Development 125, 2935-2942 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
DEV0175

Leaf polarity and meristem formation in *Arabidopsis*

Jane R. McConnell[1] and M. Kathryn Barton[2,*]

[1]Cellular and Molecular Biology Program and [2]Department of Genetics, University of Wisconsin-Madison, Madison, WI, USA
*Author for correspondence (e-mail: mkbarton@facstaff.wisc.edu)

Accepted 14 May; published on WWW 9 July 1998

SUMMARY

Shoot apical meristems (SAMs) of seed plants are small groups of pluripotent cells responsible for making leaves, stems and flowers. While the primary SAM forms during embryogenesis, new SAMs, called axillary SAMs, develop later on the body of the plant and give rise to branches. In *Arabidopsis* plants, axillary SAMs develop in close association with the adaxial leaf base at the junction of the leaf and stem (the leaf axil). We describe the phenotype caused by the *Arabidopsis phabulosa-1d* (*phb-1d*) mutation. *phb-1d* is a dominant mutation that causes altered leaf polarity such that adaxial characters develop in place of abaxial leaf characters. The adaxialized leaves fail to develop leaf blades. This supports a recently proposed model in which the juxtaposition of ad- and abaxial cell fates is required for blade outgrowth. In addition to the alteration in leaf polarity, *phb-1d* mutants develop ectopic SAMs on the undersides of their leaves. Also, the *phb-1d* mutation weakly suppresses the *shoot meristemless* (*stm*) mutant phenotype. These observations indicate an important role for adaxial cell fate in promoting the development of axiallary SAMs and suggest a cyclical model for shoot development: SAMs make leaves which in turn are responsible for generating new SAMs.

Key words: Meristem, Leaf development, *PHABULOSA* (*PHB*), Branching, *Arabidopsis*

INTRODUCTION

The shoot apical meristem (SAM) of angiosperm plants is the site at which new leaves and stem are made. The primary SAM is formed during embryogenesis and gives rise to the main axis of the plant. New SAMs, axillary SAMs, develop on the main axis and give rise to branches. These SAMs usually develop in the axils of leaves; the axil is the junction between leaf and stem. The subtending leaf may be required in some species for axillary SAM formation: when the subtending leaf is surgically removed the associated axillary SAM often fails to form (Snow and Snow, 1942). In *Arabidopsis*, two observations support a connection between the subtending leaf and the axillary meristem. First, the axillary SAM appears to arise on the adaxial leaf base (Talbert et al., 1995). Second, the subtending leaf and its associated axillary bud are clonally related (Furner and Pumfrey, 1992; Irish and Sussex, 1992).

One model for the development of axillary SAMs (reviewed by Steeves and Sussex, 1989) proposes that fragments of the main meristem, so called detached meristems, remain associated with each axil. These detached meristems become activated and form buds when the leaf and its associated axil are some distance from the main SAM. In this model, SAM fate is acquired only once in the development of the plant. Alternatively, axillary SAMs may form from cells that have lost SAM identity, partially differentiated, and then been instructed to regain SAM fate.

The first indication of leaf primordium formation in *Arabidopsis* occurs while the presumptive leaf primordium resides entirely within the SAM. Loss of *SHOOT MERISTEMLESS (STM)* expression in the presumptive leaf distinguishes it from other regions of the SAM (Long et al., 1996). Subsequently, these groups of cells grow outward from the SAM as small bumps, or leaf primordia. As the leaf develops, the adaxial side of the primordium (the side towards the center of the plant) grows more than the abaxial side. This unequal growth causes the leaf to bend outward and away from the long axis of the plant such that the adaxial side of the leaf primordium becomes the top of the leaf and the abaxial side of the leaf primordium becomes the bottom of the leaf.

The *Arabidopsis* leaf consists of a short petiole, connecting the leaf to the stem, and an entire, flattened blade. The leaf blade is polarized along its ad/abaxial axis (Telfer and Poethig, 1994). The adaxial epidermis is glossy, dark green and trichome-rich while the abaxial epidermis is matte, grey-green and, especially in the early leaves, trichome-poor. Internal tissues are also polarized: a layer of closely packed palisade cells underlies the adaxial epidermis while a loosely packed layer of spongy mesophyll cells lies adjacent to the abaxial epidermis. In addition, within the vascular strand, xylem is located adaxial to the phloem.

Waites and Hudson (1995) have proposed a model for leaf development in which the juxtaposition of ad- and abaxial leaf cell fates is required for the development and outgrowth of the leaf blade (Fig. 1). This model is based on observations of snapdragons homozygous for the recessive *phantastica* (*phan*) mutation; *phan* mutants possess radially symmetric leaves with abaxial characters around their circumference. An important prediction of this model is that in a mutant with the opposite phenotype (one in which abaxial leaf fates are transfomed to

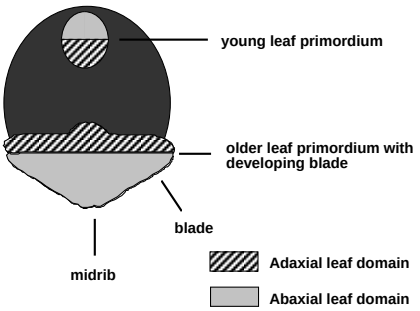

Fig. 1. Schematic of shoot apical meristem shown from above, illustrating model for leaf development proposed by Waites and Hudson (1995). In this model, ad- and abaxial domains of the leaf are specified early in the development of the leaf primordium, while the primordium still resides within the SAM. The juxtaposition of ad- and abaxial cell fates causes the subsequent development and outgrowth of the leaf blade.

adaxial leaf fates) the leaf will again fail to form a blade and develop with radial symmetry.

We have recently isolated an *Arabidopsis* mutant the phenotype of which satisfies this prediction. The dominant *phabulosa-1* (*phb-1d*) mutation causes both sides of the leaf to develop with adaxial characters. *phb-1d* leaves fail to develop blades and are frequently radially symmetric. Additionally, the *phb-1d* mutant provides evidence that adaxial leaf identity plays a critical role in the development of axillary SAMs.

MATERIALS AND METHODS

Plant growth conditions

Plants were grown in soil (Metromix 200) under 24 hour cool white flourescent light at either 24°C or 17°C (all plants used as pollen donors were grown at 17°C). All phenotypes were scored at 24°C unless otherwise noted.

Genetics

Seeds of ecotype Landsberg *erecta* (Ler) were mutagenized by soaking them in 0.4% EMS in 100 mM phosphate buffer (pH 7) for 8 hours. The self-progeny of individual plants (M_1 plants) grown from mutagenized seed (n=1800) were harvested, planted in soil and screened for mutants defective in vegetative growth. A single M_1 plant segregated *phb-D* mutants in its progeny. The mutant was recovered by crossing a *phb-1d* mutant male to Ler. At least five backcrosses to Ler were performed prior to any genetic or phenotypic analysis.

To determine the phenotype of plants doubly mutant for *stm-1* and *phb-1d, stm-1*/+; *phb-1d*/+ individuals were crossed to *stm-1*/+*;* +/+ individuals. In the cross progeny 21 plants had the *stm* single mutant phenotype (presumed genotype *stm-1/stm-1*), 52 plants had the *phb-1d* single mutant phenotype (presumed genotype *stm-1*/+ or +/+*; phb-1d*/+), 57 plants were wild-type (presumed genotype *stm-1*/+ or +/+*;* +/+) and 20 plants were both *phb-1d* (as judged by their cotyledons) and *stm* (presumed genotype *stm-1/stm-1;phb-1d*/+). The observed progeny are consistent with the presumed genotypes (χ^2=0.684; P<0.8).

STM-GUS/phb-1d transgenic plants were generated by crossing *phb-1d*/+ mutants to individuals homozygous for the STM-GUS construct. *phb-1d*/+ individuals in the cross progeny were then examined for GUS expression.

CAPS mapping was carried out as described by Konieczny and Ausubel (1993). *phb-1d*/+ individuals (Ler background) were crossed to wild-type Columbia (Col) individuals. Mutant cross progeny were crossed back to Col individuals. 2/32 mutant progeny of this second cross were found to carry recombinant chromosomes between the *PHB* locus and the *m429* CAPS marker corresponding to a recombinant frequency of approximately 6%. 2/34 mutant progeny from the same cross carried recombinant chromosomes between the *PHB* locus and the GPA1 marker corresponding to a recombinant frequency of approximately 6%. This map data places the *PHB* locus at an approximate position of 59.8 on chromosome 2.

Microscopy

Samples were prepared and visualized by scanning electron microscopy as described by McConnell and Barton (1995). Tissues for thin sections were fixed overnight in a solution containing: 3% glutaraldehyde, 1.5% acrolein, 1.6% paraformaldehyde, and postfixed in 1% osmium tetroxide. Fixed material was dehydrated through an ethanol series and embedded in Spurr's medium. Approximately 1 μm sections were cut and stained with 0.2% toluidine blue in 2.5% sodium carbonate (pH 11). Histological staining of β-glucuronidase (GUS) activity was performed overnight on seedlings at 37°C in 2 mM 5-bromo-4-chloro-3-indolyl β-D-glucuronic acid, 100 mM $NaPO_4$, pH 7, 0.1% Triton X-100, 1 mM FeCN and 10 mM EDTA. Samples were then washed with 70% ethanol for 2 days and visualized using a dissecting microscope.

RESULTS

The dominant *phb-1d* mutation causes a transformation of abaxial cell fates to adaxial cell fates in leaves and floral organs and alters organ shape. The mutation was isolated in a screen for EMS-induced mutants altered in SAM development and maps to chromosome II between CAPS markers m429 and GPA1. Crosses of *phb-1d*/+ pollen onto Ler carpels resulted in 47.1% *phb-1d* mutants (n=191: χ^2=0.6335; P<0.5). The wild-type siblings were never observed to segregate *phb-1d* mutants in their self-progeny (n=50) indicating that the *phb-1d* mutation is completely penetrant. Since the *phb-1d*/+ mutants are sterile as females at 24°C, the *phb-1d* mutation is propagated through the pollen (except at low temperatures as described below).

phb-1d heterozygotes have leaves with adaxial epidermal characters (dark green, glossy, trichome-rich surfaces) around their circumference and exhibit varying degrees of radial symmetry and loss of blade outgrowth (Fig. 2). The expressivity of the *phb-1d* mutant phenotype is variable. Some leaves are rod-like while others are shaped like trumpets. The latter have adaxial tissue on their outer surfaces and abaxial tissue on the inside of the 'bell' of the trumpet. *phb-1d* leaves grow nearly vertically rather than bending away from the plant. The upright posture of mutant leaves is consistent with equal growth rates of both sides of the leaf, as expected if identical cell fates are specified on both faces of the developing primordium.

Wild-type epidermal leaf cell shape varies according to the surface upon which it is located as well as its position along the leaf. Fig. 3 shows ad- and abaxial epidermal cells from three positions on the leaf: petiole, midrib midway along the length of the blade, and blade midway up the length of the blade and midway between the margin and the midrib. Wild-type petiole cells are long and rectangular with straight anticlinal walls. This is true of both surfaces though the adaxial

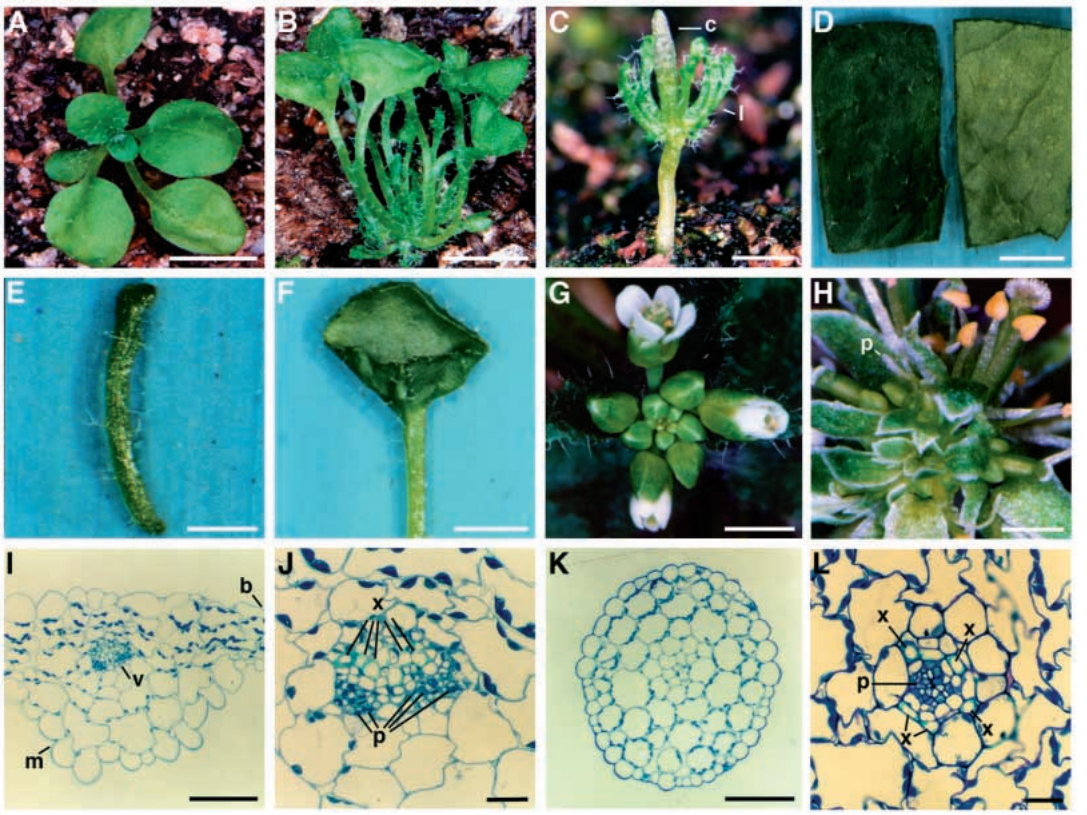

Fig. 2. (A) Wild-type *Arabidopsis* plant viewed from above; leaves bend away from center of the plant. (B) *phb-1d*/+ plant viewed from the side. Leaves grow upward and vary in the degree to which they are radialized: some are trumpet-shaped, others are rod-like. (C) *phb-1d/phb-1d* plant viewed from the side. Leaves and cotyledons are extremely radialized and grow vertically. c, cotyledons; l, leaves. (D) Adaxial (left) and abaxial (right) surfaces of a wild-type *Arabidopsis* leaf. The adaxial surface is glossy, dark-green and trichome-rich while the abaxial leaf surface is matte, grey-green, and (especially in the early leaves) trichome-poor. (E) Severely adaxialized leaf. The glossy, dark-green, trichome-rich surface characteristic of the adaxial leaf surface extends around the circumference of the radialized leaf. The petiole is often highly reduced in such extremely affected leaves. (F) Less severely adaxialized leaf. This trumpet-shaped leaf exhibits adaxial characters on the outside of the 'bell' and abaxial characters on the inside of the 'bell'. (G) Wild-type inflorescence viewed from above; young flowers are fully enclosed within the sepals. (H) *Phb-1d*/+ inflorescence; the sepals fail to enclose the developing flower. Note 'stringy' nature of floral organs. p, petal. (I) Cross section through wild-type leaf; adaxial surface is up. b, blade; m, midrib; v, vascular strand. (J) Close-up of wild-type vascular strand; adaxial surface is up. x, xylem cells; p, phloem cells. (K) Cross section through extremely radialized *phb-1d*/+ mutant leaf. (L) Close-up of vascular strand from a moderately radialized *phb-1d*/+ mutant leaf. Xylem cells surround phloem cells. x, xylem; p, phloem. Scale bars: (A,B) 5 mm; (C,H) 1.25 mm; (D) 1.75 mm; (E,F) 1 mm; (G) 2 mm; (I, K) 100 µm; (J,L) 20 µm.

petiole surface also contains stomates along its margin (Fig. 3A,D). Continuing up the midrib, midway along the length of the blade, ad- and abaxial cell morphologies differ significantly. The abaxial epidermal cells at this position are long and rectangular, similar to petiole cells. The corresponding adaxial cells are more complex and resemble blade cells (Fig. 3B,E). Epidermal blade cells are jig-saw shaped on both leaf surfaces, but adaxial blade cells are larger, more uniform in size, and less complex than the corresponding abaxial cells (Fig. 3C,F).

Comparing wild-type and *phb-1d* epidermal leaf cell shapes further supports the conclusion that *phb-1d* leaves are adaxialized. Trumpet-shaped leaves have larger, less complex cells on the outside of the 'bell' than on the inside (Fig. 3H-J). This is similar to the difference seen between the wild-type adaxial and abaxial blade epidermis. The cells are smaller overall in the *phb-1d* mutant than in the wild type. This is likely due to less organ expansion in the mutant as *phb-1d* organs are smaller than wild-type organs (Fig. 2A-C). It is more difficult to assign ad- versus abaxial fates to the epidermal cells of rod-shaped leaves based on cell shape. Rod-shaped leaves have cells that are similar in size to blade epidermal cells but they are less jigsaw-shaped and more rectangular (Fig. 3G). Stomates are distributed throughout the rod-shaped leaves as in wild-type blade tissue. Epidermal cells in young, wild-type leaf primordia are simple in shape and only achieve their complex, jigsaw shapes upon leaf expansion. The rod-shape of the extreme *phb-1d* leaves does not allow for normal blade expansion and this may affect cell shape.

Internal tissues are also affected in *phb-1d* mutants (Fig. 2I-L). The more extremely radialized leaves either entirely lack a vascular strand or possess single xylem elements. In such leaves, phloem (normally found in the abaxial pole of the vascular strand) was not seen. In less extremely affected leaves, vascular tissue in which xylem surrounds phloem tissue has been observed; the latter arrangement is the opposite of what is seen in the abaxialized snapdragon *phan* mutant where phloem surrounds xylem (Waites and Hudson, 1995).

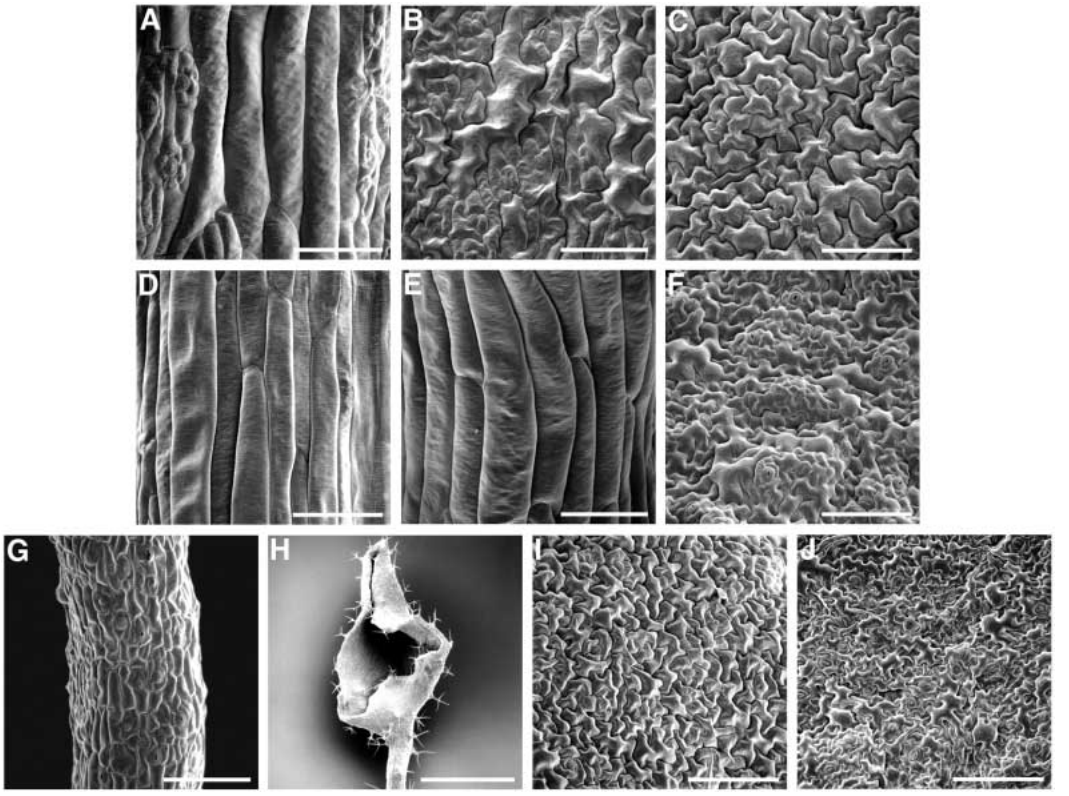

Fig. 3. (A) Wild-type adaxial petiole epidermis. Cells have straight anticlinal walls and are larger than blade cells (see C,F). (B) Wild-type adaxial epidermis above midrib, halfway along the leaf blade. Cells are jig-saw shaped. (C) Wild-type adaxial epidermis of blade. Cells are jig-saw shaped and relatively uniform in size. (D) Wild-type abaxial petiole epidermis. Similar to comparable cells on the adaxial petiole surface, these cells have straight anticlinal walls and are longer than typical blade cells. (E) Wild-type abaxial epidermis below midrib, halfway along the leaf blade. Cell shape and size is similar to those of the abaxial petiole (see Fig. 3D). (F) Wild-type abaxial epidermis of blade. Cells are jig-saw shaped. However, unlike adaxial blade cells, these cells are not uniform in size. (G) Surface of a radialized leaf from an individual homozygous for the *phb-1d* mutation. These cells are simpler in shape but are similar in size to blade cells (see Fig. 3I,J). (H) Trumpet-shaped leaf from an individual heterozygous for the *phb-1d* mutation. (I) Outer surface of trumpet-shaped leaf. Though these cells are smaller than wild-type blade cells, they have characteristics typical of adaxial blade cells: they are jig-saw shaped and uniform in size. (J) Inner surface of trumpet-shaped leaf. Again, while these cells are overall smaller than wild-type abaxial cells, they have characteristics of abaxial blade cells: they are jig-saw shaped and are not uniform in size. Scale bars: (A-G, I,J) 100 μm; (H) 1.50 mm.

All four types of floral organs – sepals, petals, stamens and carpels – are affected in *phb-1d* heterozygotes to some degree (Figs 2, 4). Both sepals and petals of *phb-1d*/+ individuals frequently develop with what appears to be radial symmetry (Fig. 4B,H). Sepals may be filamentous, trumpet-shaped, or may appear wild-type in shape. When trumpet-shaped, cells on the outside exhibit adaxial epidermal fates while cells on the inside exhibit abaxial epidermal fates (Fig. 4C-E). Petals are most commonly filamentous and have cell types characteristic of the wild-type adaxial epidermis around their circumference (cone-shaped cells with straight cuticular ridges) (Fig. 4F-I).

The reproductive organs are also affected in *phb-1d* mutants. In wild-type stamens, the pollen sacs form laterally and rotate to the adaxial side through unequal growth of the ad- and abaxial surfaces (Fig. 4J,K) (Bowman, 1994). In *phb-1d*/+ mutants, the pollen sacs often remain in lateral positions indicating a lack of unequal growth consistent with a failure to specify different cell types on the two surfaces (Fig. 4L,M). The ovules, which normally originate from the interior (adaxial side) of the carpel, develop ectopically in the *phb-1d* mutant from the abaxial base of the carpel (Fig. 4N). Furthermore, instead of displaying the curvature seen in the wild-type ovule where the unequal growth of the integuments and the nucellus generates a curved structure, *phb-1d*/+ ovules are frequently linear (Fig. 4O,P).

The *phb-1d* mutation affects sepals developing in the abaxial region (relative to the inflorescence axis) more than sepals in the adaxial region of the flower. The sepal closest to the inflorescence meristem is least likely to develop as a filament (Fig. 5). The shape of the adaxial sepals is most frequently either wild-type or trumpet-shaped. While other floral organs did not show any obvious position-dependent variation in phenotype, we cannot rule out minor differences since quantitative comparisons were not made.

The *phb-1d* mutant phenotype is temperature sensitive. At low temperatures (17°C), the *phb-1d* phenotype is somewhat alleviated and *phb-1d*/+ plants are weakly self-fertile. This has allowed us to recover homozygous *phb-1d* plants. Heterozygous *phb-1d* plants raised at 17°C produce wild-type (+/+; n=67), moderately affected (*phb-1d*/+; n=123) and severely affected seedlings (presumed *phb-1d/phb-1d*; n=59) in their self-progeny. The presumed homozygotes have more fully

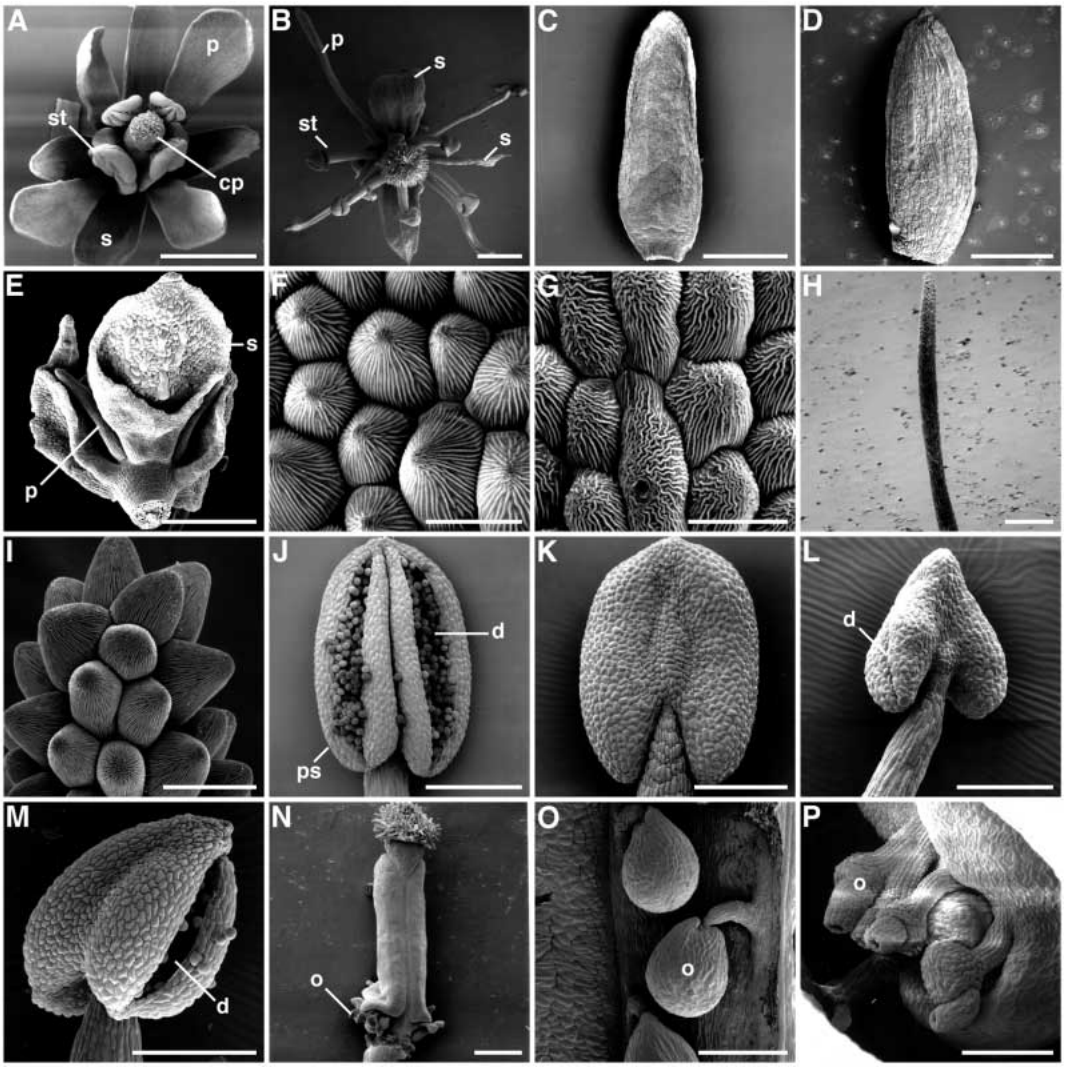

Fig. 4. (A) Wild-type flower. (B) *phb-1d*/+ flower; sepal nearest inflorescence axis is up. Floral organs frequently develop as bladeless organs with radial symmetry. (C) Wild-type adaxial surface of sepal. (D) Wild-type abaxial surface of sepal. (E) Adaxial sepal of a flower from a *phb-1d*/+ mutant. The trumpet-shape of this sepal resembles that of the leaf shown in Fig. 3H. (F) Wild-type adaxial petal epidermis showing cone-shaped cells with straight cuticular ridges. (G) Wild-type abaxial petal epidermis showing cobble-stone shaped cells with wavy cuticular ridges. (H) *phb-1d*/+ mutant petal. (I) Tip of *phb-1d*/+ mutant petal. Cells around circumference of mutant petal are cone-shaped and have straight cuticular ridges similar to adaxial epidermal cells. (J) Wild-type adaxial stamen surface. Each pollen sac splits along the line of dehiscence to empty its contents of pollen grains. (K) Wild-type abaxial stamen surface. (L) Adaxial surface of *phb-1d*/+ mutant stamen. Pollen sacs develop with their lines of dehiscence oriented laterally. (M) Abaxial surface of *phb-1d*/+ mutant stamen. (N) *phb-1d*/+ mutant carpel showing ectopic ovules developing from the base. (O) Wild-type ovules. The wild-type ovule develops as a curved structure in which the inner and outer integuments enclose the nucellus. Differential growth of the integuments result in the curvature observed in the mature ovule. (P) Ectopic ovules from a *phb-1d*/+ mutant. The ovules develop as linear structures due to a lack of unequal growth. cp, carpel; d, line of dehiscence; o, ovule; p, petal; ps, pollen sac; s, sepal; st, stamen. Scale bars: (A) 0.75 mm; (B) 0.5mm; (C) 500 μm; (D) 500 μm; (E) 300 μm; (F,G) 15 μm; (H) 220 μm; (I) 20 μm; (J-L) 176 μm; (M) 136 μm; (N) 0.38 mm; (O) 270 μm; (P) 127 μm.

radialized and adaxialized leaves than the heterozygotes (Fig. 2C). This is especially striking in the cotyledons which are often only weakly affected in the heterozygote. The presumed homozygotes are very small and grow slowly. These individuals are sterile as the floral organs produced are completely radialized.

Normally, axillary SAMs develop on the adaxial leaf base, initially oriented toward the stem (Talbert et al., 1995; Fig. 7C). Later growth of the developing bud obscures this early relationship making the branch appear to emanate from the stem side of the axil. Although axillary buds normally develop on the adaxial leaf base, it is unclear what aspects of position are assessed by these cells in making developmental decisions. If adaxial leaf fate is a critical determinant of axillary SAM formation we would expect *phb-1d* mutants to develop SAMs ectopically. Indeed, ectopic SAMs form on the undersides of *phb-1d* mutant leaves (Fig. 6). To determine the origin of the ectopic buds in *phb-1d* mutants, we observed the expression of an *STM* promoter-GUS reporter construct in *phb-1d* mutants. This construct is expressed in the SAM (Long and

sepal characteristics	sepal position relative to inflorescence meristem (n = 52 flowers) 1	2	3	4
normal	35 (67%)	5 (10%)	3 (6%)	11 (21%)
filament	1 (2%)	37 (71%)	42 (81%)	29 (56%)
other	16 (31%)	10 (19%)	7 (31%)	12 (23%)

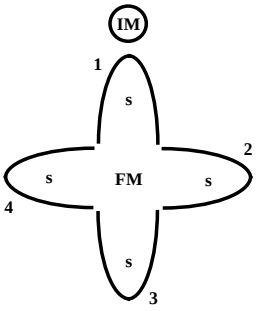

Fig. 5. Position-dependent sepal development in *phb-1d*/+ mutants. 'Normal' indicates sepals with normally shaped blades; 'filament' indicates sepals with radial symmetry; 'other' indicates sepals intermediate in phenotype. IM, inflorescence meristem; FM, floral meristem; s, sepal.

Barton, unpublished). Regions of dark staining indicative of early steps in SAM formation were found on the undersides of leaves in *phb-1d* mutant seedlings but not in wild-type seedlings (Fig. 7A,B). In the most severely adaxialized mutant leaves, reporter gene expression could be observed in a ring around the entire base of the leaf (Fig. 7D). Thus, the development of the axillary bud behaves like other adaxial characters in that additional buds develop on the underside of leaves in *phb-1d* mutants.

During embryogenesis the SAM develops at the bases of and between the adaxial surfaces of the cotyledons. This position in some ways parallels the environment in which the axillary meristem develops. If adaxial cotyledon fate is responsible for promoting the development of the embryonic SAM, we might expect the *phb-1d* mutation to affect the embryonic SAM. We have detected a positive effect of *phb-1d* on the development of the embryonic SAM in two situations. First, the SAM of *phb-1d/+* mutant embryos is enlarged relative to wild-type indicating that a stronger 'meristem promoting' signal may be present in *phb-1d* mutant embryos (Fig. 8A,B). Second, the *phb-1d* mutation partially suppresses the *stm* mutant phenotype. In mutants homozygous for *stm-1*, a strong but non-null allele of the *STM* gene, the SAM fails to form and cells at the site of the presumptive SAM terminally differentiate (Barton and Poethig, 1993). Thus, *stm-1* homozygotes entirely lack any structures at the site normally occupied by the SAM. A week or two following germination, *stm-1* mutants form leaves ectopically from a region below the point of cotyledon fusion (Barton and Poethig, 1993; Fig. 8C). In *stm-1*; *phb-1d* double mutants, an abnormal determinate structure is present at the site normally occupied by the SAM at germination (Fig. 8D). The position of this structure and its early appearance are consistent with it forming directly from the presumptive SAM.

DISCUSSION

The model for leaf development put forth by Waites and Hudson (1995; Fig. 1) proposes that the juxtaposition of ad- and abaxial cell fates is required for outgrowth of the leaf blade. The phenotypes of two leaf mutants described to date support the model. In the snapdragon *phan* mutant, adaxial characters are transformed into abaxial characters (Waites and Hudson, 1995). In the *Arabidopsis phb-1d* mutant, the opposite transformation of cell fate occurs: abaxial characters are replaced with adaxial characters. In neither mutant are ad- and abaxial cell fates juxtaposed; under this model lack of juxtaposition causes the leaves of both mutants to develop as radially symmetric, bladeless organs.

The snapdragon *phan* mutation is recessive and is therefore likely caused by a loss-of-function mutation in the *PHAN* gene. If so, the wild-type role of the *PHAN* gene is to specify adaxial cell fates since these are missing in the *phan* mutant. Since the *phb-1d* mutation is dominant, the role of the wild-type *PHB* gene in the specification of ad/abaxial leaf cell fates is uncertain. One possibility is that *phb-1d* represents a gain-of-function mutation in a gene normally required to specify adaxial fate, and thus plays a similar role in *Arabidopsis* to that played by the *PHAN* gene in snapdragon. Interestingly, both *phan* and *phb-1d* mutant phenotypes are temperature sensitive, exhibiting more abaxialized phenotypes at low temperature and more adaxialized phenotypes at high temperature. Like *PHAN*, the action of *phb-1d* is cell autonomous, or at least limited in its range, because we have obtained wild-type sectors on otherwise *phb-1d* mutant plants after treatment of *phb-1d*/+ seeds with EMS (McConnell and Barton, unpublished). Taken together these observations are consistent with *PHAN* in snapdragon and *PHB* in *Arabidopsis* controlling similar processes in leaf development.

However, it is also possible that the wild-type *PHB* gene plays no role in the development of leaf polarity. The dominant *phb-1d* mutation may have altered the pattern of *PHB*

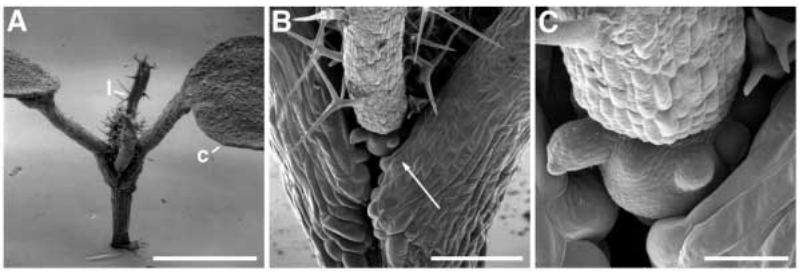

Fig. 6. (A) *phb-1d*/+ seedling. (B) Close-up of seedling in A showing the presence of an ectopic bud (arrow) developing from the underside of the leaf. (C) Higher magnification view of ectopic bud in B. c, cotyledon; l, leaf. Scale bars: (A) 1.5 mm; (B) 250 μm; (C) 75 μm.

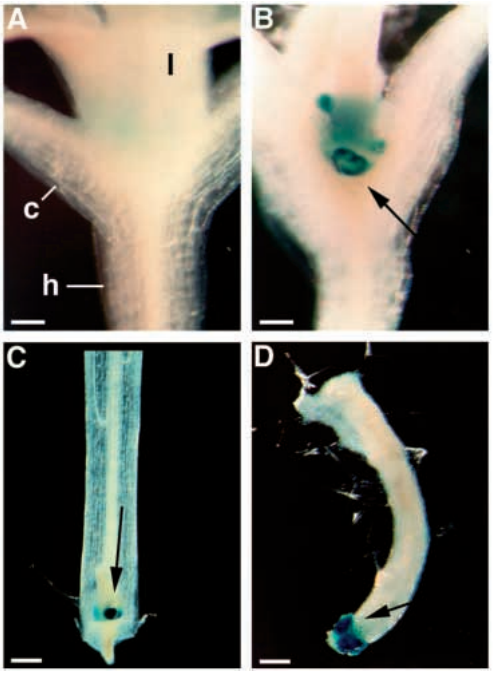

Fig. 7. Expression of an *STM* promoter β-glucuronidase reporter construct in wild-type and *phb-1d*/+ mutants. (A) Wild-type seedling. Undersides of leaf bases show no expression of the reporter. The SAM is evident as a blue haze that shows through the cleared leaves. (B) *phb-1d*/+ mutant. A dark patch of reporter expression is evident on the underside of the leaf (arrow). (C) Development of an axillary bud (arrow) from the wild-type adaxial leaf base. (D) Radialized *phb-1d*/+ leaf showing a ring of reporter gene expression around the circumference of the leaf base (arrow). c, cotyledon; h, hypocotyl; l, leaf. Scale bars: (A,B) 200 μm; (C) 150 μm; (D) 300 μm.

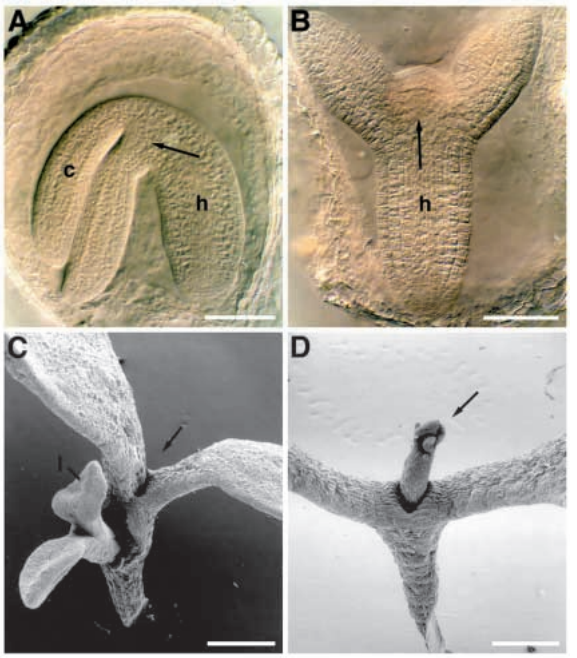

Fig. 8. Effect of *phb-1d* on the development of the embryonic SAM. (A) Wild-type embryo. (B) *phb-1d*/+ mutant embryo. The SAM (arrow) in mutant embryos appears larger than that found in the wild-type embryo. Also note that the cotyledons grow straight out from the embryonic axis. (C) *stm-1/stm-1* mutant. No SAM is formed during embryogenesis (site where SAM would normally be located is marked with an arrow). Ectopic leaves are frequently made postembryonically from a region below the apex. (D) *stm-1/stm-1 phb-1d*/+ double mutant. An abnormal determinate structure (arrow) is present at the site normally occupied by the SAM. Such structures were not seen in *stm* single mutants. c, cotyledon; h, hypocotyl; l, ectopic leaves. Scale bars: (A,B) 100 μm; (C,D) 0.86 mm.

expression or the activity of the *PHB* gene product to allow it to take on an activity it does not normally possess. Even if this is true, the results presented here still show a correlation between adaxial transformation and lack of blade formation and a correlation between adaxial transformation and ectopic SAM formation.

Our results show a correlation between the acquisition of adaxial leaf fate and the development of ectopic buds. While it is possible that the *phb-1d* mutation acts to generate these two phenotypes independently of one another, a simpler explanation is that the ectopic SAMs on the underside of the *phb-1d* leaf are a consequence of the transformation of this tissue into adaxial leaf tissue. In this model, adaxial leaf fate is a major factor in determining whether an axillary SAM forms or not. We propose that, in *Arabidopsis*, leaf and meristem development are linked through a cycle in which SAMs make leaves, the adaxial sides of which in turn induce the development of new SAMs from their bases (Fig. 9A). This is in contrast to a model in which axillary SAMs are derived from remnants, so-called detached meristems, of the primary SAM left behind in the leaf axil. For the detached meristem model to hold, such remnants of the SAM would have to be left behind on the underside of the leaf in the *phb-1d* mutant and, in the extremely radialized *phb-1d* leaves, around their entire circumference. This would require a more complex model for *PHB* action than proposed here.

In dicot species, numerous observations indicate a link between adaxial leaf cell fate and competence to develop axillary meristems. For instance, begonia leaves regenerate SAMs from the adaxial but not the abaxial leaf surface. Transgenic tobacco plants that ectopically express the *knotted1* gene (Sinha et al., 1993) or ectopically synthesize cytokinin (Estruch et al., 1991) develop SAMs on the adaxial but not the abaxial leaf surfaces. Also, after surgical manipulation, Epilobium and potato leaf primordia developed as abaxialized, radially symmetric organs. In these situations, axillary meristems often did not develop (Snow and Snow, 1959; Sussex, 1955). These observations suggest that the adaxial leaf environment is *required* for the development of an axillary SAM. Consistent with this, functional axillary meristems are lacking in the axils of the most severely affected leaves in snapdragon *phan* mutants (A. Hudson, personal communication). Our observations of the *phb-1d* mutant phenotype suggest that adaxial, basal leaf fate is also *sufficient* to direct axillary SAM formation.

There are many possible mechanisms through which the adaxial leaf environment might be responsible for the development of the axillary bud. For instance, a cell type present in the adaxial portion of the leaf may promote axillary SAM development; or a cell type present in the abaxial portion of the leaf may inhibit axillary SAM development; or cells in

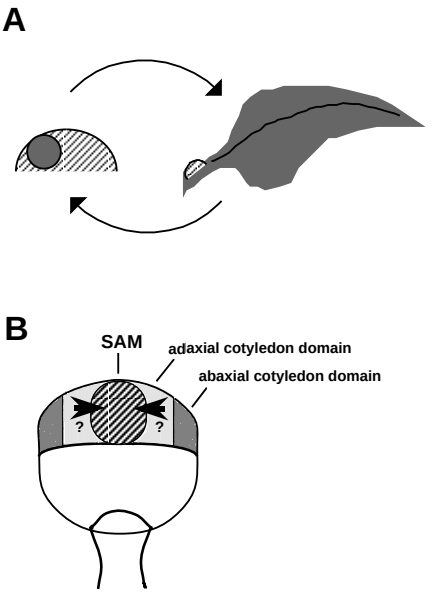

Fig. 9. (A) Leaf - meristem - leaf cycle model for branch development. The SAM (hatched) generates leaf primordia (grey). The adaxial domain of the leaf primordium subsequently induces the formation of axillary SAMs that make branches. (B) Schematic showing the presumptive cotyledon and SAM domains in the late globular/early transition stage embryo. The embryonic SAM develops close to the adaxial domain of the cotyledons in an arrangement that resembles the arrangement of the axillary SAM and its subtending leaf. The arrows indicate a hypothetical interaction between the adaxial domain of the developing cotyledons and more central cells that could act in promoting the formation of the SAM during embryogenesis.

the adaxial portion of the leaf may express a molecule, for instance a receptor or a transcription factor, that confers competence to respond to SAM-inducing signals.

Once the proposed leaf-meristem-leaf cycle is initiated, it can be propagated indefinitely – but where does it start? The position at which the primary SAM develops, at the adaxial bases of the cotyledons, is reminiscent of the position at which the axillary SAM develops, at the adaxial leaf base. The *phb-1d* mutation positively affects the development of the embryonic SAM in two situations. It partially suppresses the phenotype of mutants homozygous for the non-null *stm-1* allele, causing the development of determinate structures at the site of the presumptive SAM. Since the *stm-1* allele retains some activity, the suppression by *phb-1d* could act by boosting the levels of this residual activity. In addition, *phb-1d/+* mutant embryos have larger SAMs than wild-type embryos. These results can be explained by a model in which the adaxial sides of the presumptive cotyledons positively influence development of the presumptive SAM (Fig. 9B). While the evidence for this model of embryonic SAM development is limited, it provides a novel conceptual framework for apical embryonic development.

Adaxial leaf fate need not play a similar role in SAM formation in other types of vascular plants. For instance, in several grass species, the axillary SAM develops not on the adaxial leaf base but in closest association with the stem side of the axil (Sharman, 1945). Furthermore, the axillary SAM is clonally related to the leaf and stem segment above it (Johri and Coe, 1983; Poethig et al, 1986) suggesting that positional signals related to stem fate rather than adaxial leaf fate may be important in axillary SAM formation in this group.

Branch formation is a fundamental process in the development of plant form. The finding that a subregion of the leaf may play a critical role in the formation of SAMs, and therefore branches, sheds light on the way that plants make buds and also may change the way we think about the development of the embryonic SAM.

This work was supported by the National Science Foundation and the US Department of Agriculture. This is paper number 3506 from the Laboratory of Genetics. We thank A. Hudson and M. Evans for helpful discussions and M. Evans for critical reading of the manuscript. We also thank R. Fisher, E. Moan, G. Heck and J. Long for providing the STM-GUS reporter line and Heidi Barnhill for excellent technical assistance with the SEMs.

REFERENCES

Barton, M. K. and Poethig, S. (1993). Formation of the shoot apical meristem in *Arabidopsis thaliana*:: an analysis of development in the wild type and in the *shoot meristemless* mutant. *Development* **119**, 823-831.

Bowman, J. (1994). In *Arabidopsis, an Atlas of Morphology and Development* (ed. J. Bowman), pp. 156-161. New York: Springer-Verlag.

Estruch, J. J., Prinsen, E., Onckelen, H. V., Schell, J. and Spena, A. (1991). Viviparous leaves produced by somatic activation of an inactive cytokinin-synthesizing gene. *Science* **254**, 1364-1367.

Furner, I. J. and Pumfrey, J. E. (1992). Cell fate in the shoot apical meristem of *Arabidopsis thaliana. Development* **115**, 755-764.

Irish, V. and Sussex, I. M. (1992). A fate map of the *Arabidopsis* embryonic shoot apical meristem. *Development* **115**, 745-753.

Johri, M. M. and Coe, E. H. Jr. (1983). Clonal analysis of corn plant development I. The development of the tassel and ear shoot. *Dev. Biol.* **97**, 154-172.

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

Long, J. A., Moan E. I., Medford, J. I. and Barton, M. K. (1996). A member of the KNOTTED class of homeodomain proteins encoded by the *STM* gene of *Arabidopsis thaliana*. *Nature* **379**, 66-69.

McConnell, J. R. and Barton, M. K. (1995). Effect of mutations in the *PINHEAD* gene of *Arabidopsis* on the formation of the shoot apical meristem. *Dev. Genet.* **16**, 358-366.

Poethig, R. S., Coe, E. H. Jr. and Johri, M. M. (1986). Cell lineage patterns in maize embryogenesis: a clonal analysis. *Dev. Biol.* **117**, 392-404.

Sharman, B. C. (1945). Leaf and bud initiation in the Gramineae. *Bot. Gazette.* **106**, 269-289.

Sinha, N. R., Williams, R. E. and Hake, S. (1993). Overexpression of the maize homeobox gene, *Knotted-1*, causes a switch from determinate to indeterminate cell fates. *Genes Dev.* **7**, 787-795.

Snow, M. and Snow, R. (1942)**.** The determination of axillary buds. *New Phytol.* **41**, 13.

Snow, M. and Snow, R. (1959). The dorsiventrality of leaf primordia. *New Phytol.* **58**, 188-207.

Steeves, T. A. and Sussex, I. M. (1959). *Patterns in Plant Development* 2nd edition, pp. 139-141.

Sussex, I. M. (1955). Morphogenesis in *Solanum tuberosum* L. : Experimental investigation of leaf dorsiventrality and orientation in the juvenile shoot. *Phytomorphology* **5**, 286-300.

Talbert, P., Adler, H. -T., Parks, D. W. and Comai, L. (1995). The *REVOLUTA* gene is necessary for apical meristem development and for limiting cell divisions in the leaves and stems of *Arabidopsis thaliana. Development* **121**, 2723-2735.

Telfer, A. and Poethig, S. (1994). In *Arabidopsis* (eds. E. M. Meyerowitz and C. R. Sommerville), pp. 379-401. New York: Cold Spring Harbor Laboratory Press.

Waites, R. and Hudson, A. (1995). *phantastica*: a gene required for dorsiventrality of leaves in *Antirrhinum majus*. *Development* **121,** 2143-2154.