RESEARCH PAPER

Flower primordium formation at the Arabidopsis shoot apex: quantitative analysis of surface geometry and growth

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Received 28 June 2005; Accepted 31 October 2005

Abstract

Geometry changes, especially surface expansion, accompanying flower primordium formation are investigated at the reproductive shoot apex of Arabidopsis with the aid of a non-invasive replica method and a 3-D reconstruction algorithm. The observed changes are characteristic enough to differentiate the early development of flower primordium in Arabidopsis into distinct stages. Primordium formation starts from the fast and anisotropic growth at the periphery of the shoot apical meristem, with the maximum extension in the meridional direction. Surprisingly, the primordium first becomes a shallow crease, and it is only later that this shape changes into a bulge. The bulge is formed from the shallow crease due to slower and less anisotropic growth than at the onset of primordium formation. It is proposed that the shallow crease is the first axil, i.e. the axil of a putative rudimentary bract subtending the flower primordium proper, while the flower primordium proper is the bulge formed at the bottom of this axil. At the adaxial side of the bulge, the second axil (a narrow and deep crease) is formed setting the boundary between the flower primordium proper and the shoot apical meristem. Surface growth, leading to the formation of the second axil, is slow and anisotropic. This is similar to the previously described growth pattern at the boundary of the leaf primordium in Anagallis.

Key words: Arabidopsis, flower primordium, reproductive shoot apex, surface curvature, surface growth.

Introduction

A fundamental process in shoot morphogenesis is the formation of lateral organs, such as leaves and flowers, which takes place at the shoot apical meristem (SAM). SAM growth is indeterminate and changes of SAM shape and size are cyclical, with plastochron (time lapse between successive lateral organ initiation) being the main cycle (Lyndon, 1998). Shape and size changes in the course of leaf or flower formation are directional and growth of these organs is regarded as determinate, at least in the case of flowers and simple leaves (Lyndon and Battey, 1985). This means that early in lateral organ formation, the SAM surface has to be divided into two portions of different fate and growth (determinate versus indeterminate), which is the process of SAM surface partitioning (Lyndon, 1998). In the course of partitioning, a portion of SAM periphery becomes the lateral organ primordium, while the remaining portion maintains the SAM character. A number of genes are known to be involved in the regulation of this process, and the gene interactions underlying SAM function and, in particular, partitioning, have been characterized in detail for Arabidopsis thaliana (L.) Heynh. (reviewed recently in Golz and Hudson, 2002; Aida and Tatsaka, 2005).

In plants with an apical inflorescence the switch from the vegetative to the reproductive phase of development is often accompanied by a change in lateral organ identity from leaves to bracts or flowers. It is known from anatomical sections that, in Anagallis arvensis L. and Arabidopsis, the first periclinal divisions observed during primordium formation are in different tunica layers depending on the primordium identity (Vaughan, 1955). This is L2 in the case of leaf primordia and L3 in the case of flower primordia. Nevertheless, lateral organ primordia of different identities often appear first as bulges on the SAM periphery (compare, for example, leaf and flower primordia in Arabidopsis shoot apex shown in SEM micrographs by Long and Barton, 2000), and the question arises as to what is the difference in the growth leading to their formation. Another question is on the differences in growth leading to

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SAM surface partitioning when primordia of various identities are formed. One way in which these two questions can be addressed is the quantitative analysis of organogenesis at the SAM periphery. Since the geometry (shape) changes mark stages in lateral organ primordium formation, the geometry has to be quantified (Silk, 1984) in order to study organogenesis. This can be achieved with the aid of curvature computation (Dumais and Kwiatkowska, 2002). Growth quantification, in turn, has to account for the fact that plant cell growth is often anisotropic (Hernández et al., 1991; Tiwari and Green, 1991), i.e. growth rate is different in different directions. Growth anisotropy is manifested in principal growth rates, which are characterized by principal growth rate directions and values (Hejnowicz and Romberger, 1984). The principal directions are the directions in which growth rates attain their extreme values (minimal or maximal in the case of surface growth). While growth anisotropy refers to variation in linear growth rates in different direction at one point, growth heterogeneity means that areal or volumetric growth rate, which is a scalar value assigned to each point, is different at different points. Because of anisotropy, it is not sufficient to quantify growth just with measures of cell proliferation like the Mitotic Index, because such parameters can only be informative of growth in terms of heterogeneity.

A number of methods have been developed to facilitate plant growth quantification accounting for both heterogeneity and anisotropy (reviewed in Coen et al., 2004; Prusinkiewicz, 2004). In some, the computation of growth variables is based on sequential in vivo observations of a growing plant organ, like a leaf (Silk, 1983; Wolf et al., 1986; Schmoldt et al., 1998) or a flower (Hernández et al., 1991). In other methods growth variables are inferred from the analysis of cell wall pattern, as has been done for roots (Silk et al., 1989), or from clonal analysis as in the case of petal growth (Rolland-Lagan et al., 2005). The method developed by Green and collaborators (Goodall and Green, 1986; Williams and Green, 1988; Green et al., 1991; Hernández et al., 1991) is applicable to surface growth and geometry analysis at the shoot apex (Dumais and Kwiatkowska, 2002). Using this method, the early development of the leaf primordium and the accompanying SAM partitioning have been studied at the vegetative SAM of Anagallis (Kwiatkowska and Dumais, 2003). For the same species, flower development stages following the SAM partitioning have been also studied (Hernández et al., 1991). However, such an approach has not yet been used to investigate the earliest stages of flower primordium development, although major differences are expected between growth leading to the formation of flower and leaf primordia even at the earliest stages, since flowers are believed to be shoots, and flower organs, rather than the whole flowers, are homologues to leaves.

In the model plant Arabidopsis, for which so much molecular data are available, neither anisotropy of surface growth nor geometry changes accompanying leaf and flower primordium formation have been investigated, although novel techniques for in vivo observation of the Arabidopsis shoot apex have been developed (Dumais and Kwiatkowska, 2002; Grandjean et al., 2004; Kwiatkowska, 2004; Reddy et al., 2004). During the vegetative phase of Arabidopsis development, rosette leaves are formed. When the reproductive phase begins some of the pre-existing leaf primordia become bracts subtending lateral inflorescence shoots (paraclades), while the shoot apex starts to initiate flowers (Hempel and Feldman, 1994). Flowers in Arabidopsis are not subtended by bracts although gene expression patterns indicate that rudimentary bracts are formed (Long and Barton, 2000). The geometric aspects of the formation of rudimentary bracts remain unknown.

This paper presents an analysis of surface growth and geometry at the reproductive shoot apex of Arabidopsis, using a non-invasive replica method (Williams and Green, 1988) and the computational approach that includes 3-D reconstruction of the apex surface (Dumais and Kwiatkowska, 2002). The data collected are used to address the two questions on the morphogenesis of reproductive shoots stated above.

Materials and methods

Plant material and growth conditions

Seeds of Arabidopsis thaliana Columbia ecotype were obtained from the Nottingham Arabidopsis Stock Centre. The plants were grown in pots, in temperatures ranging from 21 °C (night minimum) to 28 °C (day maximum), with an illumination of 9 W m⁻². They were kept in long days (16/8 h day/night) throughout the experiment. The observation of apices started 5 weeks after germination, when the length of inflorescence axes varied between 4–40 mm. The oldest flower bud of the inflorescence had just opened in the longest of the analysed shoots and was still closed in the remaining shoot, i.e. the inflorescences were before or at stage 13 of their development according to Smyth et al. (1990). Seven inflorescence shoots were studied. Three exemplary shoot apices (apices I–III) are shown in the Results. The length of the shoot with apex I was 28 mm; with apex II=4 mm; and apex III=8 mm.

Data collection

Material was collected in essentially the same way as described by Kwiatkowska (2004) for the pin-formed Arabidopsis. Briefly, replicas (dental polymer moulds) were taken from the surface of each individual shoot apex (Williams and Green, 1988). Taking of the replica required only a temporary bending outward of young flower buds overtopping the apex, or sometimes removal of one or two of the oldest flowers. Replicas were filled with epoxy resin in order to prepare casts, which were sputter coated and observed in scanning electron microscopy (SEM) LEO435VP. From each of the individual apices replicas were taken at c. 12 h intervals for 24–36 h. With this material, morphogenesis of 15 flower primordia at different developmental stages could be followed.

Computational protocol for data analysis

Computer programs (available at: http://culex.biol.uni.wroc.pl/instbot/dorotak) written in Matlab (The Mathworks, Natick, MA, USA) were used to analyse the data (Dumais and Kwiatkowska,
Flower primordium formation: geometrical aspects

In the SEM image of the apex I, shown in Fig. 1A, flower primordia P2 and P3 are recognizable. However, a plot of curvature directions on the same apex surface also allows the younger primordium P1 to be recognized, because of the zero or negative (concave) meridional curvature of its surface. After 23 h all the three primordia (P1–3) become recognizable without the curvature plot (Fig. 1B). During this time interval P1 in Fig. 1B attained a developmental stage slightly more advanced and of a size slightly larger than P2 in Fig. 1A, while P2 in Fig. 1B was slightly more advanced and larger than P3 in Fig. 1A. This means that the plastochron for this apex is a bit shorter than 23 h. The same is true for apex II shown in Fig. 2. The time interval used for the apex III, i.e. 11 h, is, in turn, shorter than the plastochron duration. P3 from Fig. 3B does not attain the stage of P4 in Fig. 3A. Thus for the apices analysed the plastochron duration is between 11 h and 23 h.

Based on the geometry changes, the early flower primordium development can be divided into distinct stages. At first, a lateral outgrowth (bulge) is formed at the SAM periphery (P1 in Fig. 1A; P2 in Fig. 2A; P1 in Fig. 3A). The meridional curvature of the portion of primordium surface, which is visible in the top view of the shoot apex, is lower than the meridional curvature of the SAM surface. An adaxial boundary of the primordium, which can be drawn based on this difference in curvature, is not apparent in SEM micrographs (e.g. P1 in Fig. 3A). During the following 11–23 h the meridional curvature of the primordium is decreasing and the primordium becomes more prominent being extended in a meridional direction. The portion of the primordium visible in the top view of the apex attained the shape of a shallow crease (P2 in Fig. 1A; P1 in Fig. 3B), concave in a meridional direction (negative meridional curvature) and convex in a latitudinal direction (positive latitudinal curvature). This developmental stage, leading to the formation of a shallow crease, will be referred to as an ‘initial bulging’ stage. At the end of this stage the adaxial boundary of the primordium is still not recognizable in SEM micrographs, unless the curvature is computed.

Statistics

Analysis of variance (ANOVA) was performed for values of cell areas on SAM and flower primordium surface as well as for the areal growth rates computed for the SAM and various flower developmental stages. This was followed by a multiple comparison of means using a Tukey’s HSD test for unequal sample sizes. Statistica (Statsoft Inc.) software was used for this analysis.

Results

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Within the next 23 h the major portion of the shallow crease changes its shape to bulge, and its curvature is positive in both curvature directions (P3 in Fig. 1B; P3 in Fig. 3B). Only a band of cells at the abaxial margin of the shallow crease retains the negative meridional curvature, i.e. retains the crease shape (compare P2 in Fig. 1A and B). In the course of this stage a new crease also develops. This is the axil between the bulge and the SAM, i.e. at the adaxial side of the primordium (e.g. along P3 in Fig. 1B). It is a narrow region of negative meridional and positive latitudinal curvature, which develops from a band about two cells wide. The majority of these cells have not been located earlier in the shallow crease region, but originate from the adjacent portion of the SAM (compare P3 in Fig. 1A and B; P3 in Fig. 3A and B). This developmental stage will be referred to as ‘bulging in the shallow crease’. Starting from this stage the flower primordium is discernible from the SAM in SEM micrographs. During this and the following stage it was often difficult to obtain replicas from the entire upper primordium surface. The majority of these cells have not been located earlier in the shallow crease region, but originate from the adjacent portion of the SAM (compare P3 in Fig. 1A and B; P3 in Fig. 3A and B). This developmental stage will be referred to as ‘bulging in the shallow crease’. Starting from this stage the flower primordium is discernible from the SAM in SEM micrographs. During this and the following stage it was often difficult to obtain replicas from the entire upper primordium surface. This is because the primordium was overtopped by older flower primordia, which were often in direct contact with the primordium under consideration, obstructing the access of the dental polymer (hence the damaged replica surface of P3 in Fig. 2B or of the primordium shown in Fig. 4A).

After the bulging in the shallow crease, for at least 24 h, the bulge-shaped primordium increases in size while the axil on its adaxial side deepens (Fig. 4A–C). This stage will be called the ‘bulge’ stage. Initially the abaxial remnants of the shallow crease are maintained, as at the end of the previous stage. This region is concave in a meridional direction, and convex in a latitudinal direction (Fig. 4A). This adaxial crease flattens out during later primordium growth (compare Fig. 4A and B).

When the bulge attains a certain size two pairs of sepals are formed. This is the ‘sepal formation’ stage (Fig. 5). The first pair to appear are sepals located on the latitudinal sides of the primordium (defined with respect to the SAM), like S1a in Fig. 5A. These sepals appear as ‘folds’. Sepals of the second pair are formed slightly above the first pair and become apparent within the next 12 h on the meridional sides of the primordium (S2a and S2b in Fig. 5B). The first sepal of this pair emerges on the abaxial primordium side. The older sepals (i.e. of the first pair) remain smaller than the younger ones (compare S1a, b with S2a, b in Fig. 5B, C). Because of the limited access to the sepals of the first
pair, their development was not studied here. During the formation of the second pair of sepals, a crease (concave along the meridional direction and convex along latitudinal) appears between each of the sepals and the remaining primordium portion, i.e. the central bulge, which maintains positive curvatures in both directions (Fig. 5B, C).

Flower primordium formation: growth aspects

The consecutive stages of primordium development are characterized by a specific growth pattern. At the region of the SAM periphery, where the initial outgrowth is formed, areal growth rates are elevated (Table 1) and growth is strongly anisotropic (P1 formation site in Fig. 1C, D). The direction of maximal growth is meridional. In the latitudinal direction extension also takes place, but growth rate is much lower. The same growth pattern persists until the shallow crease is formed (P1 in Fig. 3C, D). Obviously, the formation of any bulge on the apex surface is also due to growth in a direction perpendicular to this surface. This growth component, however, cannot be studied with the present method.

Fig. 4. Scanning electron micrographs with curvature plots (A–C), and growth rate plots (D, E) for the flower primordium from the shoot apex II (P5 of Fig. 2A). Labelling as in Fig. 1. Growth rates are shown for cells on the plot of cell outlines as they appeared at the beginning of the considered time intervals. The sequence covers 23 h. Flower primordium is in the sepal formation stage throughout the sequence. Bars=15 μm.

Fig. 5. Scanning electron micrographs with curvature plots (A–C), and growth rate plots (D, E) for the flower primordium from the shoot apex I. Labelling as in Fig. 1. Red star is on the side of the primordium at which it contacts with the SAM (i.e. adaxial side of the primordium). Sepals are numbered in order of their appearance (S1a, S1b and S2a, S2b). Sepal boundaries identified at consecutive instants are marked on all the plots with the same colour: white for time 11 h; black for 23 h. Growth rates are shown for cells on the plot of cell outlines as they appeared at the beginning of the considered time intervals. The sequence covers 23 h. Flower primordium is in the bulge stage throughout the sequence. Bar=15 μm.
During the bulging in the shallow crease the future bulge cells grow fast and anisotropically (e.g. P2 and P3 in Fig. 1C, D). The direction of maximal growth is often meridional. Where the axil is formed, the above-mentioned band of cells composed of cells from the adaxial primordium portion and of adjacent SAM cells, starts to grow with low areal rates and high anisotropy (e.g. along P3 in Fig. 1C, D). The direction of maximal growth of these cells is latitudinal, while in the meridional direction contraction may occur. Consequently, the primordium becomes a bulge separated from the SAM by the axil.

Growth rates of the bulge surface are initially very high (P3 in Fig. 3C, D; Fig. 4D, E). At this developmental stage areal growth rates are the highest of all the stages analysed (Table 1). The direction of maximal growth rate is meridional, but growth is not strongly anisotropic (Fig. 4D, E). When sepal formation begins, areal growth rates decrease (Table 1) and the primordium surface becomes differentiated into variously growing regions (Fig. 5D, E). During the formation of the second pair of sepals, two bands of cells (one parallel to the adaxial, and the other to the abaxial margin of the bulge) start to grow more slowly than the remaining portion of the primordium (central bulge). These two regions give rise to two creases separating the sepals from the central bulge (like bands separating S2a and S2b from the central bulge in Fig. 5D, E). Sepal surface growth has not been analysed because, in the course of their formation, the surface, which could be tracked on preceding replicas, becomes hidden (Fig. 5C). Further growth of the central bulge is also not uniform (Fig. 5E). It is faster on the bulge periphery than in its centre, presumably because stamen formation begins (Vaughan, 1955).

**Changes of the mean cell size (surface area) during primordium development**

For the SAM and flower primordia of various stages, mean cell size was estimated as the mean area of the outer periclinal wall of the surface cells (Table 1). A comparison of mean cell areas revealed statistically significant differences between SAM cells and the flower primordium cells at different developmental stages. During the initial bulging stage, primordium cells are the smallest of all the cells considered, including the SAM cells. In the bulge stage, the primordium cells are the biggest of all the stages considered. The statistically significant difference is also between the cells of the bulge stage primordium and the sepal formation stage. In the latter, the cells are smaller, although still significantly bigger than the SAM cells.

**Discussion**

**Interpretation of geometry changes in the course of early flower primordium development in Arabidopsis**

According to the classical interpretation (Esau, 1953; Lyndon, 1998), a flower appearing as a lateral organ (like in the raceme-type inflorescence) develops as an axillary shoot, i.e. in the axil of a subtending leaf – a bract. Several papers report that the potential for the formation of a bract subtending the flower is also sometimes revealed in *Arabidopsis*. In certain growth conditions single flowers are formed instead of paracles in the axils of well-developed bracts (Hempel et al., 1998). Moreover, ‘empty bracts’ replace flowers if flower primordia are genetically ablated in transgenic lines with the cell-autonomous diphtheria toxin expressed under the control of the LEAFY (LFY) promoter (Nilsson et al., 1998). Certain traces of the development of *Arabidopsis* flowers in putative bract axils can also be found with the aid of curvature computation. It shows that, initially, the flower primordium in *Arabidopsis* does not appear as a bulge, but that bulge formation is preceded by the formation of a shallow crease. Next, bulging in the shallow crease takes place. Although during this stage the large adaxial portion of the shallow crease changes into a bulge, the remaining smaller abaxial portion retains the shallow crease shape. This abaxial crease, however, does not persist and is soon incorporated into the bulge. When bulging occurs in the shallow crease, adjacent SAM cells form one more crease, i.e. the axil at the adaxial flower primordium boundary. Such a developmental sequence could be interpreted in the way shown in Fig. 6. It can be assumed that the shallow crease is the first axil between the SAM and the lateral organ primordium, i.e. the axil of a putative rudimentary bract (Br in Fig. 6B, C).
The bulge, in turn, is the flower primordium proper (Fl in Fig. 6D), formed at the bottom of the putative bract axil. The second axil would then be the axil of the flower primordium proper, formed at the boundary between the flower primordium proper and the SAM (adaxial flower primordium boundary). In Fig. 6D the remnants of the first formed axil are visible on the right side of the flower primordium proper, while the second formed axil is on its left.

A similar sequence of stages of flower primordium development in Arabidopsis has been proposed earlier by Long and Barton (2000), who based their interpretation on gene expression patterns. They distinguished the initial stage 0 preceding the floral buttress stage (Smyth et al., 1990). The stage 0 is characterized by the appearance of the SHOOT MERISTEMLESS (STM) negative region on the meristem flanks. Comparison of the anatomical sections presented by Long and Barton (2000) with SEM micrographs, suggests that this stage is most likely the initial bulging stage of the present paper. Long and Barton (2000) showed that AINTEGUMENTA (ANT) and STM expression patterns change during the primordium development in such a mode that the rudimentary (‘cryptic’) bract subtending the flower primordium proper is manifested. This takes place during the stage which most likely corresponds to the early bulge stage of the present paper, when the remnants of putative bract axil (the first formed axil) are apparent on the abaxial side of the flower primordium proper.

The interpretation presented here is also supported by Grandjean et al. (2004), who observed that, during the early flower primordium development, new cells are recruited to the primordium from the SAM, as has been recognized from in vivo analysis of the ANT::GFP expression pattern. This recruitment stage of Grandjean et al. (2004) may be the stage when the flower primordium proper is formed. The recruited ANT expressing cells would be the flower primordium proper cells, which would be added to the cells earlier expressing ANT, i.e. those which already have the bract primordium identity. The next stage described by Grandjean et al. (2004) is the proliferation stage when the recruitment has already stopped and the number of cells comprising the primordium increases due to cell proliferation. This, in turn, would correspond to the bulge stage.

Finally, since the curvature is computed in the present analysis, shape changes accompanying primordium formation can be detected earlier here than in developmental studies in which the shape has not been quantified. Thus the first sign of primordium formation should be detectable earlier than when other methods are used. The number of cells, which can be assigned to the flower primordium in the present analysis is, nevertheless, larger than the number of the so-called founder cells, namely four, estimated on the basis of sector boundary analysis (Bossinger and Smyth, 1996). There is also a similar discrepancy between the sector boundary analysis (Bossinger and Smyth, 1996) and the results of Grandjean et al. (2004). A possible explanation for these differences could be that not all of the cells involved in the earliest primordium development, but only the portion of the shallow crease forming the bulge, contribute to flower formation, while other cells are ‘left behind’ at the base of the primordium as a putative rudimentary bract.

The observed changes in geometry and growth are distinct enough to resolve the earliest stages of flower primordium development defined by other authors. A comparison of SEM micrographs reveals that the flower buttress stage of Smyth et al. (1990) is presumably
comprised of the initial bulging and bulging in the shallow crease stages defined here. It is, however, questionable whether the whole initial bulging stage of the present paper has been recognized by Smyth et al. (1990). This may explain why the duration of the flower buttress stage estimated by these authors is shorter than the first two stages described here. The next two stages distinguished in the present paper and their duration correspond to those described by Smyth et al. (1990). These corresponding stages are the bulge stage and the hemispherical flower primordium; the sepal formation stage and primordium with sepalps, defined in the present paper and by Smyth et al. (1990), respectively. On the other hand, the initial bulging leading to the shallow crease formation is most likely equivalent to the P1 stage of Reddy et al. (1990), respectively. On the other hand, the initial bulging leading to the shallow crease formation is most likely equivalent to the P1 stage of Reddy et al. (2004) defined on the basis of in vivo observations using confocal laser scanning microscopy; while bulging in the shallow crease and bulge stages is equivalent to the P2 of the same authors.

Growth and cell divisions during the early flower development in Arabidopsis

Areal growth rates change significantly during flower primordium development. They are high and uniform over the primordium surface during the initial bulging stage, and lower and non-uniform during bulging in the shallow crease. During the bulge stage, in turn, the rates are the highest and again uniform, while the growth slows down and becomes non-uniform during the sepal formation stage. Remarkably, during the stage of the most rapid growth, i.e. the bulge stage, primordium surface cells are the largest, as if the division rates were not fast enough to partition the new surface available due to growth. When growth rates are again slower, i.e. during the sepal formation stage, the mean cell size diminishes.

Observed changes in areal growth rates are concurrent with the earlier reports on cell division distribution in the reproductive shoot apex of Arabidopsis. The elevated areal growth rates at the site of initial bulging and shallow crease formation are concurrent with the increase in the Mitotic Index preceding and accompanying the formation of primordium outgrowth observed by Laufs et al. (1998). Interestingly, the intensified cell proliferation has not been recognized when the Cumulative Mitotic Index (CMI) was estimated for these stages of primordium development by Reddy et al. (2004). A reason for this could be in the cumulative character of this index. The present study has revealed that growth of the flower primordium slows down during early bulging in the shallow crease stage, after the initial fast growth period. Including this stage of slower growth in the time interval for CMI calculation would mean an averaging of the high and low cell division rates.

During the bulging in the shallow crease and bulge stages the portion of shoot apex surface where the floral primordium is formed becomes differentiated into the fast growing primordium bulge and the slowly growing axil. Such a growth distribution is also manifested in the cell proliferation pattern as revealed by both CMI estimation (Reddy et al., 2004) and the 5-bromo-2′-deoxyuridine (BrdU) incorporation/immunodetection procedure (Breuil-Broyer et al., 2004). Later differentiation of the primordium surface into slowly growing sepal boundaries and the faster growing central bulge is again in agreement with the results of BrdU labelling technique (Breuil-Broyer et al., 2004).

The present investigation also reveals a remarkable variation of growth anisotropy in both time and space. The most striking temporal changes take place at the beginning of primordium development, when strongly anisotropic growth, with a meridional direction of maximal growth rate changes to less anisotropic and later on to nearly isotropic growth during the passage from the initial bulging to the bulge stage. Initially, the meridional direction of maximum growth rate is most likely manifested in the longitudinal cell files oriented across the boundary between the SAM and flower primordium proper, which has been observed by Reddy et al. (2004). Spatial variation in growth anisotropy becomes prominent, starting from the bulging in the shallow crease stage, when the axil region and the flower primordium proper differ not only in the degree of anisotropy but also in the direction of maximal growth rate. The same is true for the sepal formation stage.

Growth patterns in the course of the SAM surface partitioning

In is assumed that partitioning of the SAM surface can be regarded as complete as soon as the lateral organ boundary is settled, i.e. it ceases to move with respect to cells (Kwiatkowska and Dumais, 2003). In the case of the leaf primordium in Anagallis this is the time when a crease (the axil) is formed at the adaxial primordium boundary (Kwiatkowska and Dumais, 2003). Starting from this moment, the boundary is stable with respect to cells, although the boundary recognized from the curvature in preceding developmental stages was moving. The partitioning of the SAM surface in the course of formation of the flower primordium proper in Arabidopsis is similar. In the case of both the leaf and flower primordium, growth at the axil formation site is slow and anisotropic, with extension taking place in a latitudinal direction, and contraction in a meridional direction. In both the cases, the axil is formed at least partly by SAM cells adjacent to the primordium.

Future axil or boundary cells presumably express specific genes (Aida and Tasaka, 2005), although the exact spatial and temporal relationships between gene expression domains and these cells remain unknown. The expression domains of CUP-SHAPED COTYLEDON (CUC) genes and LATERAL ORGAN BOUNDARIES are related to primordium boundaries in Arabidopsis (Aida et al., 1999; Traas and Doonan, 2001; Shuai et al., 2002; Vroemen et al., 2004).
et al., 2003). The CUC genes are thought to act as a local growth suppressor (Traa and Doonan, 2001; Vorstenbosch et al., 2003), which theoretically can lead to the crease formation (Todd, 1986). CUC2 expression is strictly opposite to cell-cycle gene expression profiles in flower primordium (Breuil-Broyer et al., 2004). It also overlaps with boundaries between sepal and other floral organ whorls.

These observations together with the observations presented here are in favour of the argument that locally changed (decreased) growth rate rather than the appearance of a crease-like region is a crucial factor for setting the primordium boundary. Crease-like regions can also be formed without growth suppression, as in the case of the primordium boundary. Crease-like regions can also be presented here are in favour of the argument that locally

Acknowledgements

The author thanks Professor Zygmunt Hejnowicz (Silesian University, Poland) and Dr Mitsuhiro Aida (Nara Institute of Science and Technology, Japan) for critical reading of the manuscript, and Drs Zofia Czarna and Krystyna Heller (Electron Microscopy Laboratory, Wroclaw University of Agricultural Sciences, Poland) for help in preparing the scanning electron micrographs used in this work. Thanks are also to Dr Mitsuhiro Aida for making the manuscript available prior to its publication. The final part of this research was financed by the Marie-Curie Research-Training Network SY-STEM.

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