3*, *ICU6–ICU9*
Total		94

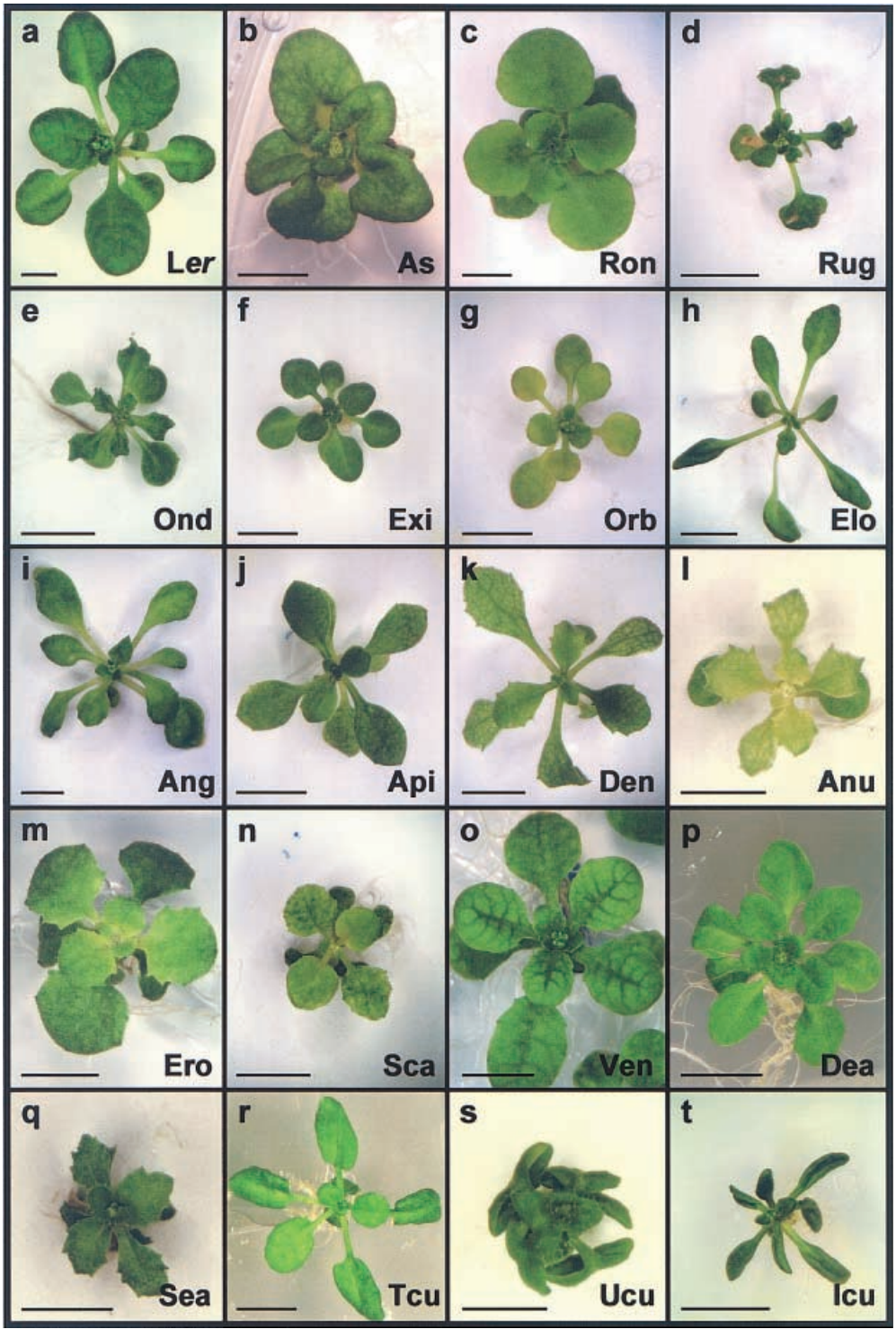

Fig. 1a–t Leaf form in the wild type (ecotype L*er*; **a**) and representatives of the phenotypic classes defined during the complementation analysis of EMS-induced mutants with abnormal leaves mapped in this work (**b–t**). See Table 1 for the full name and a brief description of each phenotypic class. Photographs were taken 21 days after sowing. The *scale bars* indicate 5 mm

den16, and *sca2*). Mutants belonging to four other complementation groups had already been found to be alleles of genes mapped by other authors (Berná et al. 1999): *ASYMMETRIC LEAVES1* (*AS1*; Rédei 1965a, 1965b; Barabas and Rédei 1971), which maps on chromosome 2, *AS2* (Fabri and Schaffner 1994), on chromosome 1, and *CURLY LEAF* (*CLF*; Goodrich et al. 1997) and *RETICULATA* (*RE*; Rédei and Hirono 1964), two genes that we initially called *INCURVATA1* (*ICU1*) and *VENOSA2* (*VEN2*), respectively, both of which map on chromosome 2. Other genes not mapped in this work were *ICU3* (Serrano-Cartagena et al. 2000), which has been named *HASTY* (*HST*) by Telfer and Poethig (1998), and *ICU6*, *ICU7*, *ICU8*, *ICU9* and *UCU1*, the genetic analyses of which will be published elsewhere.

Outcrosses were performed between Col-0 and 81 mutant strains in the L*er* background, each representative of a different complementation group. The resulting F_1 populations were wild type in all cases, the only exceptions being those involving the semi-dominant mutations *ven3-2* and *ven6*. Even though monogenic inheritance for all such mutants, as well as recessiveness for 79 of them, had previously been demonstrated in the progeny of backcrosses to the L*er* ecotype (Berná et al. 1999), we wanted to test their inheritance patterns in the F_2 populations of the outcrosses to Col-0. More mutant F_2 plants than expected for a 3:1 segregation were found for five of the outcrosses – those involving *anu5-1b*, *anu9-1b*, *den13*, *exi7*, and *ero3-1a*. In contrast, fewer phenotypically mutant individuals than expected for 3:1 segregation were found in the F_2 progenies of 32 mutant strains (*ang2*, *ang3*, *ang4*, *anu1-1*, *anu2*, *anu3*, *anu4*, *anu6*, *anu8-1b*, *anu10-1a*, *anu11*, *anu12*, *api3*, *api5*, *api7-1a*, *den1*, *den2*, *den8*, *den10*, *den17*, *elo1-1a*, *elo4*, *ero1*, *exi2-1a*, *ond1*, *ond2*, *ond3*, *ron1*, *ron3*, *tcu1-1a*, *tcu2-1b* and *tcu3-1a*). A similar observation was made by Koornneef and Hanhart (1988), who proposed the existence of certation – a reduced rate of transmission of mutant alleles through the gametophyte – as the most likely explanation for the reduced proportion of phenotypically mutant individuals that they found in nine of the F_2 progenies of the outcrosses to the Niederzenz (Nd) ecotype of 18 mutants obtained in a L*er* background. Alternatively, this finding may indicate the influence of the genetic background on the phenotype associated with these recessive mutations, whose penetrance and/or expressivity in the F_2 of the outcrosses was lower than that of the corresponding backcrosses. This could also be the reason why the mutations *ven3-2* and *ven6* mutations, which are semi-dominant in the L*er* background, behaved as recessives in the F_2 populations of their outcrosses to Col-0.

Some mutant strains (*ron3*, *sca4*, *sca5* and *tcu3*) were found to be carriers of additional mutations, other than the mutation responsible for the phenotype of interest, since we found unexpected phenotypes in the F_2, apparently caused by recessive mutations. The *den3* strain was not subjected to linkage analysis since some Exigua individuals but no Denticulata plants were found among its F_2 progeny. Neither *exi8-1*, *dea1* nor *exi4* was mapped, since the F_2 seeds of *exi8-1* did not germinate and only wild-type individuals were found among the progeny of the others.

Table 2 presents a few representative examples from the entire set of raw recombination data that are obtained when the method used in this paper is applied to mapping on a one gene-one gel basis. For each mutation, only those recombination frequencies considered as unequivocal evidence of linkage are listed in Table 3, where only distances to the microsatellites flanking each gene are given. Most of the map positions presented here were determined from DNA samples extracted from 19 F_2 plants (see Materials and methods). However, it was not possible to detect linkage on a one gel one-gene basis for some mutations. We studied linkage on more than one gel (i.e., genotyping more than 19 F_2 plants) in those cases where fewer than 30 chromosomes could be genotyped for the two nearest microsatellites, due to their poor amplification (*ANG2*, *ANG4*, *ANU4*, *ANU6*,

Table 2 Recombination percentages obtained in mapping experiments performed in single gels

Microsatellite	Chr.	Locus[a]				
		ANG1	*ANU2*	*API5*	*SCA3*	*VEN6*
AthACS	1	44.4 (36)	63.9 (36)	50.0 (38)	42.1 (38)	31.3 (32)
AthZFPG	1	39.5 (38)	63.9 (36)	55.3 (38)	60.5 (38)	44.7 (38)
T27k12-Sp6	1	42.9 (28)	58.3 (36)	47.2 (36)	82.1 (28)	60.0 (20)
AthGENEA	1	47.1 (34)	61.1 (36)	50.0 (38)	44.1 (34)	58.82 (34)
nga111	1	44.7 (38)	63.9 (36)	52.6 (38)	60.5 (38)	79.0 (38)
nga1145	2	36.8 (38)	47.2 (36)	47.4 (38)	34.2 (38)	47.4 (38)
nga1126	2	**3.1 (32)**	47.2 (36)	44.7 (38)	**6.3 (32)**	57.1 (28)
nga361	2	**2.6 (38)**	28.6 (28)	44.7 (38)	**11.11 (36)**	52.8 (36)
nga168	2	**7.7 (26)**	45.0 (20)	55.3 (38)	**11.11 (36)**	52.9 (34)
AthCHIB	3	21.9 (32)	31.3 (16)	35.3 (34)	21.9 (32)	35.7 (28)
nga162	3	36.8 (38)	47.2 (36)	60.5 (38)	41.7 (36)	**10.5 (38)**
AthGAPab	3	44.7 (38)	67.7 (34)	42.1 (38)	50.0 (34)	**0.0 (38)**
nga6	3	52.6 (38)	47.2 (36)	83.3 (36)	50.0 (36)	63.3 (30)
nga12	4	57.9 (38)	58.3 (36)	65.8 (38)	47.4 (38)	52.6 (38)
nga1111	4	52.6 (38)	58.3 (36)	57.9 (38)	50.0 (38)	44.8 (38)
nga1139	4	57.9 (38)	44.4 (36)	68.4 (38)	60.5 (38)	52.6 (38)
nga1107	4	60.5 (38)	69.4 (36)	57.9 (38)	52.8 (36)	38.9 (36)
AthCTR1	5	34.2 (38)	**8.3 (36)**	31.6 (38)	34.2 (38)	61.8 (34)
nga139	5	29.0 (38)	**11.1 (36)**	**7.9 (38)**	30.6 (36)	52.8 (36)
AthPHYC	5	44.7 (38)	30.6 (36)	**10.5 (38)**	38.9 (36)	55.6 (36)
MBK5	5	60.5 (38)	50.0 (36)	60.5 (38)	50.0 (36)	52.8 (36)

[a]The figures indicate raw recombination percentages, while the absolute number of chromosomes analyzed is given in *parentheses*. Values highlighted in *bold* are those considered as unequivocal evidences of linkage

Table 3 Map distances between mutations studied in this work and their neighboring markers

Gene	Chr.	Flanking markers
ANG1	2	nga1126 (32), 3.13 ± 3.09; nga1145 (38), 47.18 ± 17.12
ANG2	4	nga1107 (60), 11.89 ± 4.38; nga1139 (60), 13.66 ± 4.72
ANG3	5	MBK5 (36), 35.53 ± 12.25; AthPHYC (34), 11.99 ± 5.85
ANG4	2	nga168 (16), 6.28 ± 6.15; nga361 (52), 15.90 ± 5.53
ANU1	1	AthZFPG (36), 14.26 ± 6.25; AthACS (36), 23.89 ± 8.63
ANU2	5	nga139 (36), 11.30 ± 5.51; AthCTR1 (36), 8.41 ± 4.74
ANU3	5	nga139 (68), 11.99 ± 4.14; AthCTR1 (66), 38.51 ± 9.76
ANU4	3	nga162 (38), 7.96 ± 4.49; AthCHIB (22), 0.00 ± 0.21
ANU5	2	nga1126 (32), 9.49 ± 5.34; nga1145 (38), 41.85 ± 14.47
ANU6	4	nga1139 (54), 9.37 ± 4.08; nga1111 (58), 42.36 ± 11.90
ANU7	5	MBK5 (38), 16.35 ± 6.57; AthPHYC (38), 41.85 ± 14.47
ANU8	5	MBK5 (36), 17.33 ± 6.99; AthPHYC (36), 23.89 ± 8.63
ANU9	5	nga139 (36), 17.33 ± 6.99; AthCTR1 (36), 11.30 ± 5.51
ANU10	1	T27k12Sp6 (28), 14.69 ± 7.20; AthZFPG (38), 7.96 ± 4.49
ANU11	5	AthCTR1 (38), 7.96 ± 4.49
ANU12	1	AthGENEA (34), 15.15 ± 6.65; T27k12Sp6 (32), 16.17 ± 7.11
API1	2	nga168 (34), 0.00 ± 0.17; nga361 (34), 38.51 ± 13.80
API3	4	nga1139 (32), 16.17 ± 7.11; nga1111 (36), 31.32 ± 10.80
API4	1	nga111 (64), 1.56 ± 1.55; AthGENEA (62), 16.73 ± 5.21
API5	5	AthPHYC (38), 10.69 ± 5.21; nga139 (38), 7.96 ± 4.49
API6	5	MBK5 (30), 17.33 ± 7.65
API7	4	nga1107 (32), 9.49 ± 5.34
DEN1	5	MBK5 (36), 8.41 ± 4.74
DEN2	2	nga168 (42), 4.78 ± 3.32; nga361 (68), 15.15 ± 4.70
DEN4	3	nga6 (38), 0.00 ± 0.16; AthGAPab (36), 27.47 ± 9.62
DEN5	2	nga1145 (36), 8.41 ± 4.74
DEN6	4	nga1139 (102), 42.04 ± 8.89; nga1111 (102), 24.30 ± 5.19
DEN7	1	nga111 (74), 6.80 ± 2.97; AthGENEA (62), 13.20 ± 4.56
DEN8	2	nga168 (62), 22.35 ± 6.27
DEN9	1	AthZFPG (72), 27.47 ± 6.80; AthACS (70), 26.52 ± 6.71
DEN10	5	MBK5 (30), 0.00 ± 0.18; AthPHYC (28), 44.79 ± 18.49
DEN11	5	nga139 (36), 11.30 ± 5.51; AthCTR1 (36), 5.58 ± 3.87
DEN12	2	nga361 (38), 0.00 ± 0.16; nga1126 (34), 0.00 ± 0.17
DEN13	3	nga6 (70), 28.43 ± 7.10; AthGAPab (72), 25.65 ± 6.44
DEN15	3	nga6 (62), 6.49 ± 3.17
DEN17	4	nga1107 (38), 2.63 ± 2.60
ELO1	3	AthCHIB (59), 5.38 ± 3.04
ELO2	5	nga139 (42), 2.38 ± 2.36; AthCTR1 (30), 6.71 ± 4.64
ELO3	5	MBK5 (34), 25.54 ± 9.34; AthPHYC (36), 11.30 ± 5.51
ELO4	1	AthZFPG (32), 6.28 ± 4.35; AthACS (32), 12.77 ± 6.24
ERO1	1	T27k12Sp6 (28), 18.68 ± 8.30; AthZFPG (38), 19.33 ± 7.28
ERO2	1	AthZFPG (38), 10.69 ± 5.21; AthACS (34), 25.54 ± 9.34
ERO3	3	nga6 (36), 5.58 ± 3.87
EXI1	4	nga1107 (64), 31.82 ± 8.22; nga1111 (64), 31.82 ± 8.22
EXI2	5	MBK5 (36), 23.89 ± 8.63; AthPHYC (30), 17.33 ± 7.65
EXI3	5	MBK5 (90), 17.33 ± 4.42
EXI5	5	nga139 (32), 19.71 ± 8.03; AthCTR1 (32), 23.46 ± 9.04
EXI6	1	nga111 (30), 10.14 ± 5.71; AthGENEA (26), 24.96 ± 10.50
EXI7	5	AthPHYC (38), 41.85 ± 14.47; nga139 (38), 16.35 ± 6.57
OND1	3	nga162 (68), 10.44 ± 3.85; AthCHIB (34), 0.00 ± 0.17
OND2	1	nga111 (72), 15.78 ± 4.68
OND3	5	MBK5 (34), 15.15 ± 6.65; AthPHYC (34), 33.75 ± 11.95
OND4	2	nga1126 (36), 2.78 ± 2.75; nga1145 (38), 41.85 ± 14.47
ORB1	5	AthCTR1 (34), 5.91 ± 4.09
ORB2	3	AthGAPab (60), 3.34 ± 2.33; nga162 (70), 24.68 ± 6.34
RON1	5	MBK5 (36), 8.41 ± 4.74
RON2	4	nga1107 (36), 11.30 ± 5.51; nga1139 (32), 23.46 ± 9.04
RON3	4	nga1107 (34), 25.54 ± 9.34; nga1139 (34), 15.15 ± 6.65
RUG1	5	nga139 (32), 27.47 ± 10.21; AthCTR1 (32), 3.13 ± 3.09
RUG2	4	nga1111 (36), 20.52 ± 7.77; nga12 (26), 3.85 ± 3.77
SCA1	2	nga168 (34), 11.99 ± 5.85
SCA3	2	nga1126 (32), 6.28 ± 4.35; nga1145 (38), 41.85 ± 14.47
SCA4	3	AthCHIB (36), 8.41 ± 4.74
SCA5	5	AthPHYC (32), 36.66 ± 13.45; nga139 (38), 0.00 ± 0.16
SEA1	4	nga1111 (38), 7.96 ± 4.49; nga12 (38), 5.28 ± 3.66
SEA2	2	nga168 (38), 5.28 ± 3.66
SEA3	1	AthZFPG (72), 22.18 ± 5.79; AthACS (70), 19.50 ± 5.39
SEA4	1	AthZFPG (36), 27.47 ± 9.62; AthACS (36), 14.26 ± 6.25
TCU1	4	nga1139 (36), 8.41 ± 4.74

Table 3 (Contd.)

Gene	Chr.	Flanking markers
TCU2	5	MBK5 (34), 0.00 ± 0.17; AthPHYC (36), 35.53 ± 12.25
TCU3	4	nga1107 (32), 6.28 ± 4.35; nga1139 (32), 9.49 ± 5.34
VEN1	5	nga139 (38), 16.35 ± 6.57; AthCTR1 (38), 7.96 ± 4.49
VEN2	2	nga168 (30), 0.00 ± 0.18; nga361 (34), 21.89 ± 8.35
VEN3	1	T27k12Sp6 (48),19.71 ± 6.56; AthZFPG (60), 11.88 ± 4.38
VEN4	5	AthPHYC (36), 2.78 ± 2.75; nga139 (36), 20.52 ± 7.77
VEN5	2	nga361 (30), 0.00 ± 0.18
VEN6	3	AthGAPab (38), 0.00 ± 0.16; nga162 (38), 10.69 ± 5.21

[a]The figures in *parentheses* indicate the number of chromosomes analyzed to test for linkage to a given microsatellite marker

API4, *DEN2*, *DEN7*, *OND1*, *ORB2* and *VEN3*), or when the map distance to the nearest microsatellite was greater than 20 cM (*DEN6*, *DEN8*, *DEN9*, *DEN13*, *EXI1*, *EXI3*, *OND2* and *SEA3*). In this latter group, although a second gel helped to refine map positions, five of the eight genes studied still fell more than 20 cM away from their closest neighboring microsatellites (*DEN6*, *DEN8*, *DEN9*, *DEN13* and *EXI1*). The distances between the markers flanking four of these genes (*DEN6*, *DEN9*, *DEN13* and *EXI1*) are longer than 34 cM (nga1111 and nga1139, AthACS and AthZFPG, AthGAPab and nga6, separated in the last RI map by 53.77, 34.05 and 42.64 cM, respectively). The remaining one (*DEN8*) was distal to the nga168 marker, which lays 23.68 cM from the telomere.

A map is shown in Fig. 2 indicating the positions of the microsatellites used as markers and those of the 76 genes subjected to linkage analysis in this work. To test the robustness of our method, we pooled all the mapping data to calculate map distances between the markers themselves. The distances obtained in this way between adjacent microsatellites were very close to those given in the last version of the Lister and Dean RI map (see

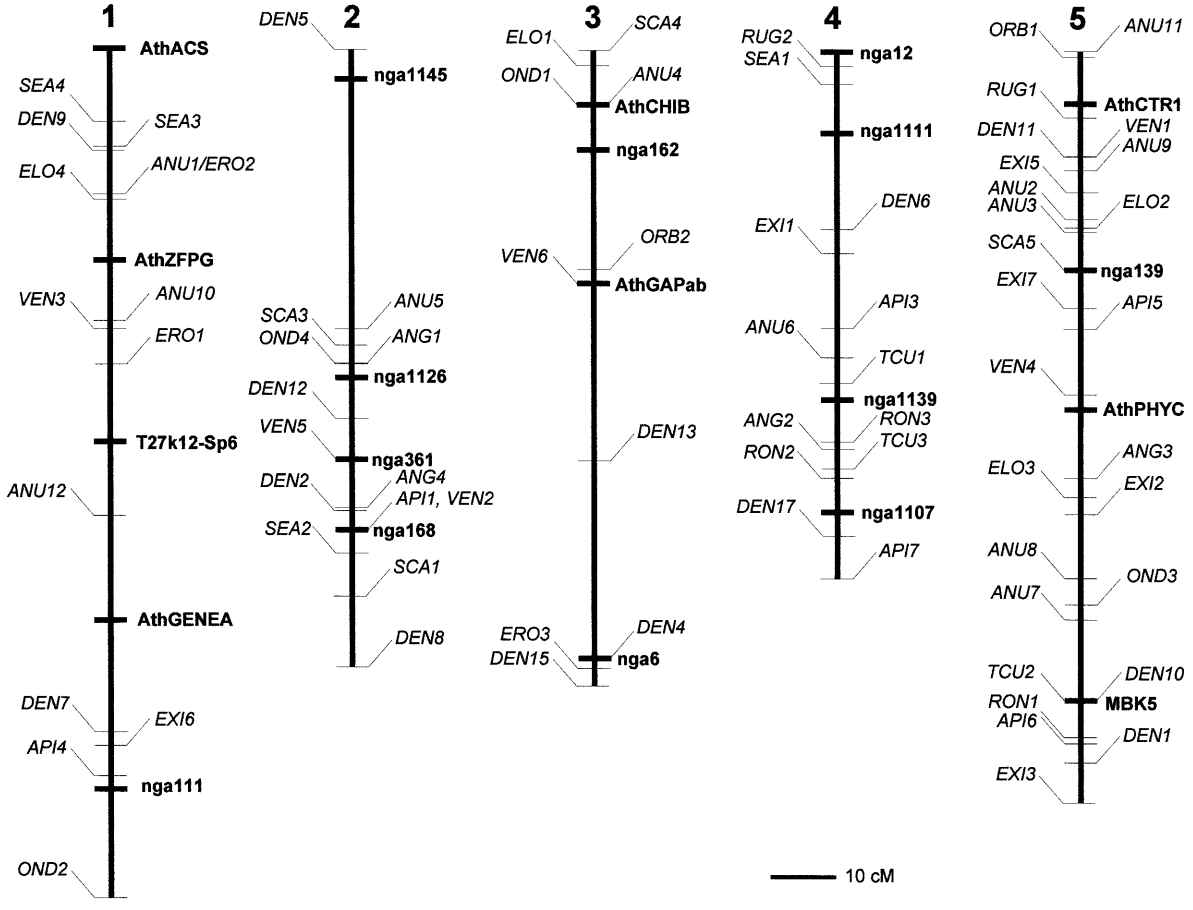

Fig. 2 Genetic map of *A. thaliana*, showing the positions of the genes mapped in this work. The microsatellite markers used are indicated in *bold*. Given that allelism tests clearly indicated allelism between *ero2* and *anu1* (see text), they are represented here as a single locus on Chr. 1 (named *ANU1-ERO2*), whose position was determined by merging the linkage data obtained for both mutations

Materials and methods), with an average deviation of 4.76 cM. The greatest differences found were the distance between AthGAPab and nga6, and that between AthCTR1 and nga139, which diverged 15.1 and 15.6 cM respectively, with respect to the RI map. Such deviations are likely to be due to the different types of mapping populations used, since the map of Lister and Dean was obtained from recombinant inbred lines and ours from the F_2 progeny of outcrosses.

Allelism tests performed after linkage analysis

We performed some allelism tests between mutant strains that share some phenotypic traits and carry mutations that map to similar positions, such as some of the *ero*, *anu* and *den* mutants that have toothed leaves. Only two of the mutants that were crossed were shown to carry allelic mutations: *ero2* and *anu1*, both mapping on chromosome 1, at 27.00 and 20.57 cM respectively. Complementation was found in the progeny of the remaining crosses, which were the following (indicated as female × male, each mutation shown with its chromosome and approximate map position, in cM), *ero2-1a* (1; 27.00) × *anu10-1a* (1; 40.59), *ero2-1a* (1; 27.00) × *anu12* (1; 74.06), *ang1-1* (2; 43.25) × *den5-1* (2; 0.00), *api3* (4; 42.39) × *den6-1* (4; 27.29), *den17* (4; 74.84) × *ang2* (4; 59.81), *den17* (4; 74.84) × *api7-1a* (4; 81.08), *api7- 1a* (4; 81.08) × *ang2* (4; 59.81), *den11* (5; 18.52) × *ven1* (5; 18.43), *sca5* (5; 35.64) × *anu3* (5; 30.55), *sca5* (5; 35.64) × *anu2* (5; 27.75) and *api6* (5; 109.02) × *den10* (5; 101.94).

Discussion

Much information can be gained from large-scale mutant screens aimed at identifying the genes controlling developmental processes (Nüsslein-Volhard and Wieschaus 1980; Jürgens et al. 1991; Haffter et al. 1996; Berná et al. 1999). Such screens usually yield a large number of mutants, which must then be subjected to complementation and linkage analyses in order to ascertain whether they carry mutations in as yet undescribed genes or new alleles of already known genes. This can only be done for a large group of new mutants in a short period of time if a high-throughput procedure such as the one used in this paper is available.

Almost twenty years have passed since the first large *Arabidopsis* genetic map, which included 76 loci, was obtained (Koornneef et al. 1983). Such pioneering work was based on the use of classical genetic markers, which were later used to map more than two hundred *EMBRYO DEFECTIVE* (*EMB*) genes (Patton et al. 1991; Castle et al. 1993; Franzmann et al. 1995), obtained in the laboratory of D. Meinke in an attempt to saturate the genome for mutations affecting embryonic development. Allelism tests involving members of such a large collection of *emb* mutants were limited to those with neighboring map positions (Franzmann et al. 1995). In this case, proximity between genetic map positions was considered to be a better criterion than an apparent resemblance between phenotypes on which to base the choice of candidates for allelism tests. More recently, molecular markers have been used to map nine genes that cause organ fusion (Lolle et al. 1998), nine *SENSITIVE TO FREEZING* (*SFR*) genes (Thorlby et al. 1999), and nine *RESPONSIVE TO DEHYDRATION* (*RD*) and 16 *EARLY RESPONSIVE TO DEHYDRATION* (*ERD*) genes (Taji et al. 1999).

We contribute here to enriching the genetic map of *A. thaliana* by determining the positions of 76 genes identified by mutation, whose activity is required for the leaf to acquire its wild-type architecture. The mapping of this large collection of mutations that perturb leaf development establishes the basis for future advances that will shed light on the processes involved in the making of a leaf. On the other hand, our collection of mutants, together with their map positions presented here, will facilitate studies of leaf mutants isolated by others.

Although until recently map-based cloning has been considered time-consuming and expensive in *A. thaliana*, techniques developed in recent years, and the information provided by the *Arabidopsis* Genome Initiative, now make it possible to complete positional cloning projects in a short period of time (Lukowitz et al. 2000). The mapping data, as well as the F_2 mapping populations obtained in this work, lay the foundations for attempts to positionally clone the mutated genes, which will be identified by narrowing down the genetic intervals presented in this paper. In fact, efforts to clone some of the genes mapped in this work are already underway, in collaboration with other groups.

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