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Genetic analysis of leaf form mutants from the *Arabidopsis* Information Service collection

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Abstract Although a vast inventory of morphological mutants of *Arabidopsis thaliana* is available, only some have been used for genetic studies of leaf development. Such is the case with the *Arabidopsis* Information Service (AIS) Form Mutants collection, assembled by A. R. Kranz and currently stored at the Nottingham *Arabidopsis* Stock Centre, which includes a large number of mutant lines, most of which have been little studied. With the aim of contributing to the genetic dissection of leaf ontogeny, we have subjected 57 mutant lines isolated by others to genetic analysis; 47 of which were from the AIS collection. These are characterized by vegetative leaves of abnormal shape or size, and were chosen as candidates for mutations in genes required for leaf morphogenesis. The mutant phenotypes studied were shown to be inherited as single recessive Mendelian traits and were classified into 10 phenotypic classes. These mutant strains were found to fall into 37 complementation groups, 7 of which corresponded to known genes. Results of the phenotypic analysis and data on the genetic interactions of these mutants are presented, and their possible developmental defects discussed.

Key words *Arabidopsis* leaf mutants · Leaf morphogenesis · *asymmetric leaves* · *compact rosette* · *denticulata*

Introduction

The isolation of morphological mutants has played a crucial role in the identification of genes controlling developmental processes, as well as in the elucidation of their functions and regulatory interactions. Major insights have been obtained by isolating and studying morphological mutants in animals. Indeed, the work of Lewis (1978) and Nüsslein-Volhard and Wieschaus (1980) in *Drosophila melanogaster*, as well as that of Haffter et al. (1996) in *Danio rerio*, has been epoch-making in the field of developmental biology. In plant developmental biology, some notable examples of analyses based on the study of large groups of morphological mutants are those on flower morphogenesis (reviewed in Weigel and Meyerowitz 1994), embryonic development (Jürgens et al. 1991) and trichome cell morphogenesis (Hülskamp et al. 1994) in *Arabidopsis thaliana*. In all these cases, the genetic and molecular characterization of morphological mutants has allowed the identification of genes that specify developmental cell fates and determine the final architecture of specific organs.

From a developmental genetic point of view, current knowledge of the leaf is far inferior to that of other plant organs or tissues, such as flowers and roots. Many mutations affecting leaf architecture are known in several plant species, some of which have been analyzed at both the genetic and molecular level (reviewed in Hake and Sinha 1991; Smith and Hake 1992; Sinha et al. 1993; Telfer and Poethig 1994; Tsukaya 1995; Hall and Langdale 1996; Sylvester et al. 1996; Poethig 1997; Brutnell and Langdale 1998; Van Lijsebettens and Clarke 1998), but many fundamental questions concerning the ontogeny of leaves remain unanswered. With the aim of contributing to the dissection of the mechanisms underlying leaf ontogeny, we are using a genetic approach to the causal analysis of leaf morphogenesis, based on the isolation of *A. thaliana* mutants with abnormalities in the venation pattern, marginal configuration, shape or size of their rosette leaves as a starting point. We have screened for such variations in ecotypes (Candela et al. 1999; Serrano-Cartagena, Pérez-Pérez and Micol, submitted), among mutants from already existing collections (this work; Serrano-Cartagena, Candela, Robles and

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Micol, submitted), and among newly isolated mutants (Berná, Robles and Micol 1999).

One of the largest collections of plant morphological mutants available is the *Arabidopsis* Information Service (AIS) Form Mutants collection, assembled by A. R. Kranz and currently stored at the Nottingham Arabidopsis Stock Centre (NASC). It includes mutants isolated by G. Röbbelen during the period 1964–1969 (NASC stock numbers N300-N429) and by A. R. Kranz from 1980 to 1986 (N430-N462) (Bürger 1971; Kranz and Kirchheim 1987, 1990; Anderson 1993). Such a large collection of morphological mutants represents an indisputable treasure for research projects devoted to the genetic dissection of leaf morphogenesis. However, the usefulness of this collection is seriously limited by the paucity of information available on the phenotypes, allelic relationships, map positions and genetic interactions of its mutant lines. Some of this information is provided in this paper, in which we present the results of a genetic analysis of 57 leaf mutants, 47 of which belong to the AIS Form Mutants collection.

Materials and methods

Plant materials and growth

A. thaliana (L.) Heyhn. seeds used in this work were supplied by the Nottingham *Arabidopsis* Stock Centre (NASC) and the *Arabidopsis* Biological Resource Center (ABRC, Columbus, Ohio) (stock numbers starting with "N" or "CS" were obtained, respectively, from NASC or ABRC). 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 1:1:1 mixture of perlite, vermiculite and sphagnum moss) cultures were grown as described in Ponce et al. (1998), at 20 ± 1° C and 60–70% relative humidity under continuous illumination with 7000 lx. For gibberellic acid (GA_3; Sigma G1025) treatment, the hormone was added to the medium at a concentration of 500 μM.

Crosses

Immature flower buds to be used as female parents were emasculated with forceps and cross-pollinated under a binocular microscope using mature anthers as pollen donors. Each cross was attempted at least three times by using as pollen receptors several flowers of the same plant. For outcrosses and backcrosses, recessive mutants were used as female parents to permit easy discrimination of successful intercrosses from self-pollination events.

Genetic mapping

Representative alleles of some of the genes identified after complementation analysis were used to perform linkage analysis using SSLP markers (Bell and Ecker 1994). All the mutant lines subjected to linkage analysis in this work were derived from the Enkheim-2 (En-2) ecotype, which is known to be polymorphic with respect to Landsberg *erecta* (L*er*) for most SSLP molecular markers (Ponce et al. 1999). Hence, mutant lines were first crossed with L*er* and phenotypically recessive individuals among the F_2 progeny were collected and their DNA isolated as described in Edwards et al. (1991). Multiplex PCR amplification conditions, fluorescently labelled oligonucleotide sets and automated fragment sizing of amplified microsatellites were as described in Ponce et al. (1999). Map distances were determined using the Kosambi (1944) map function.

Results

Phenotypic classification of mutant lines

We requested a total of 172 strains from the NASC and the ABRC, which were described as leaf mutants in the respective catalogs; 161 of these belong to the AIS collection of Form Mutants. Of these, a total of 57 mutant lines could not be studied, as they did not germinate after several sowings (10 lines), were not available from the NASC for more than a year (10 lines), or displayed only subtle mutant phenotypes under our growth conditions (37 lines) (Table 1). The remaining 115 strains were subjected to genetic analysis, as they presented mutant phenotypes that were clearly distinguishable from the wild type, and appeared consistently and with complete penetrance and only small variations in expressivity in at least two consecutive generations of selfing. In order to facilitate such an analysis, mutants were grouped into phenotypic classes defined on the basis of the kind of morphological alteration displayed by their leaves (Table 1 and Fig. 1). Such phenotypic classes were named using Latin words referring to the most conspicuous trait of each mutant phenotype, following Berná, Robles and Micol (1999), except for the already assigned class names Asymmetric leaves (Rédei and Hirono 1964) and Tortifolia (Reinholz 1947). Two class names, Compact rosette and Filiforme, are used for the first time in this work (see below). Some complementation groups established in this work were assigned provisional gene numbers (*ANG5–ANG7*, *DEN18–DEN28*, *TCU4* and *EXI8*), given that the corresponding AIS lines displayed phenotypes similar to those of EMS-induced mutants previously isolated in our laboratory (*ang1–ang4*, *den1–den17*, *exi1–exi7* and *tcu1–tcu3*; Berná, Robles and Micol, 1999). Linkage and complementation analyses are in progress in order to make these assignments definitive or eventually modify the gene numbers used in this paper.

A study of the phenotypic class that we named Incurvata, because of their involute leaves, was considered to be beyond the scope of this work. This class includes 22 strains (Table 1) that were shown to fall into 10 different complementation groups, two of which correspond to the previously described genes *CURLY LEAF* (Goodrich et al. 1997) and *HASTY* (Telfer and Poethig 1998). Details of this study will be published elsewhere (Serrano-Cartagena, Candela, Robles and Micol, submitted).

Determination of inheritance patterns

A total of 93 mutant lines were backcrossed to their ecotype of origin, which in most cases was En-2 (Fig. 1A). CS3240, CS3250, CS3257, CS3138, CS3397

Table 1 Phenotypic classification of mutant lines with abnormal leaves studied in this work

Affected structure	Phenotype	Phenotypic class	Mutant lines[a]
Leaf lamina	Involute	Incurvata (Icu)	N313, N314, N328, N329, N345, N346, N347, N348, N350, N351, N419, N242, N311, N330, N349, N353, N357, N373, N379, N400, N401, N431 [22]
	Revolute	Transcurvata (Tcu)	N423 (*tcu4*), N424 [2]
	Spirally rolled downwards	Ultracurvata (Ucu)	CS3397 (*inl*[b]) [1]
	Rotated round the petiole	Tortifolia (Tor)	N378 [1]
	Heart-shaped	Asymmetric leaves (As)	N230 (*as2-13*), N321 (*as1-14*), N444 (*as1-15*), N463 (*as2-12*), CS3240 (*as1-16*), CS3250 (*as1-17*) [6]
	Pointed	Angusta (Ang)	N241 (*ang5*), N333 (*ang6*), N336 (*ang7*) [3]
	Needle-shaped	Filiforme (Flr)	N325 (*flr*), CS3254 (*flv*[b]) [2]
	Small	Exigua (Exi)	N438 (*exi8*), N442, N450, N461 [4]
Leaf margin	Serrated	Denticulata (Den)	N243 (*den18*), N308, N316 (*den19*), N320 (*den20*), N322, N323 (*den21*), N343 (*den22*), N360, N363, N371 (*fas1-11*), N381, N383, N384, N405 (*den23*), N407 (*den24*), N408, N415, N421, N429 (*den25*), N436, N437, N443 (*den26*), N447(*yi-2*), N451(*den27-1*), N452 (*den27-2*), N457, N458, CS3257 (*se*[c]), CS3138 (*den28*) [29]
Rosette	Compact, with bushy inflorescence	Compact rosette (Cro)	N303 (*cro1-1*; *det2-11*), N317(*cro4-1*), N318 (*cro2-1*), N319 (*cro1-2*; *det2-12*), N331 (*cro7*), N340, N352 (*cro1-3*; *det2-13*), N355 (*cro2-2*), N356 (*cro2-3*), N359 (*cro1-4*; *det2-14*), N365 (*cro3-1*; *dwf4-3*[d]), N374 (*cro3-2*; *dwf4-4*[d]), N387 (*cro1-5*; *det2-15*), N388 (*cro4-2*), N389 (*cro1-6*; *det2-16*), N390 (*cro1-7*; *det2-17*), N398 (*cro6-1*), N399 (*cro5*), N402 (*cro6-2*), N409 (*cro3-3*), N416 (*cro1-8*; *det2-18*), N417 (*cro4-3*) [22]
	Compact, with nonbushy inflorescence	Compact rosette (Cro)	N254 (*cro10*), N307, N334 (*cro11*), N341, N342, N354, N368 (*cro12*), N370, N375, N377, N380, N403 (*cro9*), N411, N420, N422, N425, N427, N428, N445 (*cro8-1*), N446 (*cro8-2*), N454, N455, N462 (*cro13*) [23]
Other	Wild-type		N300, N302, N304, N305, N312, N315, N326, N327, N335, N337, N338, N339, N344, N361, N362, N364, N367, N369, N376, N385, N386, N392, N393, N396, N397, N410, N412, N413, N430, N435, N439, N440, N441, N448, N453, N459, N460 [37]
	Not germinated		N301, N306, N309, N310, N372, N391, N395, N404, N406, N434 [10]
	Not available		N332, N358, N382, N394, N414, N418, N432, N449, N456, N464 [10]

[a] Mutant lines are designated by their NASC/ABRC stock numbers. Allele names are indicated in *parentheses* for those strains that were subjected to genetic analysis (see text). Most allele names were assigned in this work. Exceptions are noted below. The total number of mutant lines in each class is shown in brackets. Of the 172 lines listed, 41 affect the leaf lamina, 29 the leaf margin and 45 the rosette. The remaining 57 were not studied further

[b] Name assigned by Rédei (cited in ABRC catalog 1995)

[c] Rédei and Hirono (1964)

[d] Azpiroz et al. (1998)

and CS3254 were backcrossed to Col-1, N243 to Dijon-G, N303 and N308 to An-1, and N241 and N254 to S96. Despite repeated attempts no viable offspring were obtained from 20 such backcrosses – those involving N307, N322, N340, N363, N378, N380, N381, N383, N384, N408, N420, N421, N424, N436, N437, N442, N450, N454, N457 and N461. This reduced to 73 the number of lines under study. All the F_1 progenies obtained were shown to be wild-type, suggesting that all the mutant phenotypes had been transmitted as recessive traits. Self-pollination of such F_1 individuals yielded 3:1 (wild-type:mutant) F_2 phenotypic segregations in 56 cases, indicating that the mutant phenotypes were monogenic traits, while a 15:1 ratio was more likely for one line, N325. The mutant phenotype was absent or appeared with variable expressivity in 13 F_2 populations (N308, N341, N342, N354, N360, N370, N415, N422, N425, N427, N428, N455 and N458), whereas more than one mutant phenotype was found in the remaining three (N375, N377 and N411).

The Asymmetric leaves (As) class

Mutant strains in this group are phenotypically similar to those previously termed Asymmetric leaves by Rédei and Hirono (1964) (Fig. 1B, C). The lamina of the vegetative and cauline leaves is broad and weakly wrinkled, with the margins unevenly curled downwards; this slightly modifies the bilateral symmetry of the organ with respect to the axis defined by the midvein, which is prominent. Adult vegetative leaves are more lobed and

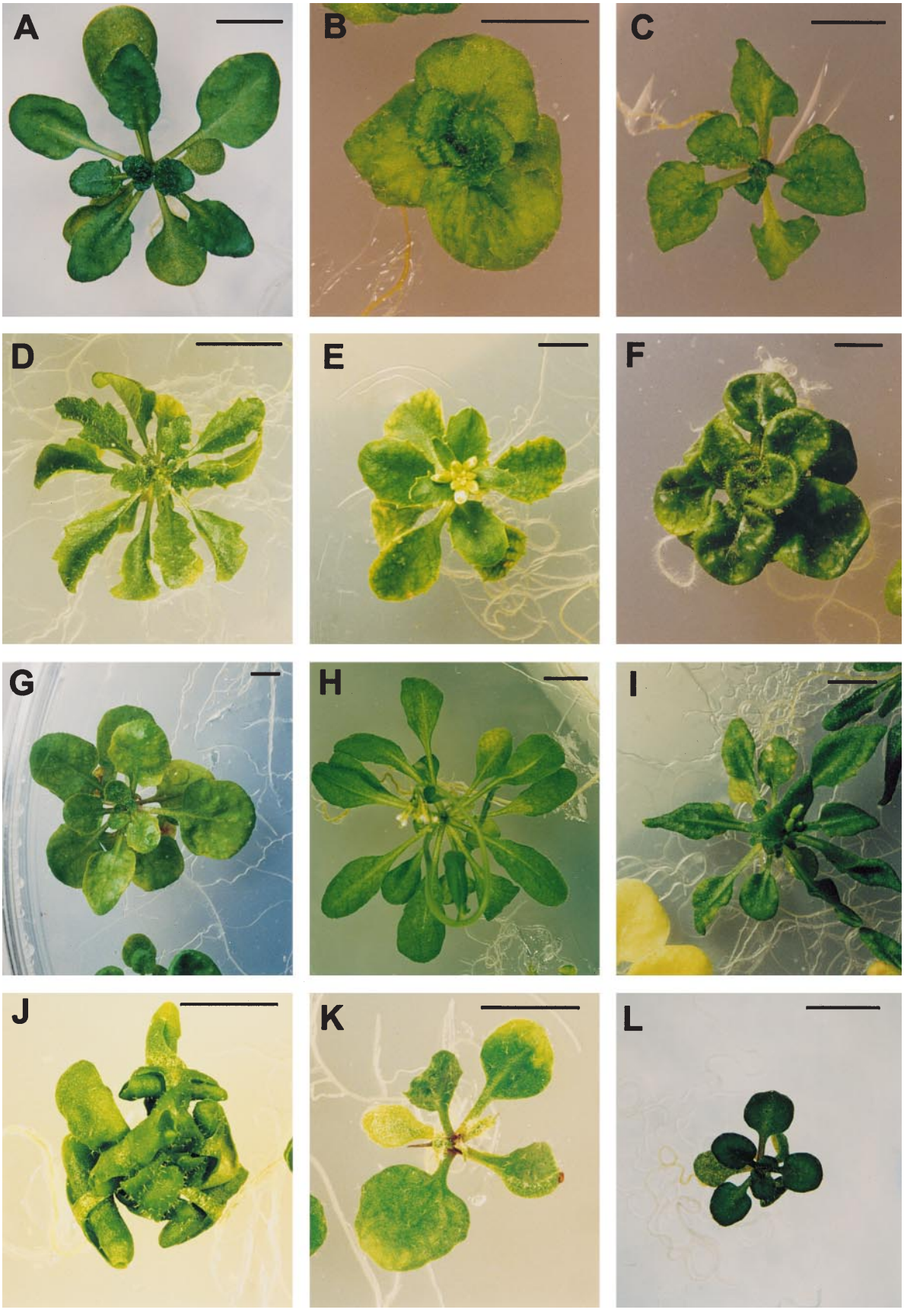
A
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Fig. 1A–L Representative examples of the phenotypic classes of mutants studied in this work. **A** En-2 wild-type plant. **B** N321 (*as1-14*/*as1-14*). **C** N463 (*as2-12*/*as2-12*). **D** N451 (*den27-1*/*den27-1*). **E** N447 (*yi-2*/*yi-2*). **F** N417 (*cro4-3*/*cro4-3*). **G** N334 (*cro11*/*cro11*). **H** N241 (*ang5*/*ang5*). **I** N424, a mutant representative of the Transcurvata phenotypic class. **J** CS3397 (*inl*/*inl*). **K** CS3254 (*flv*/*flv*). **L** N461, a mutant representative of the Exigua phenotypic class. Scale bars indicate 5 mm. Photographs were taken 21 (**A**), 22 (**B** and **C**), 32 (**D**, **E**, **G** and **I**), 27 (**F**), 42 (**H**), 35 (**J**), 15 (**K**) and 24 (**L**) days after sowing

asymmetric than the juvenile ones. Mutants in this group also display reduced fertility and abnormal flowers with short petals and a prominent pistil. The inflorescence is convoluted and the siliques are curled.

Six mutant lines were grouped in this class (Table 1), which in turn can be phenotypically classified into two groups. The first one includes N321 (Fig. 1B), N444, CS3240 and CS3250, which display a dense rosette due to the reduced length of the petiole of the leaves. The second group includes N230 and N463 (Fig. 1C), which display a loose rosette composed of leaves whose margins show a more pronounced downward curl in the apical region. Mutants in these two groups are very similar to the previously described *asymmetric leaves 1* (*as1*; Reinholz 1947; Barabás and Rédei 1971) and *asymmetric leaves 2* (*as2*; Fabri and Schäffner 1994), respectively. After the monogenic and recessive nature of the *as* mutations had been established, complementation analysis, including the strains NW146, NW151, CS3374, CS3283 (all previously described alleles of *AS1*) and CS3117 (a mutant line isolated by Rédei and later found to be allelic to *AS2* by Fabri and Schäffner; Schäffner, personal communication), revealed the existence of only two complementation groups, corresponding to the previously known genes *AS1* and *AS2* (Table 2).

In order to identify double mutants, we studied about 200 individuals of the inbred F_2 progeny of phenotypically wild-type F_1 plants from two crosses involving lines representative of the two complementation groups: N321 (*as1-14*/*as1-14*) × N230 (*as2-13*/*as2-13*) and N463 (*as2-12*/*as2-12*) × N321 (*as1-14*/*as1-14*). However, no individuals with an extreme phenotype were found among these populations. On the contrary, the number of plants displaying the As2 phenotype was significantly higher than that of As1 individuals, suggesting an epistatic effect of *as2* mutations upon *as1*, which yields a 9:4:3 phenotypic segregation. In order to confirm that the excess of As2 plants was due to recessive epistasis, F_3 inbred families were established from six F_2 plants displaying the As1 phenotype, chosen from among the progeny of a cross between N463 (*as2-12*/*as2-12*) and N321 (*as1-14*/*as1-14*). Four of these six families included plants displaying an As2 phenotype (Fig. 2A), the most likely genotype for such individuals being *as1-14*/*as1-14;as2-12*/*as2-12*. This was confirmed by crossing them with either *as1-14*/*as1-14;AS2*/*AS2* or *AS1*/*AS1;as2-12*/*as2-12* individuals.

The Denticulata (Den) class

In this class we included lines displaying alterations in the marginal configuration of leaves, leading to incised, toothed or serrated margins (Table 1 and Fig. 1D, E). This is a large and heterogeneous group, some of whose members show long leaves (N308, N316, N320, N343, N371, N381, N383, N405, N429, N451, N452 and N457), long and glabrous leaves (CS3138, named Edward by Rédei), late flowering (N371, N381, N405, N407, N421, N451 and N452), fasciated stems (N371), pigmentation alterations such as a pale colour (N343, N360, N405, N407, N436, N437 and N447), and albino seedlings which progressively take on a green colour (N415 and N458). One of the strains included in this group, CS3257 (Fig. 2C), which is known as *serrate* (*se*; Rédei and Hirono 1964), is early flowering, with narrow cotyledons and trichomes on both the adaxial and abaxial surfaces of all the vegetative leaves, including the juvenile ones.

Backcrosses performed with lines N322, N363, N381, N383, N384, N408, N421, N436, N437 and N457 were not successful, so that it was impossible to determine the inheritance patterns of the underlying mutations. Four

Table 2 Complementation analysis of *asymmetric leaves* mutants

Female	Male										
	NW146	NW151	N321	N444	CS3374	CS3283	CS3240	CS3250	CS3117	N230	N463
NW146			−*		−	−			+		+*
NW151				−			−*	−*	+	+	+
N321	−			−			−	−	+	+	+
N444		−*	−						+	+	+
CS3374	−*					−*			+*	+	
CS3283	−*				−				+		+*
CS3240		−	−*					−			+
CS3250		−	−*				−				+
CS3117	+*	+*	+*	+*	+	+				−	−*
N230		+*	+*	+*	+*				−*		−*
N463	+	+	+	+		+	+	+	−	−	

Complementation or non-complementation is indicated by + or −. An *asterisk* indicates that the reciprocal cross was tested

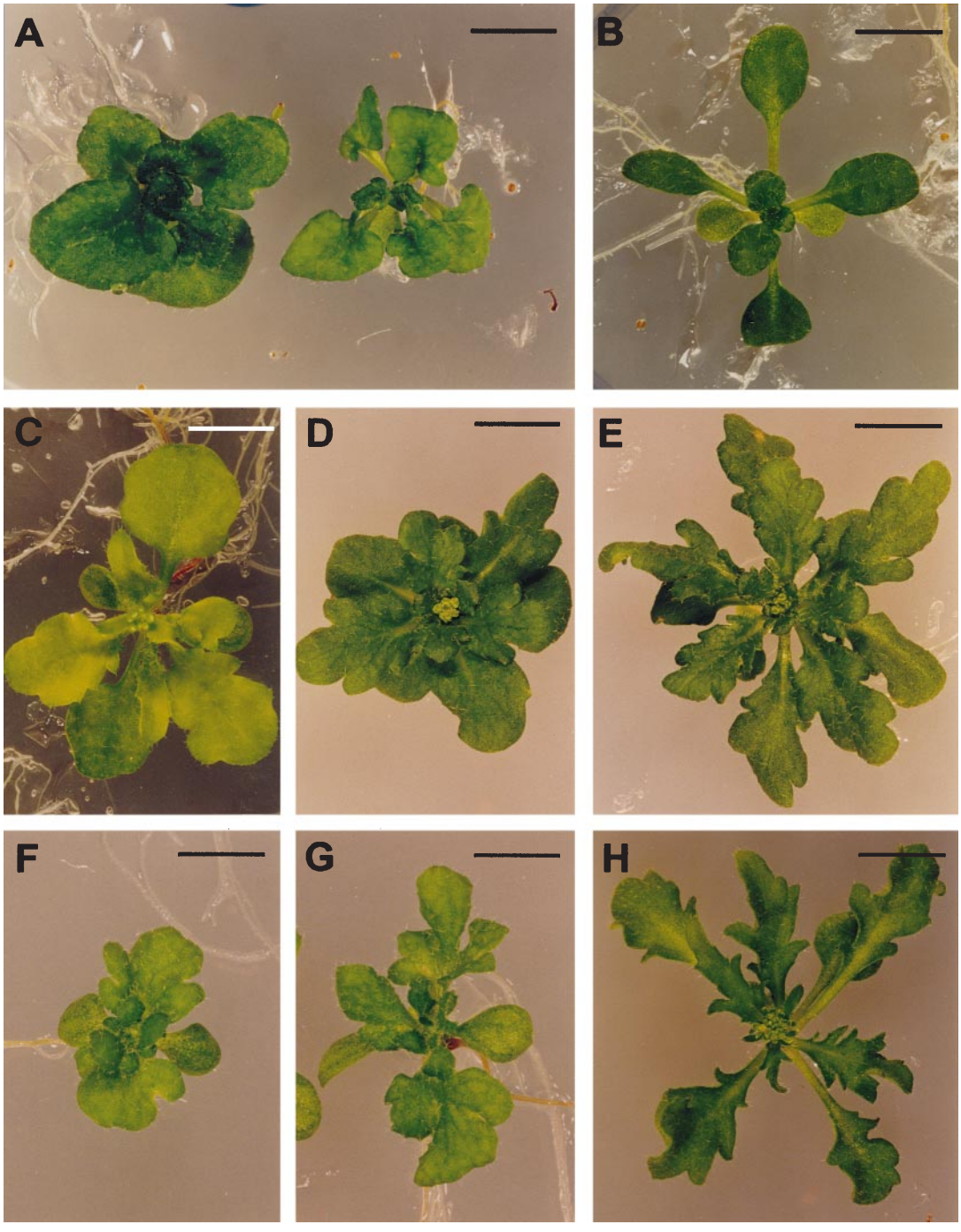

Fig. 2A–H Rosettes of some single and double mutants. **A** *as1-14*/*as1–14* (N321; left) and *as1-14*/*as1-14; as2-12*/*as2-12* (right) individuals. **B** CS3400 (*an-1*/*an-1*). **C** CS3257 (*se*/*se*). **D** *an-1*/*an-1; as1*/*as1*. **E** *an-1*/*an-1; as2*/*as2*. **F** *as1*/*as1; se*/*se*. **G** *as2*/*as2; se*/*se*. **H** *se*/*se; an-1*/*an-1*. Scale bars indicate 5 mm. Photographs were taken 26 (**A**), 22 (**B**, **E**, **F** and **G**) and 34 (**C**, **D** and **H**) days after sowing

strains (N308, N360, N415 and N458) yielded heterogeneous F_2 populations in which it was not possible to distinguish wild-type from mutant plants. The remaining *denticulata* strains were shown to carry monogenic recessive mutations. Since one of the lines included in this group, N371, showed fasciated stems, we tested for allelism with several other mutants displaying such a phenotypic trait, *miniature* (*min-1*; CS74; Koornneef et al. 1983), *fasciata1* (*fas1-1*; N265; Reinholz 1966), *fasciata2* (*fas2-1*; N266; Leyser and Furner 1992) and *clavata1* (*clv1-1*; NW45; Koornneef et al. 1983). In addition, *min-1*, *fas1-1* and *yellow inflorescence* (*yi-1*; CS91; Koornneef et al. 1983), which exhibit toothed leaves, were included in complementation tests with *den* mutants. The complementation analysis (Table 3) revealed

Table 3 Complementation analysis of *denticulata* mutants

Female	Male[a]																								
	N243	N316	N320	N323	N343	N371	N405	N407	N429	N443	N447	N451	N452	CS3257	CS3138	*yi-1*/*yi-1*	*min-1*/*min-1*	*fas1-1*/*fas1-1*	*fas2-1*/*fas2-1*	*clv1-1*/*clv1-1*
N243		*	*	*	*	*	*		*			*		*	*	*				
N316		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
N320	+		*	*	*	*	*	+	+	*	*	*	*	*	*	*	+			
N323	+	+	+	*	*	*	*	+	+	+	+	+	+	*	*	+	+			
N343	+	+	+	+	*	*	*	+	+	+	+	+	+	*	*	*				
N371	+	+	+	+	+	*	*	+	+	+	+	+	+	*	*	+				
N405	+	+	+	+	+	+	*	+	+	+	+	+	+	*	*			*		
N407	+	+	+	+	+	+	+	*	+	+	+	+	+	*	*	*				
N429	+	+	+	+	+	+	+	+	*	+	+	*	+	*	*	*				
N443	+	+	+	+	+	+	+	+	+	*	+	+	+	+	*	*				
N447	+	+	+	+	+	+	+	+	+	+	*	*	+	+	*	*				
N451	+	+	+	+	+	+	+	+	+	+	+	*	–	+	*	*				
N452	+	+	+	+	+	+	+	+	+	+	+	–	*	+	+	+				
CS3257	+	+	+	+	+	+	+	+	+	+	+	+	+	*	*	*				
CS3138	+	+	+	+	+	+	+	+	+	+	–	+	+	+	*	*				
yi-1/*yi-1*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*				
min1-1/*min1-1*		*	*				*					+					*			
fas1-1/*fas1-1*						–												*		
fas2-1/*fas2-1*						+													*	
clv1-1/*clv1-1*						+														*

[a] Complementation or non-complementation is indicated by + or –. An *asterisk* indicates that the reciprocal cross was tested

the existence of 12 complementation groups: *DEN18–DEN28*, and *SE*. We found that the mutant phenotypes of N371 and N447 (Fig. 1E) are due to alleles of the *FAS1* and *YI* genes, respectively, while no mutant alleles of the genes *FAS2*, *MIN1* and *CLV1* were found among the progeny of the crosses performed (Table 3).

We also studied the effects of gibberellin (GA_3) upon the Serrate phenotype, since this phytohormone is thought to promote phase changes and to anticipate the beginning of reproductive development in maize (Evans and Poethig 1995). Moreover, *Arabidopsis* mutants such as *spindly* (*spy*; Jacobsen and Olszewski 1993) display characteristics of wild-type plants that have been treated with gibberellin, such as early flowering and an elongated phenotype. However, *Arabidopsis* mutants affected in the synthesis or signal transduction of this hormone, such as the GA-responsive mutants *ga1–ga5* (Koornneef and van der Veen 1980) and the GA-insensitive *gai* (Koornneef et al. 1985), show a delay in phase change (Telfer et al. 1997). Seeds of the strain CS3257 and its ecotype of origin, Col-1, were sown in medium supplemented with 500 μM GA_3. We used the time of appearance of abaxial trichomes and floral primordia as developmental stage markers. As shown in Table 4, the addition of GA_3 to the medium anticipates the development of the wild-type Col-1 strain, producing partial phenocopies of the *serrate* mutant. Furthermore, the phenotype of the mutant is enhanced in the presence of the hormone.

The bushy Compact rosette (Cro) class

A large number of mutants from the AIS collection produce a dense rosette due to a reduction in the length of the petioles of their vegetative leaves. Some also shared other traits, such as late flowering and a very bushy inflorescence, so that it was possible to distinguish two different phenotypic classes within this group: the bushy Compact rosette, and non-bushy Compact rosette phenotypes (Table 1).

All bushy *compact rosette* mutants display a similar phenotype, which is clearly distinguishable from the wild-type (Fig. 1F). The rosette is small and very compact, with dark green leaves. These are dwarf and late flowering, bolting about 2 months after sowing. The inflorescence is short, without apical dominance or cauline leaves, and the stems are unbranched, abundant and stiff. As regards the flowers, they present hanging pedicels and shortened petals and stamens, which results in reduced fertility. The study of the progeny of backcrosses revealed the monogenic and recessive nature of all the bushy Cro phenotypes, except for those of strains N319 and N399, which segregated additional phenotypes, presumably caused by mutations unlinked to those causing rosette compactness: *transparent testa glabra* (Koornneef 1981) and *stickle* (Hülskamp et al. 1994), respectively.

The inheritance pattern of the mutant phenotype of N340 could not be established, since all backcross trials

Table 4 Effects of gibberellin on Col-1 and CS3257 lines

Character	Col-1		CS3257	
	$-GA_3$	500 μM GA_3	$-GA_3$	500 μM GA_3
Cotyledons	Dark and rounded	Narrow and pale	Narrow	Large and narrow
Hypocotyl length	Short	Intermediate	Intermediate	Long
Petiole	Normal	Elongated	Elongated	Elongated
Earliest rosette leaf to show abaxial trichomes	Sixth	Third	First	First[a]
Time of appearance of floral primordia (days)	27	20	20	16
Number of rosette leaves	8–10	6–8	5–7	2–4

[a] Showing many abaxial trichomes

were unsuccessful. The complementation analysis established seven complementation groups (Table 5): *CRO1–CRO7*. In addition, a phenotypic gradation was observed among the strains carrying *CRO4* alleles. As regards rosette compactness, height of the inflorescence and fertility, an ordered allelic series can be defined, with *cro4-2* being the strongest allele, *cro4-1* the weakest and *cro4-3* (Fig. 1F) between the two. Such homozygous *cro4* individuals and the hybrid F_1 progeny of their intercrosses can be ordered in a descending series of mutant phenotypic strength as follows: *cro4-2*/*cro4-2* > *cro4-2*/*cro4-3* > *cro4-2*/*cro4-1* ≈ *cro4-3*/*cro4-3* > *cro4-3*/*cro4–1* > *cro4-1*/*cro4-1*.

In an attempt to isolate double mutants, we studied F_2 progenies obtained by selfing F_1 plants heterozygous for non-allelic *cro* mutations (Table 6). We did not find any F_2 individual with an extreme phenotype that could be considered as a putative double mutant. Since parental phenotypes were similar, it was not possible to establish an epistatic effect of one phenotype on the other. However, the 9:7 (wild-type:mutant) F_2 phenotypic ratios that were found clearly suggested the existence of duplicate recessive epistasis; only plants carrying at least one wild-type allele of each of the genes studied displayed the wild-type phenotype.

The rosette phenotype of previously described mutants, which are disturbed in their photomorphogenetic responses, is similar to that of bushy *cro* mutants. For instance, *de-etiolated* (*det*) mutants have short hypocotyls and develop leaves in the dark, displaying a dwarf, dark and compact rosette when grown in the light (reviewed in Wei and Deng 1996; Fankhauser and Chory 1997). Hence, we studied rosette compactness and hypocotyl length, respectively, in light- and dark-grown bushy *cro* mutants, together with the wild-type strain En-2 and the photomorphogenetic mutants *det1-1* (CS6158), *det2-1* (CS6159) and *det3-1* (CS6160) (Chory et al. 1989, 1991; Cabrera y Poch et al. 1993). All the bushy *cro* mutants displayed an abnormal photomorphogenetic response characterized by expanded cotyledons and reduced hypocotyl length after growth in the dark for 10 days. In addition, the phenotype of *det2* mutants was found to be very similar to that of the strongest *cro* mutants, both in the presence and absence of light. Allelism tests revealed that lines belonging to the *CRO1* complementation group were alleles of the *DET2* gene (Table 7).

Mutants deficient in gibberellin biosynthesis, such as *ga1–ga5*, are known to show dwarf compact rosettes, a phenotype that is restored to wild-type by treatment with GA_3 (Koornneef and van der Veen 1980). We therefore studied the effects of this phytohormone upon *cro* mutants and found that the presence of GA_3 did not modify the phenotype of any of them, as is also the case with the gibberellin-insensitive mutant *gai*, which is assumed to be affected in GA perception (Koornneef et al. 1985). The allelism tests performed did not reveal any allelic relationship between *cro1–cro7* mutants and *gai-1*, *ga4-1* or *ga5-1* (Table 7).

CRO2 was shown to map on chromosome 3, at 24.96 ± 10.49 cM from the AthCHIB marker; *CRO4* on chromosome 5, at 17.33 ± 5.41 cM from AthCTR1 and not linked to nga139; *CRO5* on chromosome 4, 5.57 ± 5.46 cM and 12.77 ± 4.71 cM below nga1107 and nga1139, respectively; *CRO6* on chromosome 1, 5.28 ± 3.66 cM and 15.02 ± 5.57 above nga128 and AthGENEA, respectively; and *CRO7* on chromosome 1, between AthGENEA (at 2.57 ± 2.54 cM) and nga111 (at 18.78 ± 7.04 cM). The *cro3* mutations, whose map position was not determined in this work, are alleles of the *DWARF4* (*DWF4*) gene, as recently reported by Azpiroz et al. (1998), who determined that N365 and N374 carry *dwf4* mutations.

The non-bushy Compact rosette (Cro) class

Strains in this group (Table 1) also show a dense rosette due to the reduced length of petioles, but they are not dwarf (Fig. 1G). This is a phenotypically heterogeneous group, since some lines show a yellowish colour (N427 and N428), undulate leaves (N334, N368, N403, N411 and N462), or late flowering (N342, N354, N368, N370 and N377). Backcrosses involving N307, N380, N420 or N454 were unsuccessful. In most of the F_2 populations obtained from backcrosses (N341, N342, N354, N370, N422, N425, N427, N428 and N455), the non-bushy Cro phenotype reappeared with variable expressivity, and in some cases, a second mutant phenotype was found (albino individuals from N375, N377 and N411, as well as glabrous plants from N422). None of these strains were included in the complementation tests (Table 8) that revealed that the remaining ones fell into six complementation groups (*CRO8–CRO13*).

Table 5 Complementation analysis of bushy *compact rosette* mutants

Female	Male																				
	N303	N317	N318	N319	N331	N352	N355	N356	N359	N365	N374	N387	N388	N389	N390	N398	N399	N402	N409	N416	N417
N303			+	−	+	−	+	+	−		+	−	+			+	+		+	−	+
N317			+*		+*				+*				−*	+*	+*	+*	+*		+*		−*
N318	+*	+			+		−	−		+	+		+	+	+*	+	+*		+		+
N319	−*					−											+				+
N331	+*	+	+*			+	+	+		+*	+*	+	+*		+*	+	+		+*		+
N352	−			−*	+		+		−			−*		−	−	+	+	+	+		+*
N355	+		−*		+*	+		−*	+	+	+*		+	+*	+	+	+		+		+
N356	+*		−*		+*		−		+*	+	+		+		+*	+	+*		+*		+*
N359	−	+				−	+	+		+							+				
N365			+*		+		+*	+*	+*		−		+		+	+	+*		−*		+*
N374	+*		+*		+		+	+*		−*						+	+		−*		+
N387	−				+*	−											+		+	−	
N388	+*	−	+*		+		+*	+*		+*					+	+	+*		+		−
N389		+	+*			−*	+									+	+	+	+		+*
N390		+	+		+	−*	+*	+		+			+*				+*		+*		+
N398	+*	+	+		+*	+*	+*	+*		+*	+		+	+*			+	−	+*		+*
N399	+	+	+	+*	+*	+	+	+	+	+	+*	+	+	+	+	+		+	+	+	+
N402						+*								+*		−*	+*		+*		+*
N409	+	+	+*		+	+*	+*	+		−	−	+*	+*	+	+	+	+*	+		+*	+
N416	−*											−*					+*		+		
N417	+*	−	+*	+*	+*	+	+*	+		+	+*		−*	+	+*	+	+*	+	+*		

Complementation or non-complementation is indicated by + or −. An *asterisk* indicates that the reciprocal cross was tested

Table 6 Interactions between some bushy *compact rosette* mutations

Cross (Female × Male)	F_1		F_2		Segregation	
	Wild type	Cro	Wild type	Cro	χ^2 value (9:7)	*P*
N390 (*cro1-7*/*cro1-7*) × N356 (*cro2-3*/*cro2-3*)	23	0	104	94	0.97	0.32
N352 (*cro1-3*/*cro1-3*) × N409 (*cro3-3*/*cro3-3*)	14	0	51	40	0.00	1
N319 (*cro1-2*/*cro1-2*) × N417 (*cro4-3*/*cro4-3*)	34	0	84	82	1.93	0.16
N303 (*cro1-1*/*cro1-1*) × N399 (*cro5*/*cro5*)	22	0	50	40	0.00	1
N398 (*cro6-1*/*cro6-1*) × N416 (*cro1-8*/*cro1-8*)	20	0	64	42	0.58	0.45
N356 (*cro2-3*/*cro2-3*) × N365 (*cro3-1*/*cro3-1*)	35	0	102	90	0.64	0.42
N356 (*cro2-3*/*cro2-3*) × N388 (*cro4-2*/*cro4-2*)	33	0	110	82	0.05	0.82
N399 (*cro5*/*cro5*) × N356 (*cro2-3*/*cro2-3*)	12	0	76	66	0.33	0.57
N409 (*cro3-3*/*cro3-3*) × N417 (*cro4-3*/*cro4-3*)	23	0	62	60	1.25	0.26
N399 (*cro5*/*cro5*) × N409 (*cro3-3*/*cro3-3*)	21	0	61	43	0.16	0.69
N399 (*cro5*/*cro5*) × N417 (*cro4-3*/*cro4-3*)	15	0	106	70	0.98	0.32

Table 7 Complementation analysis of bushy *compact rosette* mutants and mutants with altered photomorphogenetic or gibberellin responses

Female	Male					
	ga4-1/*ga4-1*	*ga5-1*/*ga5-1*	*gai-1*/*gai-1*	*det1-1*/*det1-1*	*det2-1*/*det2-1*	*det3-1*/*det3-1*
N319 (*cro1-2*)					–	
N352 (*cro1-3*)	+	+	+			
N389 (*cro1-6*)				+		
N355 (*cro2-2*)	+	+	+		+	
N356 (*cro2-3*)				+		
N409 (*cro3-3*)	+	+		+	+	+
N417 (*cro4-3*)	+	+	+	+	+	+
N399 (*cro5*)	+	+	+	+	+	+
N398 (*cro6-1*)				+	+	+
N331 (*cro7*)		+				

Table 8 Complementation analysis of non-bushy *compact rosette* mutations

Female	Male								
	N254	N334	N368	N403	N445	N446	N462	*cp2*/*cp2*	*cp3*/*cp3*
N254		+	+	+		+*	+		
N334	+		+*	+	+*	+*	+		
N368	+*	+		+	+		+		
N403	+*	+*	+		+	+*	+	+	+*
N445		+	+*	+*		–	+	+	+
N446	+	+		+	–		+		
N462	+*	+*	+*	+*	+*	+*		+	+
cp2/*cp2*				+*	+*		+*		
cp3/*cp3*				+	+*		+*		

Complementation or non-complementation is indicated by + or –. An *asterisk* indicates that the reciprocal cross was tested

Allelism tests with *compacta2-1* (*cp2-1*; CS47) and *compacta3-1* (*cp3-1*; CS48) mutants, both of which form a dense rosette (Koornneef et al. 1983), did not reveal allelism between *cro* and *cp* mutants.

Poorly represented phenotypic classes

Six of the eleven mutant phenotypic classes defined and studied here were represented by only one or a few strains. One of these was the Angusta (Ang) class, which includes three mutant lines (Table 1) whose leaves are narrow but wild-type in length (Fig. 1H). Their mutant phenotypes were inherited as monogenic and recessive traits. The CS3400 strain, carrying the *angustifolia* (*an-1*) mutation (Rédei 1962) was also included in the complementation analysis of this class, since it displays leaves similar to those of *ang* mutants. Three complementation groups were found (*ANG5–ANG7*; Table 9), none of which was allelic to *an-1*.

The Transcurvata (Tcu) class includes the strains N423 and N424 (Fig. 1I), whose leaves are folded downwards obliquely to the midvein. N423 displays in addition long serrated glabrous leaves, while N424 is

Table 9 Complementation analysis of *angusta* mutants

Female	Male			
	N241	N333	N336	*an-1*/*an-1*
N241		+*	+*	+
N333	+		+	+*
N336	+	+*		+*
an-1/*an-1*	+	+	+	

Complementation or non-complementation is indicated by + or −. An *asterisk* indicates that the reciprocal cross was tested

early flowering, and their leaves seem triangular because of the bending of the lamina. As N424 backcrosses were fruitless, only one putative complementation group could be proposed, *TRANSCURVATA4* (*TCU4*), that includes the recessive mutant strain N423.

The Ultracurvata (Ucu) phenotypic class was only represented by the CS3397 line (Fig. 1J), first named *invalida* (*inl*) by Rédei (ABRC catalog 1995). A recessive mutation causes vegetative and cauline leaves to roll downwards spirally in this line, which carries a second mutation, *gigantea*, causing extremely late flowering (Koornneef et al. 1991). Due to their extremely similar leaf phenotypes, we carried out a complementation test of CS3397 with the *ultracurvata1* (*ucu1*) mutants isolated by Berná, Robles and Micol (1999). Heterozygous strains carrying semidominant *ucu1* alleles were crossed with CS3397, but *inl* turned out not to be an allele of the *UCU1* gene.

We assigned only one strain, N378, to the Tortifolia (Tor) phenotypic class, which was given this name by Reinholz (1947). Both the petiole and the lamina of this late-flowering mutant turn counter-clockwise as it grows. All attempted backcrosses were unsuccessful under our working conditions.

Two mutant lines, N325 and CS3254 (Fig. 1K), were assigned to the Filiforme (Flr) phenotypic class. N325 exhibits dark spotted cotyledons and its first two leaves are narrow – sometimes hook-shaped – and expand late from its base to the apex, finally assuming a conical shape. Vegetative adult leaves are relatively normal and stem fasciation is sometimes observed. The CS3254 strain was named *flavodentata* (*flv*) by Rédei (ABRC catalog 1995) and shows toothed curled leaves, some of which grow mainly in the proximo-distal direction during the early stages of expansion, expanding laterally later. Both strains show leaves with a reddish adaxial surface, while the abaxial one is pale. The phenotypic ratios observed in the F_2 populations obtained from backcrosses indicate the monogenic nature of the CS3254 mutant phenotype, although we found the concurrent action of two independent recessive mutations to be the likely source of the phenotype of N325; alternatively, the viability of the homozygous individuals or some of the mutant gametes may be reduced. Reciprocal crosses involving both strains gave rise to wild-type F_1 populations, so two complementation groups were established, *FLR* and *FLV*, represented by N325 and CS3254, respectively. However, the former of these two assignments is provisional, since the possible contribution of two unlinked mutations to the phenotype of N325 cannot be discounted. No allelism was observed between these strains and others displaying fasciated stems, such as *min-1*, *fas1-1*, *fas2-1*, or *clv1-1*.

Very small leaves, initially dark but acquiring a violet colour at later stages of development characterize the four lines included in the Exigua (Exi) phenotypic class (Fig. 1L). All of them are late flowering with a small bushy inflorescence and reduced fertility. Since we did not succeed in backcrossing N442, N450 and N461, it was only possible to determine the inheritance pattern of the phenotype of N438 as a monogenic recessive trait. Hence, only a single putative complementation group could be inferred: *EXIGUA8* (*EXI8*).

Genetic interactions among mutations of different phenotypic classes

The vast majority of the crosses performed in this work involved mutants within the same phenotypic class. However, we also obtained some double mutants from strains with non-related phenotypes such as *angustifolia* (phenotypically similar to the mutants of our Angusta class; Fig. 2B), *serrate* (included in the phenotypic class Denticulata; Fig. 2C), *as1* and *as2* (from the phenotypic class Asymmetric leaves; Fig. 1B, C). Several F_2 individuals displaying additive phenotypes were chosen and their putative nature as double mutants confirmed by the absence of phenotypic segregation in their inbred F_3 progeny. In all cases we observed simple additivity of mutant phenotypes (Fig. 2D–H), which suggests that the processes perturbed in each of the mutant lines studied are independent.

Discussion

During the past four decades, experiments have been performed in many laboratories on the induction and selection of *A. thaliana* mutants. Since in most cases a large number of visible mutants of different types were isolated, researchers had to limit themselves to making only superficial observations of gross plant morphology for each mutant. Aware that their mutants would be a valuable contribution to the *Arabidopsis* community, several researchers kept and made publicly available collections of morphological mutants (examples are Cetl et al. 1969; Relichova 1976); their generous legacy is currently stored at the NASC, ABRC and SENDAI resource centers.

The largest available collection of *A. thaliana* mutants is that of the *Arabidopsis* Information Service, created from variants isolated in different mutagenesis experiments, which resulted in the selection of morphological

or colour mutants. The so-called Form Mutants make up part of this collection, many of which are characterized by their abnormal leaf morphology. AIS Form Mutants were isolated by G. Röbbelen and A. R. Kranz (see Introduction), in most cases from the Enkheim-2 (En-2) ecotype. While Kranz mainly used heavy charged particles derived from neon, krypton or cosmic rays as mutagen (Bork and Kranz 1984; Kranz and Bork 1984), and in some cases ethyl methanesulphonate, the mutagen used by Röbbelen is unknown. Since the information available for each of the AIS Form Mutant lines comprises only a brief description of the phenotypes and their genetic background, we decided to subject them to genetic analysis in order to increase the usefulness of the collection, focusing on mutants that displayed aberrant leaf morphologies, which we suspected might be affected in genes involved in leaf morphogenesis.

In order to facilitate the complementation analysis of the mutants, we first classified a total of 115 lines into 11 phenotypic groups, which were defined mainly on the basis of leaf morphology. The study of one such class, named Incurvata because of the involute leaves seen in the corresponding 22 mutant lines, will be presented elsewhere. We attempted to determine the inheritance patterns of the mutant phenotypes of the remaining 93 lines. Sixteen strains were not suitable for genetic analysis, since their mutant phenotype appeared with variable expressivity or was not observed in the F_2 progeny from backcrosses. Backcrosses were unsuccessful for a further 20 lines. The mode of inheritance was clearly established for 57 lines, the mutant phenotype of 56 of them being a monogenic and recessive trait as shown by the 3:1 (wild-type:mutant) phenotypic segregation found in the F_2 progeny of backcrosses. Only one mutant line was suspected of carrying mutations in two unlinked genes, due to the 15:1 ratio observed in the F_2.

The phenotypic groups that we named Denticulata and Compact rosette, which are characterized by leaves with toothed margins and dense rosettes, respectively, were the most common, and accounted for 74 mutant strains. As we do not know the selective criteria followed to isolate the strains studied in this work, we could not establish whether or not the large numbers of lines in these classes result from directed screening aimed at isolating mutants with these particular phenotypes. Alternative explanations for this large number of mutants are the conspicuous nature of their phenotypes (which might lead to their preferential isolation), or the existence of several different processes whose perturbation leads to such phenotypes. In fact, the Denticulata class is the most numerous group described in this work, and includes a total of 29 strains displaying pleiotropic alterations in several organs, which suggests that several processes exist whose perturbation leads to the appearance of incisions or prominences in the leaf margin. Of the 15 *denticulata* strains subjected to complementation analysis, only two carried allelic mutations, N451 and N452 (*den27-1*/*den27-1* and *den27-2*/*den27-2*). This fact suggests again that the number of genes that can be mutated to generate a Denticulata phenotype is large. Similar results have been obtained by Berná, Robles and Micol (1999), who found 19 *denticulata* mutants in a large-scale screen for EMS induced *Arabidopsis* mutants; these fell into a total of 17 complementation groups, only two of which had two alleles.

The Denticulata phenotype in the leaves could be due to localized cell death or disruption of the wild-type pattern of cell division. Thirteen of the *denticulata* mutants are characterized by serrated long leaves, an observation that can be interpreted as the anticipated presence in juvenile leaves of traits that normally appear only in adult leaves. In fact, the number of incisions in the margin of vegetative leaves of some ecotypes progressively increases in successive plastochrons, the adult rosette leaves being bigger and more serrated than the juvenile ones (Röbbelen 1957; Martínez-Zapater et al. 1994; Serrano-Cartagena, Pérez-Pérez and Micol, submitted). In this way, the expansion of the leaf along the proximo-distal axis and the increase in the number of teeth are related not only in wild-type strains but also in some *denticulata* mutants, suggesting the existence of a control shared by both processes.

We have established that two of the *den* mutants carry new alleles of previously described mutants. The fasciated stem of N371, besides being late flowering and having leaves with serrated margins, led us to test *fas1* (Leyser and Furner 1992) for allelism, and we found that the mutations were indeed allelic. On the other hand, we found that the N447 strain carries an allele of *yellow inflorescence* (*yi*), a mutation used as a phenotypic marker in linkage analysis, which causes yellow-coloured flower buds and vegetative leaves with a dentate margin (Koornneef et al. 1983). If one includes N447, seven mutants of this phenotypic class show alterations in leaf pigmentation, which suggests that some metabolic dysfunction could cause the alteration of leaf marginal configuration, among other effects.

Very little has been published about the *serrate* (CS3257) mutant, apart from the proximity of the *SERRATE* gene to *ERECTA* (Rédei and Hirono 1964). CS3257 is early flowering, shows a reduced number of vegetative leaves (5–6) compared to its ecotype of origin Col-1 (8), abaxial trichomes in all its vegetative leaves, and longer hypocotyl and petioles than the wild type. These features suggest that *serrate* is a heterochronic mutant that lacks the juvenile rosette phase, its vegetative development beginning at the adult rosette stage of the wild-type and therefore being early flowering. This effect is evident in the double mutants *se as1* and *se as2*, in which juvenile leaves are lobed, like the adult leaves of *as1* and *as2* individuals. On the other hand, we have demonstrated that GA_3 treatment enhances the phenotype of *se* individuals to an extreme degree, as well as producing partial phenocopies of the *se* mutant in Col-1. Two hypotheses could explain these observations: on the one hand, the *se* mutation could increase the sensitivity of the plant to

gibberellin, which would explain why the endogenous level of the hormone causes in the mutant a phenotype that is similar to that obtained after GA_3 treatment of the wild-type strain. On the other hand, the *se* mutation could cause an increase in the endogenous level of the hormone. The recent cloning of the *SE* gene (M. Prige and D. R. Meeks-Wagner http://genome-www.stanford.edu/Arabidopsis/madison98/abshtml/038.html), which has been found to code for a zinc finger protein, will allow elucidation of its role in leaf development.

The Compact rosette classes also account for a large number of mutants, including 45 strains that display a dense rosette due to the short petiole of vegetative leaves. The phenotype of the 22 dwarf mutants belonging to the bushy Cro class, which display a late-flowering and bushy inflorescence held together by numerous stiff and unbranched stems, is similar to those named, more than a century ago, "dwarf forms" of *Arabidopsis thaliana* (Viviand-Morel 1877–78, cited in Napp-Zinn and Bonzi 1970). Some such dwarf forms are partially normalized by treatment with GA_3 (Koornneef 1978).

The genetic analysis of bushy *cro* mutants revealed that all of them carried recessive mutations that corresponded to seven genes. Attempts to identify double mutants in the F_2 populations from dihybrid crosses revealed in every case a 9:7 (wild-type:mutant) phenotypic segregation, a typical result for mutations that affect genes whose products catalyze different steps of a linear metabolic pathway, their phenotype being due to the absence of the end product of the pathway. Hence, it is likely that *CRO1–CRO7* are involved in the same metabolic pathway. On the other hand, *cro* mutants are similar to others previously described either as disturbed in the photomorphogenetic response or affected in the synthesis, perception or signal transduction of gibberellin. These similarities led us to study the behavior of *cro* mutants in darkness and their response to GA_3, as well as to carry out allelism tests with the above-mentioned mutants. We found that all bushy *cro* mutants displayed a perturbed photomorphogenic response, and were deetiolated in the dark. However, gibberellin treatment was not able to restore the wild-type phenotype in *cro* mutants. We found that *cro1* mutants were alleles of the *DET2* gene, which is known to code for a steroid reductase involved in the biosynthesis of brassinosteroids (Li et al. 1996). In addition, two of the three allelic mutations that we assigned to the *CRO3* complementation group have recently been reported to be allelic to the *dwarf4-1* mutation, whose mutant phenotype can be rescued by exogenously supplied brassinolide (Azpiroz et al. 1998). Taken together, these results suggest that the remaining *CRO* genes might be involved in the synthesis, perception or signal transduction of steroid phytohormones. Additional support for this idea comes from the map positions of *CBB2* (Kauschmann et al. 1996; also named *BRI1* by Clouse et al. 1996, and *BIN1* by Li and Chory 1997), *CBB1* (Altmann et al. 1995; also named *DWF1* by Feldmann et al. 1989, and *DIM1* by Takahashi et al. 1995) and *CBB3* (Altmann et al. 1995; also named *CPD* by Szekeres et al. 1996, and *DWF3* by Altmann et al. 1995), which are close to those of *CRO5*, *CRO2* and *CRO4*, respectively. The phenotype of *cbb1* and *cbb3* mutants is known to be due to deficiencies in brassinosteroid biosynthesis, and that of *cbb2* to perturbations in the perception or signal transduction of these hormones, or in their further metabolic conversion.

As regards the heart-shaped leaves of the *asymmetric leaves* mutants, although the *as1* mutation has been used as a marker in mapping experiments for a long time, this is the first time that allelism has been established among the different strains in the NASC and ABRC which clearly show an Asymmetric leaves phenotype. The epistatic effect of *AS2* upon *AS1*, which we have demonstrated, suggests that both genes are involved in the same process. In contrast, the additivity of the As1 and As2 phenotypes with the Serrate and Angustifolia phenotypes in the corresponding double mutants strongly suggests that the processes in which the *SE*, *AN* and *AS* genes act are independent. The broad lamina of *asymmetric leaves* mutants suggests that they might be affected in the patterns of cell division, or in the lateral control of cell expansion, as is observed in mutants with rounded leaves such as *rotundifolia* (Tsuge et al. 1996).

Little can be said with reference to the heterogeneous group of non-bushy Cro mutants. Mapping of the six genes belonging to this group will allow us to establish their proximity to previously described loci, which will become candidates for allelism tests with *cro* mutants. On the other hand, the phenotypic class Angusta comprises mutants showing leaves that are longer and narrower than those of their ecotype of origin. They belong to three complementation groups, all of them different from *ANGUSTIFOLIA* (*AN*; Rédei 1962), whose molecular nature has not yet been determined. As proposed for the *an-1* mutation (Tsuge et al. 1996), *ang* mutants might be altered in the polar control of lateral cell expansion, so that they form narrow leaves.

The small size of *exigua* mutants suggests that they might be affected in the control of cell expansion or division, the two fundamental processes responsible for the final size of a plant. The alteration of the flattened structure in the leaves of the *transcurvata* mutants may be due to the modification of mechanisms which coordinate the division and/or expansion of the dorsal and ventral cells of the leaf. The Ultracurvata phenotype is an extreme example of deviation from the flattened structure that characterizes *Arabidopsis* vegetative leaves. We have established that the only mutant assigned to the Ultracurvata class, *invalida* (*inl*), is not an allele of the *UCU1* gene (Berná, Robles and Micol, 1999). The strong similarity between the phenotypes of these two mutants could indicate their involvement in the same biological process.

Finally, the two strains forming the Filiforme class show some needle-like leaves with an almost circular cross-section. One of these strains was first isolated and named Flavodentata by Rédei, since most of their leaves

were yellow with incised margins. Adult leaves frequently grow without expanding laterally, ultimately acquiring a conical shape. The two genes affected in these mutants could be involved in lateral expansion processes that take place after leaf primordium initiation (McHale 1993) or in the specification of dorsoventrality, as occurs in the *phantastica* mutants of *Antirrhinum majus*, which show needle-shaped leaves (Waites and Hudson 1995; Waites et al. 1998).

The *Arabidopsis* Information Service collection of form mutants is in itself a proof of the large number of genes that, when mutated, affect *Arabidopsis* leaf morphology. In fact, in a large-scale screen for viable EMS-induced mutants which approached but did not reach saturation of the genome, 94 different genes were found to yield mutations causing abnormal leaf morphology (Berná, Robles and Micol, 1999). Such a large number of available leaf mutants, many of which display pleiotropic phenotypes, might support the idea that leaf morphogenesis is a nebulous field of study, since the number of processes affecting leaf morphology seems to be considerable. However, the only way to discriminate mutations which perturb general processes and lead to alterations in leaf morphology from mutations in genes specifically involved in leaf morphogenesis is to study the available mutants that display abnormalities in their leaves. The results presented here of the phenotypic and genetic analysis of AIS Form Mutants provide useful information which will facilitate the future work of investigators interested in studying particular leaf mutant phenotypes in order to perform developmental genetic analyses of the processes contributing to the making of a leaf.

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References

Altmann T, Felix G, Jessop A, Kauschmann A, Uwer U, Peña-Cortés H, Willmitzer L (1995) *Ac*/*Ds* transposon mutagenesis in *Arabidopsis thaliana*: mutant spectrum and frequency of *Ds* insertion mutants. Mol Gen Genet 247:646–652

Anderson M (1993) The Nottingham Arabidopsis Stock Center Seed List. University of Nottingham, Nottingham

ABRC (1995) Seed and DNA stock list. Arabidopsis Biological Resource Center, Ohio State University, Columbus, Ohio

Azpiroz R, Wu Y, LoCasscio JC, Feldmann KA (1998) *Arabidopsis* brassinosteroid-dependent mutant is blocked in cell elongation. Plant Cell 10:219–230

Barabás Z, Rédei GP (1971) Facilitation of crossing by the use of appropriate parental stocks. Arabidopsis Inf Serv 8:7–8

Bell DJ, Ecker JR (1994) Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. Genomics 19:137–144

Berná G, Robles P, Micol JL (1999) A mutational analysis of leaf morphogenesis in *Arabidopsis thaliana*. Genetics, in press

Bork U, Kranz AR (1984) Early and late biological effects of heavy ions produced by accelerators on *Arabidopsis* seeds. Arabidopsis Inf Serv 21:41–45

Brutnell TP, Langdale JA (1998) Signals in leaf development. Adv Bot Res 28:36–42

Bürger D (1971) Die morphologischen Mutanten des Göttinger Arabidopsis-Sortiments, einschliesslich der Mutanten mit abweichender Samenfarbe. Arabidopsis Inf Serv 7:36–42

Cabrera y Poch HL, Peto CA, Chory J (1993) A mutation in the *Arabidopsis DET3* gene uncouples photorregulated leaf development from gene expression and chloroplast biogenesis. Plant J 4:671–682

Candela H, Martínez-Laborda A, Micol JL (1999) Venation pattern formation in *Arabidopsis thaliana* vegetative leaves. Dev Biol 205:205–216

Cetl I, Dobrovolna J, Nesrsta M (1969) Some new mutants induced by N-nitroso-N-methylurea. Arabidopsis Inf Serv 6:31

Chory J, Peto C, Feinbaum R, Pratt L, Ausubel F (1989) *Arabidopsis thaliana* mutant that develops as a light-grown plant in the absence of light. Cell 58:991–999

Chory J, Nagpal P, Peto CA (1991) Phenotypic and genetic analysis of *det2*, a new mutant that affects light-regulated seedling development in *Arabidopsis thaliana*. Plant Cell 3:445–460

Clouse SD, Langford M, McMorris TC (1996) A brassinosteroid-insensitive mutant in *Arabidopsis thaliana* exhibits multiple defects in growth and development. Plant Physiol 111:671–678

Edwards K, Johnstone C, Thompson C (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res 19:1349

Evans MM, Poethig RS (1995) Gibberellins promote vegetative phase change and reproductive maturity in maize. Plant Physiol 108:475–487

Fabri CO, Schäffner AR (1994) An *Arabidopsis thaliana* RFLP mapping set to localize mutations to chromosomal regions. Plant J 5:149–156

Fankhauser C, Chory J (1997) Light control of plant development. Annu Rev Cell Dev Biol 13:203–229

Feldmann KA, Marks MD, Christianson ML, Quatrano RS (1989) A dwarf mutant of *Arabidopsis* generated by T-DNA insertion mutagenesis. Science 343:1351–1354

Goodrich J, Puangsomlee P, Martin M, Long D, Meyerowitz EM, Coupland G (1997) A polycomb-group gene regulates homeotic gene expression in *Arabidopsis*. Nature 386:44–51

Haffter P, Granato M, Brand M, Mullins MC, Hammerschmidt M, Kane DA, Odenthal J, van Eeden FJ, Jiang YJ, Heisenberg CP, Kelsh RN, Furutani Seiki M, Vogelsang E, Beuchle D, Schach U, Fabian C, Nüsslein-Volhard C (1996) The identification of genes with unique and essential functions in the development of the zebrafish, *Danio rerio*. Development 123:1–36

Hake S, Sinha N (1991) Genetic analysis of leaf development. In: Miflin BJ (ed) Oxford Surveys of Plant Molecular and Cell Biology, Oxford University Press, London, pp 187–222

Hall LN, Langdale JA (1996) Molecular genetics of cellular differentiation in leaves. New Phytol 132:533–553

Hülskamp M, Miséra S, Jürgens G (1994) Genetic dissection of trichome cell development in *Arabidopsis*. Cell 76:555–566

Jacobsen SE, Olszewski NE (1993) Mutations at the *SPINDLY* locus of *Arabidopsis* alter gibberellin signal transduction. Plant Cell 4:1507–1518

Jürgens G, Mayer U, Torres Ruiz RA, Berleth T, Miséra S (1991) Genetic analysis of pattern formattion in the *Arabidopsis* embryo. Development Suppl 1:27–38

Kauschmann A, Jessop A, Koncz C, Szekeres M, Willmitzer L, Altmann T (1996) Genetic evidence for an essential role of brassinosteroids in plant development. Plant J 9:701–713

Koornneef M (1978) Gibberellin-sensitive mutants in *Arabidopsis thaliana*. Arabidopsis Inf Serv 15:17–20

Koornneef M (1981) The complex syndrome of *ttg* mutants. Arabidopsis Inf Serv 18:45–51
Koornneef M, van der Veen JH (1980) Induction and analysis of gibberellin-sensitive mutants in *Arabidopsis thaliana* (L.) Heynh. Theor Appl Genet 58:257–263
Koornneef M, van Eden J, Hanhart CJ, Stam P, Braaksma FJ, Fenstra WJ (1983) Linkage map of *Arabidopsis thaliana*. J Hered 74:265–272
Koornneef M, Elgersma A, Hanhart CJ, van Loenen-Martinet EP, van Rijn L, Zeevaart JAD (1985) A gibberellin-insensitive mutant of *Arabidopsis thaliana*. Physiol Plant 65:33–39
Koornneef M, Hanhart CJ, van der Veen JH (1991) A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. Mol Gen Genet 229:57–66
Kosambi DD (1944) The estimation of map distance from recombination values. Ann Eugen 12:172–175
Kranz AR, Bork U (1984) Biotests for heavy ion effects and preliminary total evaluation of cosmic radiation damage in *Arabidopsis* seeds flown during the first mission of Spacelab on STS 9. Arabidopsis Inf Serv 21:31–40
Kranz AR, Kirchheim B (1987) Genetic resources in *Arabidopsis*. Arabidopsis Inf Serv 24
Kranz AR, Kirchheim B (1990) Additions and corrections to the AIS-seed bank listing. Arabidopsis Inf Serv 27:89–200
Lewis EB (1978) A gene complex controlling segmentation in *Drosophila*. Nature 276:565–570
Leyser HMO, Furner IJ (1992) Characterisation of three shoot apical meristem mutants of *Arabidopsis thaliana*. Development 116:397–403
Li J, Chory J (1997) A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. Cell 90:929–938
Li J, Nagpal P, Vitart V, McMorris TC, Chory J (1996) A role for brassinosteroids in light-dependent development of *Arabidopsis*. Science 272:398–401
Martínez-Zapater JM, Coupland G, Dean C, Koornneef M (1994) The transition to flowering in *Arabidopsis*. In: Meyerowitz EM, Somerville CR (eds) *Arabidopsis*. Cold Spring Harbor Laboratory Press, New York, pp 403–433
McHale NA (1993) *LAM-1* and *FAT* genes control development of the leaf blade in *Nicotiana sylvestris*. Plant Cell 5:1029–1038
Napp-Zinn K, Bonzi G (1970) Gibberelin effects in dwarf mutants of *Arabidopsis thaliana*. Arabidopsis Inf Serv 7:8
Nüsslein-Volhard C, Wieschaus E (1980) Mutations affecting segment number and polarity in *Drosophila*. Nature 287:795–801
Poethig RS (1997) Leaf morphogenesis in flowering plants. Plant Cell 9:1077–1087
Ponce MR, Quesada V, Micol JL (1998) Rapid discrimination of sequences flanking and within T-DNA insertions in the *Arabidopsis* genome. Plant J 14:497–502
Ponce MR, Robles P, Micol JL (1999) High-throughput genetic mapping in *Arabidopsis thaliana*. Mol Gen Genet 261:408–415
Rédei GP (1962) Single locus heterosis. Z Vererbungs 93:164–170
Rédei GP, Hirono Y (1964) Linkage studies. Arabidopsis Inf Serv 1:9
Reinholz E (1947) X-ray mutations in *Arabidopsis thaliana* (L.) Heynh. and their significance for plant breeding and the theory of evolution. Fiat Rep 1006:1–70
Reinholz E (1966) Radiation-induced mutants showing changed inflorescence characteristics. Arabidopsis Inf Serv 3:19–20
Relichova J (1976) Some new mutants. Arabidopsis Inf Serv 13:25–28
Röbbelen G (1957) Über Heterophyllie bei *Arabidopsis thaliana* (L.) Heynh. Ber Dtsch Bot Ges 70:39–44
Sinha N, Hake S, Freeling M (1993) Genetic and molecular analysis of leaf development. Curr Top Dev Biol 28:47–80
Smith LG, Hake S (1992) The initiation and determination of leaves. Plant Cell 4:1017–1027
Sylvester AW, Smith L, Freeling M (1996) Acquisition of identity in the developing leaf. Annu Rev Cell Dev Biol 12:257–304
Szekeres M, Németh K, Koncz-Kálmán Z, Mathur J, Kauschmann A, Altmann T, Rédei GP, Nagy F, Schell J, Koncz C (1996) Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in *Arabidopsis*. Cell 85:171–182
Takahashi T, Gasch A, Nishizawa N, Chua N-H (1995) The *diminuto* gene of *Arabidopsis* is involved in regulating cell elongation. Genes Dev 9:97–107
Telfer A, Poethig RS (1994) Leaf development in *Arabidopsis*. In: Meyerowitz EM, Somerville CR (eds) *Arabidopsis*. Cold Spring Harbor Laboratory Press, New York, pp 379–400
Telfer A, Poethig RS (1998) *HASTY*: a gene that regulates the timing of shoot maturation in *Arabidopsis thaliana*. Development 125:1889–1898
Telfer A, Bollman KM, Poethig RS (1997) Phase change and the regulation of trichome distribution in *Arabidopsis thaliana*. Development 124:645–654
Tsuge T, Tsukaya H, Uchimiya H (1996) Two independent and polarized processes of cell elongation relate leaf blade expansion in *Arabidopsis thaliana* (L.) Heynh. Development 122:1589–1600
Tsukaya H (1995) Developmental genetics of leaf morphogenesis in dicotyledoneous plants. J Plant Res 108:407–416
Van Lijsebettens M, Clarke J (1998) Leaf development in *Arabidopsis*. Plant Physiol Biochem 36:47–60
Waites R, Hudson A (1995) *PHANTASTICA*: a gene required for dorsoventrality of leaves in *Antirrhinum majus*. Development 121:2143–2154
Waites R, Selvadurai HRN, Oliver IR, Hudson A (1998) The *PHANTASTICA* gene encodes a MYB transcription factor involved in growth and dorsoventrality of lateral organs in *Antirrhinum*. Cell 93:779–789
Wei N, Deng X-W (1996) The role of the *COP*/*DET*/*FUS* genes in light control of Arabidopsis seedling development. Plant Physiol 112:871–878
Weigel D, Meyerowitz EM (1994) The ABCs of floral homeotic genes. Cell 78:203–209