

Robust organ size requires robust timing of initiation orchestrated by focused auxin and cytokinin signalling

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Organ size and shape are precisely regulated to ensure proper function. The four sepals in each *Arabidopsis thaliana* flower must maintain the same size throughout their growth to continuously enclose and protect the developing bud. Here we show that DEVELOPMENT RELATED MYB-LIKE 1 (DRMY1) is required for both timing of organ initiation and proper growth, leading to robust sepal size in *Arabidopsis*. Within each *drmy1* flower, the initiation of some sepals is variably delayed. Late-initiating sepals in *drmy1* mutants remain smaller throughout development, resulting in variability in sepal size. DRMY1 focuses the spatiotemporal signalling patterns of the plant hormones auxin and cytokinin, which jointly control the timing of sepal initiation. Our findings demonstrate that timing of organ initiation, together with growth and maturation, contribute to robust organ size.

evelopment is remarkably reproducible, generally producing the same organ with invariant size, shape, structure and function in each individual. For example, mouse brains vary in size by only about 5% (ref. 1) and Arabidopsis floral organs are strikingly uniform². Defects in organ size control mechanisms contribute to many human diseases, including hypertrophy and cancer^{3,4}. Uniformity of fruit size is an important criterion for packaging and shipping of fruit to market⁵. In this context, robustness is the ability to form organs reproducibly despite perturbations, such as stochasticity at the molecular and cellular level as well as environmental fluctuations⁶. Robustness has fascinated biologists since Waddington brought the issue to prominence in 19427. One proposed scenario for achieving organ size robustness is that organs can sense their size and compensate through adjustment of their maturation time until the correct size has been attained⁸. For example, in Drosophila, damaged or abnormally growing imaginal disks activate the expression of Drosophila insulin-like peptide 8 (DILP8), which delays metamorphosis and thus allows damaged disks to reach the correct size^{9,10}. These compensatory mechanisms can mask early-stage defects but, in other cases, robustness is crucial throughout organ growth. For instance, in Arabidopsis thaliana all four sepals of the flower must maintain equal size throughout development to keep the bud closed and protect the developing reproductive organs11. Likewise in humans, two arms must maintain equal length from infancy to adulthood so that we can pick up and carry objects¹². In such cases, delaying maturation to compensate for early size defects is not effective, and how robustness is achieved is still poorly understood.

Sepals, the outermost floral organs, are a good model system for investigating the mechanisms of organ size robustness throughout development because individual plants can produce more than 50 invariant flowers. This allows statistical assessment of organ size uniformity within a single organism, which cannot be achieved in most model systems. Sepals arise from floral meristems (stem cells that give rise to floral organs), which initiate from the periphery of the inflorescence meristem (stem cells at the tip of the plant that give rise to the flowers; Fig. 1a). On the flank of the A. thaliana floral meristem, four sepals initiate and rapidly grow to cover that flower. The four growing sepals in a flower must maintain the same size and shape to enclose and protect developing reproductive organs throughout growth before the flower blooms¹¹; thus, continuous robustness of size and shape is required for sepal function (Fig. 1a). We established a nomenclature for the four sepals in a flower. The sepal closest to the inflorescence meristem is the inner sepal while the sepal opposite, farthest from the inflorescence meristem, is the outer sepal. The two sepals on the sides are lateral sepals (Fig. 1a). Here we consider the hypothesis that sepals can achieve robustness throughout their development by synchronization of initiation and maturation. This synchronization hypothesis predicts that early defects in the timing of initiation would cause cascading defects in organ size because late-initiating organs would not have as much time to grow before the organs mature synchronously.

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Fig. 1 | Mutations in the DRMY1 gene lead to increased variation in sepal size and shape. a, Anatomical diagrams. Floral meristems (FM) emerge from the inflorescence meristem (IM). Four sepals initiate evenly from the periphery of the floral meristem. The inner sepal is closest to the IM, the outer sepal farthest from the IM and the lateral sepals on the sides. Note that, throughout development, sepal sizes are always similar. The four sepals are the outermost floral organs surrounding the petals, stamens and carpels in the mature flower, as shown in the top and side views. b, WT and vos2 (drmy1-2) flowers as closed buds (stage 12, left) and after blooming (stage 15, right). Orange arrows, smaller sepals within each vos2 (drmy1-2) flower. Scale bars, 1mm. n=10 flowers for each stage and genotype. c, Sepal area distribution is wider for the vos2 (drmy1-2) mutant compared to WT. Area variability was quantified by the coefficient of quartile variation and was significantly higher in vos2 (one-sided permutation test, ***P<0.0001). The average area of vos2 sepals was significantly lower than for WT (one-sided permutation test, ***P < 0.0001). The boxes extend from the lower to upper quartile values of the data, with a line at the median. The whiskers extend past 1.5 x interquartile range. n = 400 for both WT and vos2 (drmy1-2) 10th to 25th flowers along the main branch. Outer, inner and lateral sepals were pooled. d, CV calculated for the areas of the four sepals in each single flower. Sequential flowers along the main branch of the stem (flower number on the x axis) were measured at stage 14. Three replicates are included for both WT (blue) and vos2 (drmy1-2, red) mutants, and original individual sample curves can be found in Source Data. The average CVs of the three replicates are presented as thick blue and red lines, with s.d. as a partially translucent background. e, vos2 (drmy1-2) mutants also exhibit higher variation in inner and lateral sepal shape. Superimposed outlines of stage 14 sepals from WT and vos2 (drmy1-2) mutants were normalized by sepal area. The black outline is the median. n = 60 sepals for both WT and vos2 (drmy1-2). f, Gene model for the DRMY1 gene (AT1G58220). Orange box indicates the location of the MYB domain. A G-to-A point mutation was identified at an exon-intron junction in the vos2 (drmy1-2) mutant (top, WT sequence; bottom, drmy1-2 sequence; intron in lower case, exon in upper case). drmy1-1 was reported with a tDNA insertion in the first intron. g, Expression pattern of pDRMY1::3XVENUS-N7 (white). The expression of nuclear-localized VENUS driven by the DRMY1 promoter was observed in young-stage flowers, especially in the peripheral zones. Cell walls were stained with propidium iodide (PI, red). Scale bars, 20 µm. n = 3 inflorescences. h, Scanning electron micrographs illustrating that the sepal size variability phenotype can be observed at the early stages (stage 5) and remains visible through the late stages. Red arrowheads denote delayed sepal initiation at stage 5; red arrows denote smaller sepals within the drmy1-2 flower. Scale bars: top, 30 μ m; bottom, 200 μ m. n = 2 flowers.

Results

Mutations in DRMY1 cause variability in sepal size. Robustness mechanisms can be identified by screening for mutants with increased variability^{13,14}. Accordingly, we screened for mutants exhibiting variable size or shape of the sepals, thus disrupting robustness¹⁴. Our previous analysis of the variable organ size and shape1 (vos1) mutant revealed that highly variable cell growth is averaged in time and space to create robust organs, and that synchrony of sepal maturation contributes to size robustness¹⁴. From that mutant screen, we also isolated the variable organ size and shape2 (vos2) mutant that had sepals of different sizes within the same flower. Consequently, vos2 sepals failed to form a complete barrier to protect the inner reproductive organs (Fig. 1b). To exclude the possibility that this variability arose from altered sepal number, we counted the number of sepals produced in vos2 flowers and found that it was largely unaffected, with four sepals present in >92% (164/177) of vos2 flowers compared to 100% (207/207) of wild-type (WT) flowers (Extended Data Fig. 1a). Next, we quantified the size distribution of mature sepals from many mutant plants, in flowers with four sepals. We found that vos2 mutant sepals had increased variability in area and reduced average area compared to wild type, especially for inner and lateral sepals (Fig. 1c and Extended Data Fig. 1e). We further assayed individual flowers developing sequentially along the main branch of a single plant, and found that the area of the four sepals in each vos2 flower consistently exhibited higher coefficients of variation (CV) and smaller averages in sepal area than wild type (Fig. 1d and Extended Data Fig. 1b). Additionally, the shapes of vos2 inner and lateral mutant sepals were more variable than wild-type sepals, after normalizing for area (Fig. 1e and Extended Data Fig. 1c). The defect was not restricted to sepals, as vos2 petal areas were also more variable than wild type (Extended Data Fig. 1f). VOS2 is thus required for robustness of floral organ size and shape.

Map-based cloning of vos2 identified a G-to-A point mutation in a splice acceptor site of the gene (AT1G58220) encoding the MYB domain protein DEVELOPMENT RELATED MYB LIKE 1 (DRMY1; Fig. 1f). This point mutation caused altered splicing, resulting in both premature stop codons (Extended Data Fig. 2a) and a dramatic decrease in DRMY1 transcript level (Extended Data Fig. 2b). A transfer DNA (tDNA) insertion allele, drmy1-1, was recently reported as broadly affecting cell expansion¹⁵. Thus, we renamed vos2 as drmy1-2. To verify that mutations in DRMY1 caused the variable sepal size and shape phenotype, we observed that the drmy1-1 tDNA insertion allele also exhibited sepal size variability and that drmy1-1 and drmy1-2 alleles failed to complement, indicating they were alleles of the same gene (Extended Data Fig. 2c-f). Furthermore, expression of DRMY1 under its endogenous promoter rescued the sepal variability phenotypes (20/21 rescued in T1), confirming the role of DRMY1 in the regulation of sepal robustness (Extended Data Fig. 2g).

To determine when DRMY1 functions in sepal robustness, we examined reporters for DRMY1 expression. The DRMY1-mCitrine fusion protein (*pDRMY1::DRMY1-mCitrine*) rescued the *drmy1-2* mutant phenotypes (21/23 rescued in T1), indicating that the fusion protein is functional (Extended Data Fig. 2h). The DRMY1 reporters were expressed broadly within young flowers, floral meristems and inflorescence meristems (Fig. 1g and Extended Data Fig. 2i,j). The *DRMY1* transcriptional reporter had somewhat higher expression within the periphery of developing floral and inflorescence meristems, hinting that DRMY1 might function in organ initiation.

Sepal primordium initiation is variably delayed in *drmy1-2* mutants. Since *DRMY1* reporters were expressed before and during sepal primordium initiation, we used scanning electron microscopy (SEM) to determine the stage at which the defect in sepal size robustness was first visible in *drmy1-2* mutants. Sepal variability in *drmy1-2* arose during initiation and was visible throughout flower

development (Fig. 1h). In wild type, the four sepals were the first organ primordia to initiate at the periphery of the floral meristem. At the same stage in *drmy1-2*, the flowers exhibited a normal-looking outer sepal. However, the inner and lateral sepal primordia were often absent or appeared smaller than in wild type, suggesting that their initiation was delayed (Fig. 1h). As mentioned above, >92% of *drmy1-2* flowers had four sepals at maturity, consistent with a delay rather than a blocking of sepal initiation.

To determine the extent to which the timing of sepal initiation is actually delayed in the *drmy1-2* mutant, we live-imaged wild-type and *drmy1-2* flowers throughout the initiation of sepal primordia (Fig. 2a,b and Supplementary Videos 1 and 2). We defined the bulging out of sepal primordia from the floral meristem as the morphological initiation event, which we detected by observing the Gaussian curvature of the meristem surface. A clear band of positive curvature (red on the heat map) at the flank of the floral meristem indicated the initiation of the sepal (Fig. 2c,d). The initiation of the outer sepal occurred first, and was set as the starting point, followed by the inner and then lateral sepals. For wild type, the time intervals between the initiation of outer and inner sepals were always around 6 h (Fig. 2a,c,e). Within 12 h after initiation of the outer sepal, the two lateral sepals initiated (Fig. 2a,c,f); the sepal primordia then grew rapidly to cover the floral meristem by 30 h (Fig. 2a).

In contrast, for the *drmy1-2* mutant, the time intervals for the initiation of inner and lateral sepals were elongated and more variable (Fig. 2b,d–f). In *drmy1-2*, the inner sepals initiated anywhere from 6 to 36 h after the outer sepals (Fig. 2e). Likewise, the lateral *drmy1-2* sepals initiated from 12 to 30 h after the outer sepal (Fig. 2f). Initiation of the lateral sepals occasionally occurred before the initiation of the inner sepal in *drmy1-2* flowers. Frequently, two sepal primordia appeared to form rather than one at the inner position of *drmy1-2* flowers (for example, highlighted with red arrowheads in Fig. 2b). However, further live imaging revealed that most of these fused to form a single sepal with two tips, resulting in the four sepals finally observed in *drmy1-2* flowers (Extended Data Fig. 1d). In addition, it took much more time for *drmy1-2* sepal primordia to cover the whole floral meristem (Supplementary Video 2).

We performed our analysis relative to the initiation of the outer sepal, because our hypothesis is that the delay in sepal initiation of inner and lateral sepals relative to the outer sepal accounts for their smaller size through flower development. To test whether there was also a delay in *drmy1-2* outer sepal initiation, we measured the flower radius before and at outer sepal initiation and saw no change in *drmy1-2* versus wild type (Extended Data Fig. 3a). We speculate that some positional aspect of being close to the cryptic bract, or some other remnant of floral meristem development, causes more robust outer sepal initiation. Thus, initiation of the outer sepal is a reasonable marker for staging.

Stiffer cell walls in *drmy1-2* mutants correlate with delayed sepal initiation. DRMY1 encodes a MYB domain protein, and most MYB domain proteins function as transcription factors¹⁶. To identify the biological processes that are regulated by DRMY1 to promote robust timing of sepal primordium initiation, we performed an RNA sequencing (RNA-seq) experiment comparing inflorescences and flowers of *drmy1-2* mutants to wild type. Gene ontology term analysis of the differentially expressed genes revealed an enrichment of biological processes including 'cell wall modification', 'response to hormone stimulus' and 'cellular metabolic process' (Extended Data Fig. 3e,f and Supplementary Data 1). Plant cell walls become softer through cell wall modification during primordium initiation, to allow outgrowth. Genetic stiffening of the cell wall is sufficient to block the initiation of organ primordia^{17,18}. Our RNA-seq data suggested that cell wall stiffness might be changed in drmy1-2 mutants due to altered cell wall modifications. To determine whether the delayed organ initiation in drmy1-2 might result from a stiffer cell

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Fig. 2 | Sepal initiation is variably delayed in the *drmy1-2* **mutant. a,b**, Live imaging of sepal initiation in WT (a) and *drmy1-2* (b). The plasma membrane marker (p355::mCitrine-RCl2A) is shown in greyscale. The small bulge-out is defined as sepal initiation. Blue arrowheads in WT and red arrowheads in *drmy1-2* indicate initiated sepals. Scale bars, 25 µm. n = 15 for WT and n = 18 for *drmy1-2*. **c,d**, Gaussian curvature heatmap detecting changes in curvature associated with initiation for the live-imaging sequences shown in **a,b**. Red represents the domed shape while blue represents the saddle shape. Thus, a strong red band at the periphery (white arrowheads) reveals initiated sepals. Scale bars, 25μ m. **e**, Histogram showing the time interval between the initiation of the outer and inner sepals in each flower. n = 12 for WT flowers and n = 17 for *drmy1-2* flowers. Note that WT inner sepals initiate robustly 6 h after the outer sepals, while the timing is variable and generally longer in *drmy1-2*. **f**, Histogram showing the time interval between the initiation of the outer and lateral sepals for each flower. n = 12 for WT flowers and n = 17 for *drmy1-2* flowers. Note that WT inner sepals initiate robustly 12 h after the outer sepals, while the timing is variable and generally longer in *drmy1-2* flowers. Note that WT inner sepals initiate robustly 12 h after the outer sepals, while the timing is variable and generally longer in *drmy1-2* flowers. Note that WT inner sepals initiate robustly 12 h after the outer sepals, while the timing is variable and peripheries of floral meristems where sepal primordia emerge (SP; highlighted by black dotted boxes) for WT and *drmy1-2* mutants. In the heatmap of apparent elastic modulus shown here, red indicates stiffer and blue indicates softer (n = 11). Scale bars, 10 µm.

wall, we first used atomic force microscopy (AFM) to quantify the cell wall stiffness of sepal primordia and floral and inflorescence meristems. For all three, the cell wall was significantly stiffer in

the *drmy1-2* mutant (higher average apparent elastic modulus, P=0.000618; Fig. 2g and Extended Data Fig. 3b), consistent with the delay of primordium initiation. We also used osmotic treatment

to assess cell wall stiffness by quantifying the shrinkage of cell walls when internal turgor pressure was decreased. Osmotic treatment of wild-type and *drmy1-2* developing sepals further confirmed that cell walls were stiffer in the *drmy1-2* mutant (Extended Data Fig. 3c,d). Our data are consistent with the model that stiffer cell walls in *drmy1-2* led to delayed sepal initiation and, consequently, higher sepal size variability.

Variably delayed initiation disrupts sepal size robustness throughout development. To test whether delayed sepal initiation decreases sepal size throughout flower development, we live-imaged sepals from their initiation throughout their development over 11 days (Fig. 3a,b and Extended Data Fig. 4e). In wild type, after robust initiation, the sepals maintained equivalent sizes so that flowers remained closed throughout development (Fig. 3a). At the end of our live-imaging series, sepal sizes were nearly equivalent (Extended Data Fig. 4e). In contrast, in drmy1-2 flowers, sepals with delayed initiation remained smaller than other sepals throughout development, so that flowers remained open throughout development (Fig. 3b). At the end of our live-imaging series, these sepals were of variable size (Extended Data Fig. 4e). At maturity in vivo, wild-type outer sepals were slightly larger than inner sepals which, in turn, were slightly larger than lateral sepals, corresponding with their timing and order of initiation (Fig. 3f). In wild type, outer and inner sepal sizes were correlated whereas in drmy1-2 the correlation was weaker presumably by the delayed and variable initiation (Extended Data Fig. 4f). Furthermore, the drmy1-2 inner and lateral sepals had a more severe decrease in size relative to wild type than outer sepals, correlating with their delayed initiation (Extended Data Fig. 4g). These results indicate that precisely timed initiation is crucial for robustness in organ size, consistent with the synchronization hypothesis. Our results imply that late-initiating sepals cannot catch up because they have less time to grow, resulting in variable sepal size.

Initiation is the primary cause of variability in *drmy1-2* **mutants.** We asked whether subsequent sepal growth contributes to variability in *drmy1-2* sepal size. We tracked cells and their resultant daughters in sepals after initiation, enabling us to measure cell growth and division rates. In both outer and lateral sepals, *drmy1-2* cellular growth was somewhat slower than wild type (Fig. 3c-e and Extended Data Fig. 4a-c). Cell division was reduced in the *drmy1-2* sepals (Extended Data Fig. 4d). These results indicate that *drmy1-2* sepals also exhibit growth defects. However, in both *drmy1-2* and wild type, lateral sepal growth was faster than outer sepal growth, which means that growth differences are slightly decreasing size variability rather than promoting it (Fig. 3c-e).

Previously we have shown that spatiotemporal averaging of variable cell growth results in sepal size and shape robustness and that this process is disrupted in *vos1* (ref. ¹⁴). Spatiotemporal averaging occurred normally in the *drmy1-2* mutant during early-stage growth, indicating that the loss of robustness was due to distinct

mechanisms (Extended Data Fig. 5). Thus, we conclude that variability in the timing of sepal primordium initiation in *drmy1-2* is the major source of variability in sepal size throughout development.

Weak and diffuse auxin responses in drmy1-2 mutants correlate with delayed sepal initiation. We next investigated how DRMY1 regulates the timing of sepal initiation. Auxin induces cell wall loosening, promoting cell expansion and allowing emergence of the primordium¹⁹. Before the primordium initiates or bulges, the first sign of the incipient primordium is a localized region of auxin signalling created by the polarized transport of auxin²⁰⁻²³. We examined the auxin response reporter pDR5rev::3XVENUS-N7 (DR5)^{20,24}. In wild-type floral meristems, we found that the positions of incipient sepal primordia were marked by the expression of DR5 before primordium initiation occurs (Fig. 4a). Consistent with the variably delayed primordium initiation in drmy1-2, expression of the DR5 auxin response reporter was weaker and more diffuse in drmy1-2 mutants (Fig. 4a and Extended Data Fig. 6a, quantified in Fig. 4c). Weaker and more diffuse DR5 fitted with higher stiffness and slower growth in drmy1-2 mutants. Nevertheless, sepal primordia emerged from regions of auxin signalling in drmy1-2 mutants. Positions where auxin signalling reaches sufficiently high levels to initiate primordia are determined by the polar localization of the auxin efflux carrier PINFORMED1 (PIN1)^{20,23}. PIN1 protein continued to polarize in *drmy1-2* inflorescence meristems and early flowers, so the more diffuse auxin response could not be explained readily by a loss of PIN1 polarity (Extended Data Fig. 6c,d). Consistent with a decrease in auxin signalling, drmy1-2 mutant plants exhibited a number of additional phenotypes associated with auxin signalling mutants: enhanced bushiness of the plant, shorter plant stature²⁵, smaller root meristem²⁶, shorter roots and fewer lateral roots²⁷ (Extended Data Fig. 6e-g). Together these data suggest that auxin signalling/response is reduced and more diffuse in *drmy1-2* mutants, correlating with delayed sepal primordium initiation.

Strong and diffuse cytokinin responses in drmy1-2 mutants correlate with delayed sepal initiation. Through its crosstalk with auxin, the plant hormone cytokinin controls the precise timing of flower primordium initiation within inflorescence meristems^{28,29}. Therefore, we tested whether cytokinin signalling is involved in sepal primordium initiation and is altered in drmy1-2 mutants, using the cytokinin signalling reporter pTCS::GFP (TCS)³⁰. In wild-type flowers, TCS was expressed in the four incipient sepal positions, consistent with a role for cytokinin in primordium initiation. In the drmy1-2 mutant, the expression of TCS expanded and in some flowers formed a ring shape in the periphery of the floral meristem where the sepals should initiate (Fig. 4b and Extended Data Fig. 6b, quantified in Fig. 4d). drmy1-2 mutant plants also exhibited additional phenotypes associated with increased cytokinin signalling: disordered sequence and positions of flowers around the stem (phyllotaxy)²⁸, and enlarged inflorescence meristems^{31,32} (Extended Data Fig. 6h-j).

Fig. 3 | **Sepals with delayed initiation in** *drmy1-2* **mutants remain smaller throughout development. a**,**b**, Live imaging of WT (**a**) and *drmy1-2* mutant (**b**) flowers every 24 h. The closed flower indicates robust sepal size in WT while the *drmy1-2* flower remains open due to variable sepal size (n=3). Inset, top view of the flowers for the first two time points; arrows, inner sepals; scale bars, 100 µm; scale bars in insets, 20 µm. **c**,**d**, 12-h early-stage (from stage 4 to stage 6) cellular growth heatmap for both WT (**c**) and *drmy1-2* (**d**) outer (left) and lateral (centre) sepals. The sepal cellular growth rate was quantified from live imaging of sepals immediately following initiation. For the heatmap, red and blue indicate high and low relative growth rate, respectively. Relative growth rate is defined as final cell size divided by initial cell size. Segmented cells are outlined in dashed yellow and superimposed on the meshed surface where the cell plasma membrane images are projected (greyscale). n=3 biological replicates (flowers), all showing similar trends. Scale bars, 20 µm. **e**, Average cell growth rate curves of early-stage outer and lateral sepals for WT and *drmy1-2*. Error bars, s.e.m. n=3 biological replicates (flowers), all showing similar trends. This graph shows the quantification of one flower. **f**, Sepal area distribution for outer, inner and lateral sepals. The boxes extend from the lower to upper quartile values of the data and the whiskers extend past 1.5 × interquartile range. Small dots for each box indicate outliers. Sepals from different flowers were pooled. n=48 for both WT and *drmy1-2* 10th to 25th flowers along the main branch. Two-tailed Student's t-test, *P < 0.05 (P value for the mean of sepal area, WT versus *drmy1-2* outer sepal, 1.62×10^{-12} ; WT versus *drmy1-2* inner sepal, 3.70×10^{-12} ; WT versus *drmy1-2* lateral sepal. 3.41×10^{-39}).

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Auxin and cytokinin signalling patterns are required for robust timing of sepal initiation. Based on hormone signalling reporters and hormone-related phenotypes, cytokinin response increased and auxin response decreased in *drmy1-2* mutants. More

importantly, the tight spatial localization of response reporters became more diffuse in *drmy1-2*. We therefore used three different ways to disrupt auxin or cytokinin signalling and tested whether these disruptions could cause defects in the timing of sepal primordium



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Fig. 4 | Focused auxin and cytokinin signalling are required for robust sepal initiation. a, Expression of the auxin response reporter DR5 (DR5::3XVENUS-N7, white) accumulates at the four incipient sepal initiation positions in WT. DR5 expression is lower and more diffuse in the drmy1-2 mutant. p35S::mCherry-RCI2A: red, plasma membrane; scale bars, 10 µm. n=10 flowers. b, Expression of the cytokinin response reporter TCS (pTCS::GFP, grey) accumulates at the four incipient sepal initiation positions in WT. TCS expression is enhanced and more diffuse in the drmy1-2 mutant. Red, chlorophyll autofluorescence; scale bars, 10 µm. n = 10 flowers. c, Quantification of DR5 signal in WT (blue) and drmy1-2 (red) in stage 2 flowers when no sepals have yet initiated. Signal was quantified radially for the 360° of the approximately circular flower meristem. The top-left region between the outer and lateral sepals was defined as angle 0. Angles increased in the counterclockwise direction, and normalized signal values within bins of size 1° are plotted. n = 10 for both WT and drmy1-2, and original individual sample curves can be found in the Source Data. The average signal intensity of the ten replicates is presented as thick blue and red lines, with s.d. as a partially translucent background. Note that four clear peaks of DR5 signal are present in WT. In drmy1-2 the outer sepal peak is evident, although weaker, and the remainder of the flower signal is relatively low with no evident clusters. d, Quantification of TCS signals in WT (blue) and drmy1-2 (red) flowers at stage 2 when no sepals have yet initiated. n = 10 for both WT and drmy1-2, and original individual sample curves can be found in the Source Data. The average signal intensity of the ten replicates is presented as thick blue and red lines, with s.d. as a partially translucent background. Note that four clusters of TCS signal are evident in WT, whereas in drmy1-2, TCS expression is higher and tends to surround the meristem. e, Stage 6 flowers, where the sepals have just closed, from WT inflorescences cultured in 5 µM BAP or mock media for 6 days. Blue arrowheads, delayed sepal initiation. Scale bars, 10 µm. Quantification is given in Extended Data Fig. 7c. f, Stage 6 drmy1-2 flowers from inflorescences cultured in 5 µM BAP or mock media for 6 days. Red arrowheads, smaller sepals, indicating delayed sepal initiation. Scale bars, 10 µm. Quantification is given in Extended Data Fig. 7c. Note that drmy1-2 flowers cultured in BAP exhibit phyllotaxis defects. g, The extent of disruption of auxin and cytokinin responses correlates with the degree of variability in sepal initiation timing. The mutation of AHP6, a cytokinin signalling inhibitor, causes a very mild phenotype per se but enhances drmy1-2 sepal initiation phenotypes. Quadruple mutants in the auxin receptor tir1 afb1-1 afb2-1 afb3-1 (tir1afb1-2-3 for short) exhibit a severely delayed sepal initiation phenotype. Cell walls stained with PI in greyscale. Yellow arrowheads, smaller sepals than normal, indicating delayed sepal initiation. Scale bars, 50 µm. Note that tir1 afb1-2-3 mutants exhibit phyllotaxis defects. n=3 biological replicates.

initiation: increasing cytokinin, decreasing auxin signalling and disrupting crosstalk.

First, we tested whether a broad increase in cytokinin signalling was sufficient to delay sepal primordium initiation, by external application of cytokinin to whole floral meristems in wild type. We cultured dissected wild-type inflorescences in 5μ M cytokinin (6-benzylaminopurine (BAP)) media or mock media for 6 days. Cytokinin-treated flowers exhibited delayed and more variable

sepal primordium initiation, which mimicked the phenotypes of drmy1-2 mutants (Fig. 4e and Extended Data Fig. 7a, quantified in Extended Data Fig. 7c). Similar to drmy1-2, the outer sepal was less affected (Fig. 4e and Extended Data Fig. 7a,d). Further follow-up of the development of these flowers revealed that continued cytokinin treatments severely stunted growth and that sepal size continued to be unequal (Extended Data Fig. 7g). We verified that cytokinin treatment not only increased TCS signals but also made TCS expression more diffuse (Extended Data Fig. 8a). As control, the cytokinin receptor mutant wol-1 was relatively insensitive to the treatment (Extended Data Fig. 8b) and the TCS reporter remained unaffected when the inflorescence was treated with auxin (0.1, 1 and 20µM 1-naphthaleneacetic acid (NAA)), confirming its specificity to cytokinin (Extended Data Fig. 8c). Culture of drmy1-2 mutant inflorescences in 5µM cytokinin also enhanced sepal initiation defects, even for the outer sepal (Fig. 4f and Extended Data Fig. 7b-d). This shows that the delay in organ initiation is not maximal in *drmy1-2*, and further suggests that organ initiation delays are associated with disrupted cytokinin patterns.

Having shown that enhanced cytokinin signalling could delay sepal initiation, we then checked whether reduction of auxin signalling throughout the flower was sufficient to delay sepal primordium initiation. Auxin signalling is inhibited in the auxin receptor quadruple mutant *tir1-1 afb1-1 afb2-1 afb3-1* (ref. ³³) and we observed similar, but more severe, defects in the timing of sepal primordium initiation defects compared with *drmy1-2* (Fig. 4g). Thus, auxin signalling is also required for proper timing of sepal initiation.

Finally, we tested whether crosstalk between auxin and cytokinin is necessary for robust sepal initiation. We found that 5μ M cytokinin made the DR5 auxin response reporter's signals more diffuse, similar to our observations in *drmy1-2* mutants (Extended Data Fig. 7e); this suggested that broader and increased cytokinin signalling might contribute to the diffuse DR5 auxin responses observed in *drmy1-2* mutants. However, we noted that DR5 expression levels did not decrease following cytokinin treatment, in contrast with the *drmy1-2* mutant, implying that DRMY1 has a role in promotion of auxin signalling. Furthermore, when treated with 5μ M cytokinin, the polarity pattern of PIN1-GFP appeared similar to that in *drmy1-2* mutants (Extended Data Fig. 7f).

In the inflorescence meristem, high auxin at floral primordia positions activates MONOPTEROS, which induces the expression of *Arabidopsis* HISTIDINE PHOSPHOTRANSFER PROTEIN 6 (AHP6). AHP6 acts as a cytokinin signalling inhibitor to define a brief period during which auxin and cytokinin signalling overlap to trigger primordium initiation^{28,29}. In the *ahp6* mutant, initiation of sepal primordia was mildly affected compared to *drmy1-2*

(Fig. 4g). Thus, DRMY1 plays a more prominent role than AHP6 in ensuring the robustness of sepal primordium initiation. In *drmy1-2 ahp6* double mutants, the delayed initiation phenotype was enhanced (Fig. 4g), suggesting that DRMY1 and AHP6 regulate robustness in primordium initiation synergistically. Taken together, our results suggest that DRMY1-dependent patterns of auxin and cytokinin signalling are crucial for the robust temporal pattern of sepal initiation.

To further test whether timing of initiation is crucial for robust organ size, we measured sepal size at maturity for *ahp6*, *drmy1-2 ahp6* and *tir1-1 afb1-1 afb2-1 afb3-1*. All showed more variable sepal size consistent with more variable sepal initiation (Extended Data Fig. 7h,i).

Focused auxin and cytokinin signalling regions define zones of competence for sepal initiation. How do the spatial patterns of auxin and cytokinin signalling determine the temporal pattern of sepal initiation? To answer this question, we used live imaging to track expression of the DR5 auxin response reporter and the TCS cytokinin response reporter throughout the initiation of sepal primordia in developing flowers (Fig. 5a and Supplementary Videos 3,4,6). In wild-type incipient sepal primordia before initiation, the DR5 auxin response signal accumulated first in the outermost cell layer. Simultaneously, the TCS cytokinin response reporter was expressed directly below the DR5 signal. Both signals were restricted to incipient sepal positions. Over time, the DR5 signal invaded the inner cell layers leading to the co-existence of DR5 and TCS signals. After this overlap, we observed the outward bulges of primordium initiation, then TCS and DR5 signals separated again. TCS signal remained at the base of the growing sepal, complementary to the DR5 signal which accumulated at the tip. In drmy1-2 mutants, the invasion of DR5 signal into the inner cell layers was decreased and delayed at the inner sepal (Fig. 5b,c and Supplementary Video 5). Simultaneously, TCS expression was expanded around the periphery of the floral meristem and was not limited to the incipient sepal positions in drmy1-2 (Fig. 5d,e and Supplementary Video 7). Slowly the TCS signal resolved to a sharp domain of expression at the incipient sepal position in *drmy1-2* mutants (Fig. 5e and Supplementary Video 7). Once both the sharp TCS domain and the DR5 invasion were achieved, the *drmy1-2* inner sepal bulged out, indicating initiation. Although delayed, the focused domains of reporter expression were still eventually established in drmy1-2 mutants. Our results suggest that the establishment of precisely localized and limited domains of both auxin and cytokinin signalling is required for sepal initiation, and that sepal initiation is variably delayed in *drmy1-2* until such precise domains can be established.

Fig. 5 | Spatiotemporal patterns of auxin and cytokinin signalling regulate the timing of sepal initiation. a, Dual-marker, live-imaging of DR5 (auxin, cyan nuclei) and TCS (cytokinin, yellow) signalling reporter expression throughout sepal initiation in WT. Longitudinal sections through the developing flower are shown, with the outer sepal on the right and the inner sepal on the left. Chlorophyll (grey) outlines the flower morphology. Yellow arrowheads, overlapping DR5 and TCS signals; blue arrowheads, separation between DR5 and TCS; white arrows, sepal initiation events. Scale bars, 10 µm. n = 3 live-imaging series. **b**,**c**, Live imaging of DR5 auxin signalling reporter (white) expression throughout sepal initiation in both WT (**b**) and the drmy1-2 mutant (c). Longitudinal sections through developing flowers are shown, with the outer sepal on the right and the inner sepal on the left. p355::mCherry-RCI2A, red; plasma membrane, blue; red arrows indicate sepal initiation. Note that invasion of DR5 into the inner layers is delayed for the inner drmy1-2 sepal and is followed immediately by outgrowth. Scale bars, 20 µm. n=3 live-imaging series. d,e, Live-imaging of TCS cytokinin signalling reporter expression (white) throughout sepal initiation in both WT (d) and the drmy1-2 mutant (e). Top view of the developing flower is shown, with the outer sepal at the top and the inner sepal at the bottom. Blue and red arrows indicate sepal initiation. Chlorophyll autofluorescence, red. Scale bars, 20 µm. n=3 live-imaging series. **f**,**g**, Cellular growth heatmap throughout sepal initiation in WT (**f**) and drmy1-2 (**g**). Top view of the flowers with the outer sepal at the top and the inner sepal at the bottom. For the heatmap, red indicates high relative growth rate while blue indicates low relative growth rate. Relative growth rate is defined as final cell size divided by initial cell size. White arrowheads, the band of cells with slower growth and which specify the boundary between initiating sepals and the floral meristem centre. Blue and red arrows indicate sepal initiation. Segmented cells are outlined in yellow. Scale bars, $10 \,\mu m. n = 3$ live-imaging series. For all live-imaging series, the time point when outer sepal primordium emerged was defined as time point 0. Images were staged relative to the timing of outer sepal initiation (0 h). h, DRMY1 ensures focused hormone signalling and reduced cell wall stiffness during sepal initiation, thus making the temporal pattern of sepal initiation robust. The robust timing of sepal initiation is crucial for sepal size robustness, both throughout growth and at maturity. IS, inner sepal; LS, lateral sepals; OS, outer sepal.

NATURE PLANTS

Tightly localized cell growth is associated with primordium initiation. Auxin and cytokinin regulate cellular growth^{19,34,35}. Because the spatiotemporal accumulation of hormone reporters was disrupted in *drmy1-2*, we also tested whether cell growth was affected during primordium emergence. The bulging of primordia requires both tightly localized regions of fast longitudinal



Robust sepal size throughout growth

growth at the periphery and slow latitudinal growth at the boundary between the primordium and the meristem centre^{36,37}. We analysed the cellular growth rates and growth anisotropy of emerging primordia from live imaging, which were developmentally staged by the outer sepal morphology (Extended Data Fig. 9e). In both wild type and *drmy1-2* mutants, before sepal initiation, cells grew heterogeneously with no clear spatial pattern. Primordium initiation occurred with the appearance of a coordinated zone of fast-growing cells at the periphery and a tight band of slow-growing cells at the boundary. For wild type, this switch to orchestrated spatial growth regions occurred at the inner sepal about 6h after the outer sepal (Fig. 5f and Extended Data Fig. 9a), corresponding to the previous quantification of time intervals between sepal initiation (Fig. 2e, f). For drmy1-2, although fast- and slow-growth regions began normally for the outer sepal, cellular growth within the inner sepal region remained heterogeneous 6h after initiation of the outer sepal (Fig. 5g and Extended Data Fig. 9b). As an independent test, we analysed growth anisotropy. A switch from isotropic to highly anisotropic growth led to primordium initiation in both wild type and mutant (Extended Data Fig. 9c). In drmy1-2, cellular growth at the regions where the inner sepal should initiate remained isotropic and randomly oriented during the time interval analysed (Extended Data Fig. 9d). Thus, in *drmy1-2*, the inability to create tightly localized auxin and cytokinin signalling patterns coincides with stiffer cell walls and the inability to create tightly localized growth patterns, delaying initiation.

Discussion

In this study, we report that DRMY1 ensures sepal size uniformity by coordinating the timing of sepal initiation (Fig. 5h). Because the drmy1-2 mutant delays but does not block sepal initiation, it provides insights into the mechanisms controlling the timing of organ initiation. It is well established that the pattern of auxin accumulation sets the position of organ initiation²⁰⁻²³. We observe that the sepal primordium does not emerge as soon as auxin signalling markers become apparent (Fig. 5a). Instead, stable focused regions of auxin and cytokinin signalling appear to define competency zones that give rise to the tightly localized growth patterns required for organ initiation (Fig. 5h). In the case of auxin, a key event in the establishment of this focused region appears to be the invasion of auxin signalling into underlying cell layers, which later begin vascular development³⁸. Consistent with our results, this auxin invasion has been shown to stabilize the positions at which floral primordia form in inflorescence meristems³⁸. Auxin promotes growth through loosening of the cell wall, and cell wall stiffness is hypothesized to feed back to regulate the polarity of the auxin efflux transporter PIN1 (refs. ^{39,40}). It remains for the future to determine how DRMY1 impinges on this feedback loop between cell wall stiffness, growth and hormones.

Timing is important for developmental robustness in animals⁴¹. For example, the robustness of somite size is generated by the precise timing of the somite segmentation clock⁴². An implication of this work in Arabidopsis is that developmental timing of initiation can have cascading effects on organ size. In drmy1-2 mutants, the late-initiating sepals remain smaller throughout development so that sepal size is variable. One might hypothesize that higher variance originates from altered average size. However, as shown in our previous study and confirmed here (Fig. 3f and Extended Data Fig. 1e), decreasing sepal size does not automatically lead to increased sepal size variability in mutants¹⁴. Thus, the loss of regularity is not simply a side effect of decreased average sepal size. In sepals, uniformity of size is required throughout their growth to enclose the flower bud completely, creating a barrier with the external environment¹¹. Thus, traditional compensation mechanisms that delay maturation and termination of growth until the organs reach the right size, such as DILP8 in *Drosophila*^{9,10}, serve no purpose in sepals. We conclude that mechanisms ensuring precise timing of initiation make major contributions to robustness of organ size when this is required throughout development. To explain why defects in timing of initiation cause defects in final organ size, we hypothesize that maturation and cessation of growth of the sepals occur synchronously. If maturation occurs synchronously in wild type, we would expect that flowers with slightly larger outer sepals also have slightly larger inner sepals, because all of the sepals would have grown more before maturation. We indeed observe this correlation (Extended Data Fig. 4f). We have previously shown in *vos1* mutants that disruption of synchrony in the timing of maturation causes increased sepal size variability¹⁴. Here we show in *drmy1-2* mutants that disruption of synchrony in timing of imitation also causes increased sepal size variability. Thus, our results are consistent with the hypothesis that organs can achieve robustness in size throughout their development by synchronization of initiation and maturation.

Methods

Mutations and genotyping. In this study, we primarily used A. thaliana accession Col-0 as the wild-type plants. As described in ref. 14, variable organ size and shape (vos) mutants were isolated from an M2 population of ethyl methanesulfonate-mutagenized Col-0. The vos2 (drmy1-2) mutant was back-crossed to Col-0 three times to segregate unrelated mutations before further characterization. The vos2 (drmy1-2) mutant was then crossed with an Arabidopsis Landsberg erecta accession plant to generate a mapping population. The vos2 (drmy1-2) mutated gene was identified through map-based cloning following the standard procedure described in ref. 43. The vos2 (drmy1-2) mutation was mapped to an interval containing 97 genes on chromosome 1 between 21.3 and 21.7 M. The vos2 (drmy1-2) mutant has a G-to-A mutation at the junction between the third and fourth exons within the DRMY1, AT1G58220 gene, which was predicted to cause splicing defects that were later verified experimentally. The drmy1-2 G-to-A point mutation was genotyped through PCR amplification with the dCAPs44 primers oMZ113 and oMZ114 (sequences are given in Supplementary Table 1), followed by digestion of PCR products with DdeI to produce 74-base pair (bp) wild-type and 100-bp mutant products. We crossed vos2 (drmy1-2) with drmy1-1 (allele with a tDNA insertion in the first intron; SALK012746 (ref. 15)) to test for allelism. The resulting F1 exhibited the drmy1 mutant phenotypes, indicating that these mutations failed to complement and are allelic. To verify the splicing defects, messenger RNA was extracted from the drmy1-2 mutant inflorescences followed by reverse-transcription PCR to generate complementary DNAs. Mutated DRMY1 coding sequences (CDS) were amplified with the primers listed in Supplementary Table 1 and then inserted into pENTR/D-TOPO vectors (Invitrogen). The resultant plasmids were then purified and sequenced with the commercial primer M13F.

Flower staging. All flower staging was based on Smyth et al.45.

Sepal area and shape analysis. Full-size, mature, stage 15 sepals were dissected from the flowers for analysis. For comparison of sepal area and shape variability between wild type and drmy1-2 mutants, we selected the 10th to 25th flowers from the main branch (primary inflorescence) because they are relatively consistent in wild type, as confirmed by Hong et al.14. To quantify the mean and variance of the areas of the four sepals within a single flower, we dissected sepals throughout development from the earliest flower on which we could start (ranging from the first to the sixth) to the latest flower we could get (ranging from the 42th to the 67th) on the main branch. Sepal contour extraction analysis was done as described in Hong et al.¹⁴. Briefly, the dissected sepals were flattened between slides and photographed with a Canon Powershot A640 camera on a Zeiss Stemi 2000-C stereomicroscope. Custom python scripts were then used to extract the contour from each sepal photo and quantify the variability in area and shape (scripts available in the Supplementary Material Data 1 of Hong et al.14). The CV of sepal areas was calculated by dividing the s.d. by the mean of the four sepal areas within a flower. For sepal area correlation, the outer sepal area was plotted as a function of the inner sepal area for individual flowers. In addition to plotting the values, we used a Gaussian kernel to estimate their probability density function (using the scipy.stats library). We represented the probability density function with labelled contour lines shaping the density. We used a bandwith factor of 0.5, chosen to ease the reading of data distribution.

Permutation test confirming the difference between two populations. We used the permutation test to determine whether size distributions of sepals were significantly different between wild type and *drmy1-2*. Performing the permutation test does not require knowledge of the underlying distribution functions, and was done as described in Hong et al.¹⁴.

Live imaging of sepal initiation and growth. A solution of ½ Murashige and Skoog (MS) medium containing 1% sucrose, 0.25× vitamin mix, $1 \mu lml^{-1}$ plant preservative mixture and 1% agarose (recipe modified from Hamant et al.⁴⁶)

was poured into small Petri dishes (Fisher, $60 \times 15 \text{ mm}^2$) for positioning of inflorescences and supporting growth. In this paper, we used two different methods to dissect and position *Arabidopsis* inflorescences for live imaging. The first method, viewing the inflorescence from the side, was modified from Roeder et al.⁴⁷. First, we removed the MS medium from half of the Petri dish to create space for the inflorescence. Inflorescences of plants expressing the plasma membrane marker *pLH13* (*35S:mCitrine-RCI2A*, yellow plasma membrane marker *this* a Dumont tweezer (Electron Microscopy Sciences, style 5, no. 72701-D). Overlying older flowers from one side were removed to expose the stage 4 flowers. The inflorescences were taped to a coverslip to ensure correct orientation. The coverslip was then positioned in the Petri dish with the base of the inflorescence stem inserted into the MS medium. We then taped the Petri dish to the sides of Percival growth chambers with the inflorescence vertical and the bottom of the dish facing outwards, to avoid growth bending. This method was mainly used for imaging early-stage lateral and outer sepal development.

In the second method, we imaged the inflorescence from the top, similar to the method reported in Hamant et al.⁴⁶. Primary inflorescences containing *p35S::mCitrine-RCI2A (pLH13)*, *DR5::3XVENUS-N7* (auxin response reporter²⁰), *pTCS::GFP* (cytokinin response reporter⁴⁹) or both *DR5::3XVENUS-N7* and *p35S::mCherry-RCI2A (pMZ11*, LR reaction between *pENTR/D-TOPO* with *mCherry-RCI2A (pHM52)* and *pB7WG2* (destination vector with *p35S*), red plasma membrane marker), or both *DR5::3XVENUS-N7* and *pTCS::GFP*, were dissected with tweezers and the stem was then inserted into the MS medium, positioning the inflorescence upright. Further dissection with the tip of the tweezer or a needle was done to remove all flowers older than stage 4. This method was used for imaging the initiation of sepal primordia and late-stage outer sepal development.

After dissection and positioning, the Petri dishes were maintained in the growth chamber for at least 6 h for plant recovery before live imaging. The chosen flowers were imaged every 6 h (sepal initiation), 12 or 24 h (organ growth) with a Zeiss 710 confocal microscope. For long-term live imaging, which lasted >6 d, we transferred inflorescences from the old Petri dishes to newly made fresh ones to maintain active growth every 3 d. Before imaging, the inflorescences were immersed in the water for at least 10 min and a $\times 20/1.0$ numerical aperture (NA) Plan-Apochromat water-immersion objective was used for imaging. The wavelengths used for excitation and emission for fluorescent proteins are listed in Supplementary Table 2. The depth of *z*-sections was set to either 0.5 µm (live imaging for sepal growth) or 1 µm (live imaging for sepal initiation or reporter patterning). The resultant LSM files were converted and cropped with FIJI. MorphoGraphX was used for visualization of the spatial distribution of fluorescent signals and the creation of digital longitudinal sections.

Image processing for growth quantification. Image processing and growth quantification were performed as described in Hong et al.¹⁴. Briefly, the confocal stacks collected from live imaging of sepal growth were converted from LSM to TIFF format with FIJI and the images were then imported into MorphoGraphX50. Sample surfaces were detected and meshes of the surface were generated. Fluorescent signals were then projected onto the meshes and cells were segmented on the meshes. For cellular growth, cell lineages were defined manually and cell areas between different time points were compared to quantify growth rates. Heatmaps were generated to visualize the areal growth rate, and the values for the growth rate of each cell were then exported to CSV files. Graphs of growth trends were generated with analysis of the CSV files in Microsoft Excel. The cell division heatmap was also generated based on cell lineages, demonstrating how many daughter cells originated from one mother cell. The analysis of principal directions of growth was also done with MorphoGraphX, following the user manual. Briefly, the meshes of two different time points were loaded together with the parent labels. Check correspondence was done to ensure there were no errors in cell junctions. The growth directions were then computed and visualized with a growth anisotropy heatmap and the "StrainMax" axis option.

Imaging of reporter lines. Primary branches of the reporter line plants (*DR5::3XVENUS-N7, pTCS::GFP, PIN1-GFP, pDRMY1::3XVENUS-N7, pDRMY1::DRMY1-mCitrine* and *p355::mCitrine-RCI2A*) were dissected with tweezers and inserted upright into ½ MS medium (containing 1% sucrose, 0.25×vitamin mix and 1% agar) poured into small Petri dishes. The samples were immersed in the water for 30 min and then further dissected with the tip of the tweezers to remove all unnecessary flowers. After dissection, the inflorescences were placed in the growth chamber for 6h for recovery and then imaged with a ×20/1.0 NA Plan-Apochromat water-immersion objective on a Zeiss 710 confocal microscope.

The seedlings of both wild type and *drmy1-2* with *p35S::mCitrine-RCI2A* were grown in ½ MS medium (containing 1% sucrose, 0.25× vitamin mix and 1% agar) for ~5 d, with Petri dishes placed vertically. The seedlings were then well positioned into waterdrops loaded in advance on the slides. After application of the coverslip, the roots were imaged with ×20/1.0 NA Plan-Apochromat water-immersion objective on a Zeiss710 confocal microscope.

Excitation and emission wavelengths for fluorescent proteins are indicated in Supplementary Table 2.

BAP and NAA treatments. Primary inflorescences containing target reporters were dissected and inserted into 1/2 MS medium-coated small Petri dishes. The inflorescences were then placed in the chamber for at least 6h for recovery. Cytokinin or auxin containing 1/2 MS medium was made following the 1/2 MS medium recipe mentioned above, with specific volumes of either 0.5 gl-1BAP stock solution (kept at -20°C) or 5 mM⁻¹ NAA stock solution (kept at 4°C) for specific concentrations. The inflorescences were then transferred to the cytokinin or auxin containing 1/2 MS medium for the external cytokinin or auxin treatment, respectively. Again, a ×20/1.0 NA Plan-Apochromat water-immersion objective on the Zeiss 710 confocal microscope was used for image collection. For the long-term BAP treatment, primary inflorescences containing plasma membrane markers were dissected to expose early-stage flowers for confocal imaging. The inflorescences were then transferred to fresh BAP containing medium and cultured in the chamber for a further 14d before the final round of confocal imaging. For BAP treatment, pTCS::GFP reporter lines were used as the positive control. The cytokinin receptor mutant wol was used as the negative control because it is relatively insensitive to BAP treatment.

Transgenic plants. The DRMY1 gene promoter with 5' UTR (1,535 bp before the start codon) and the terminator with 3' UTR (331 bp after the stop codon) were PCR amplified with the primers listed in Supplementary Table 1. These two pieces were fused with overlap PCR with Pfu DNA polymerase. The PCR product was adenine (A)-tailed with Taq DNA polymerase. With the help of this overhanging A, the overlap PCR product was then ligated into pGEM-T easy (Promega) to generate pMZ2. The Gateway conversion cassette was PCR amplified and digested with restriction enzymes AscI and KpnI, and then ligated into pMZ2 between the DRMY1 promoter and terminator to make pMZ3. The resulting pMZ3 plasmid and binary vector pMOA36 were digested with NotI and ligated together to make pMZ4. The final pMZ4 Gateway destination vector includes the DRMY1 promoter, a gateway cassette and the DRMY1 terminator. The DRMY1 gene CDS was PCR amplified with the primers listed in Supplementary Table 1. The DRMY-mCitrine fusion was created by amplifying the CDS of DRMY1 and the CDS of mCitrine with the primers listed in Supplementary Table 1. The DRMY1-mCitrine fusion was created through overlap PCR with a nine-alanine linker in the middle. Each of these PCR products was purified and cloned into pENTR/D-TOPO vectors (Invitrogen). The resultant vectors and pENTR 3XVENUS-N7 (pAR334) were LR recombined into the destination vector pMZ4 to generate the final constructs used in this paper: pDRMY1::DRMY1 (pMZ6), pDRMY1::DRMY1-mCitrine (pMZ18), and pDRMY1::3XVENUS-N7 (pMZ8). All of the final constructs were verified by sequencing and transformed into the drmy1-2 mutants by Agrobacterium-mediated floral dipping. All T1 plants were grown in soil for about 10 d and then selected by spraying with 100 µg ml-1 Basta. The surviving plants were then checked for sepal phenotypes.

Quantification of DR5 and TCS signals. To quantify the DR5 and TCS signals at the different positions relative to the centre of the floral meristem, we manually aligned the stacks in MorphoGraphX. These were placed with the *z* axis located at the meristem centre pointing upwards and the *x* axis representing the 0° position and pointing to the right. Angles increased in the counterclockwise direction within the *xy* plane. The images were aligned in such a way as to fix the outer sepal position at roughly 45° (the top-right direction when viewing down the *z* axis).

For quantification of the signal, we implemented a custom process in MorphoGraphX that computed a circular histogram of the signal sum around the z axis. For each voxel of the aligned image, its angle about the z axis was determined. The voxels were grouped according to the angular values in bins of size 1°, and their signal values weighted by volume were summed for each bin. To create the plot, we exported the resulting histogram to a CSV file and imported it into Microsoft Excel.

Supplementary methods. Please see Supplementary Methods for further information on plant growth conditions; photography of flowers, inflorescences and whole plants; meristem size analysis; phyllotaxy analysis; SEM observation; analysis of spatiotemporal variability in growth of cell area; Gaussian curvature measurement; PIN1 immunolocalization; AFM and data analysis; of RNA-seq data.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All other data are available in the main text, the Extended Data figures, the Supplementary Data or the Source Data.

RNA-seq data are available at NCBI BioProject PRJNA564625. Individual RNA-seq read sets are archived in SRA under the following accession numbers: WT replicate 1, SRX6821462; WT replicate 2, SRX6821463; WT replicate 3, SRX6821464; *drmy1-2* replicate 1, SRX6821465; *drmy1-2* replicate 2, SRX6821466; and *drmy1-2* replicate 3, SRX6821467. Gene information is available under the following accession numbers: DRMY1, AT1G58202; AHP6, AT1G80100; TIR1, AT3G62980; AFB1, AT4G03190; AFB2, AT3G26810; AFB3, AT1G12820; WOL, AT2G01830; and PIN1, AT1G73590. Source Data for Figs. 1–4 and Extended Data Figs. 1–7 are provided with the paper.

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Author contributions

Conception and design for experiments was carried out by M.Z., A.H.K.R, V.M., A.B., O.H., R.S.S. and C.-B.L. Isolation of the *vos2* (*drmy1-2*) mutant was performed by L.H.

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and A.H.K.R. Phenotypic analysis of the *vos2 (drmy1-2)* mutant was done by M.Z. and Z.W. Variability of organ shape analysis was carried out by C.-B.L. SEM was performed by M.Z. and A.H.K.R. Live imaging and analysis were done by M.Z. and W.C. AFM was carried out by M.Z and S.B. MorphoGraphX plug-in development for DR5 and TCS quantification was performed by S.S. and R.S.S. Computational analysis of spatial and temporal variability of growth was done by S.T. and C.-B.L. RNA-seq analysis was carried out by E.M.S., M.Z. and A.H.K.R. M.Z. and A.H.K.R. whore the manuscript. Revising and editing of the manuscript was done by M.Z., W.C., V.M., L.H., S.B., S.S., E.M.S., S.T., R.S.S, C.-B.L., O.H., A.B. and A.H.K.R.

Competing interests

The authors declare no competing interests.

Additional information

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Extended Data Fig. 1 | *drmy1-2* floral organs have enhanced sepal size and shape variability. **a**, *DRMY1* mutations have little effect on the floral organ number. Numbers of sepals, petals, stamens and carpels were quantified for both WT and the *drmy1-2* mutant. Two-tailed Student's t test * p-value < 0.05 (p-value for the mean of organ number, WT versus *drmy1-2* petal: 1.14E-03; WT versus *drmy1-2* stamen number: 2.14E-08). Measure of centre: mean. Error bars: standard error of the mean. n = 30 flowers. **b**, Quantification of the mean sepal area of the four sepals from an individual flower. Sequential flowers along the main branch of the stem (flower number on the x-axis) were measured at stage 14. Three replicates were included for both WT (blue) and *drmy1-2* (red). Original individual sample curves can be found in Source Data. The mean of the 3 replicates are presented as thick blue and red lines with the SD as partially translucent background. **c**, Quantification of the sepal shape variability for outer, inner and lateral sepals. Two-tailed Student's t test * p-value < 0.05 (p-value for the mean of shape variability, WT versus *drmy1-2* inner sepal: 2.70E-02; WT versus *drmy1-2* lateral sepal: 1.00E-07) Measure of centre: mean. Error bars: standard error of the mean. n = 60 for both WT and *drmy1-2* 10th to 25th flowers along the main branch. **d**, What at first appeared to be two sepals initiated at the inner sepal position of the *drmy1-2* flower (left panel) fused to form a single sepal with a split tip at later time points of the live imaging (right panel). Red arrowheads: initiating sepals. Red arrow: the fused sepal. Scale bar: $20 \, \mu$ m. **e**, Coefficient of variation (CV) calculated for the areas of all petals in each single flower for WT and *drmy1-2*. Two-tailed Student's t test * p-value < 0.05 (p-value for the mean of CV for petal area within individual flowers, WT versus *drmy1-2*: 1.27E-03). Measure of centre: mean. Error bars: standard error of the mean. n = 20 flowers for both WT and

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Extended Data Fig. 2 [*DRMY1* is required for sepal size robustness. a, Sequencing of *DRMY1* transcripts from the *drmy1-2* mutant verified that splicing defects occur. *DRMY1* transcripts were reverse transcribed and amplified from RNA extracted from the *drmy1-2* mutant and inserted into *pENTR/D-TOPO* for sequencing. Black shading: nucleotides remaining in the transcript after the splicing; Gray shading: nucleotides spliced out. Orange capital letter: exon. Purple lower-case letter: intron in the WT *DRMY1* transcript. Red arrowhead indicates one base pair shift. **b**, qRT-PCR measuring the expression of *DRMY1* in WT and the *drmy1-2* mutant using two pairs of primers: one before the mutation site and the other across the mutation site. The expression level in WT quantified with the primers before the mutation site was set to 1 using the Delta-delta-CT method. Two-tailed Student's t test * p-value < 0.05 (p-value for the mean of expression fold change, WT versus *drmy1-2* before mutation: 3.31E-03; WT versus *drmy1-2* across mutation: 2.01E-04). Measure of centre: mean. Error bars: standard error of the mean. n = 3 biological replicates. **c-h**, Inflorescences of WT (c), *drmy1-1* (d), *drmy1-2* (e), F1 of the cross between *drmy1-1* and *drmy1-2* for allelism test (f), T3 plants of *drmy1-2* transformed with *pDRMY1::DRMY1* (g), and T3 plants of *drmy1-2* transformed with *pDRMY1::DRMY1-mCitrine* (h). Orange arrows: smaller sepals in individual flowers. Note, open flower buds indicate unequal sepal sizes. Scale bars: 0.5 mm. n = 3 inflorescences. **i,j**, Transcriptional (i, *pDRMY1::3XVENUS-N7*, nuclear localized gray signal) and translational (j, *pDRMY1::DRMY1-mCitrine*, gray) *DRMY1* reporter expression patterns are similar. Cell walls were stained with P1 in i and plasma membranes were fluorescently labeled with *pUBQ10::mCherry-RCI2A* in j. Both *DRMY1* reporters are expressed in the inflorescence meristem, floral meristems, and initiating floral organs, with stronger expression in the periphery. Sca



Extended Data Fig. 3 | See next page for caption.

Extended Data Fig. 3 | Cell wall stiffness increases in the drmy1-2 mutant. a, Graph of flower radius used to assess developmental stage based on flower size. The radii of flowers without sepal primordia (SP = 0), with only the outer sepal primordium (SP = 1), or with outer and inner sepal primordium (SP > 1) were measured for wild-type and drmy1-2 inflorescences. The critical size threshold for outer sepal initiation is specified with a yellow dashed line. Note this size is the same for wild type and drmy1-2, indicating the stage of outer sepal initiation is not affected in drmy1-2. In contrast, the critical size threshold for inner sepal initiation (orange dashed lines), is larger for drmy1-2 than wild type, consistent with delayed inner sepal initiation. In the violin plots, the black line represents the median and the individual data points are shown. n = 46 flowers for WT SP = 0; n = 21 for WT SP = 1; n = 45 for WT SP > 1; n = 53 for drmy1-2 SP = 0; n = 63 for drmy1-2 SP = 1; n = 33 for drmy1-2 SP > 1. **b**, The average apparent elastic modulus calculated from AFM measurements of the flowers is significantly higher for the drmy1-2 mutant. Two-tailed Student's t test * p-value < 0.05 (p-value for the mean of apparent elastic modulus, WT versus drmy1-2: 6.81E-04). Measure of centre: mean. Error bars: standard errors of the mean. n = 11 samples measured for both WT and drmy1-2. c, Cell shrinkage heatmap after osmotic treatment in WT and drmy1-2. Group of cells were segmented together for area comparison. Red in the heatmap represents less shrinkage, thus stiffer cell wall. Scale bar: 50 µm. n = 3 flowers. d, Average shrinkage ratio after osmotic treatment further confirms that cells undergo less shrinkage in the drmy1-2 mutant, indicating the cell walls are stiffer. Two-tailed Student's t test * p-value < 0.05 (p-value for the mean of shrinkage ratio, WT versus drmy1-2: 5.56E-08). Measure of centre: mean. Error bars: standard errors of the mean. n = 161 cell groups for WT and n=129 cell groups for drmy1-2. e, Bar graph of GO terms that are overrepresented (against a genome wide frequency) among genes more strongly expressed in drmy1-2 inflorescences than in WT inflorescences. Genes used for this GO term analysis are listed in the 'Upreg. in drmy1-2, padj < 0.1' table of Supplementary Data 1. f, Bar graph of GO terms that are overrepresented (against a genome wide frequency) among genes more strongly expressed in WT inflorescences than in drmy1-2 inflorescences. Genes used for this GO term analysis are listed in the 'Downreg. in drmy1-2, padj < 0.1' table of Supplementary Data 1. For both (e) and (f), a subset of significant GO terms was selected for each graph (Fisher test with Yekutieli multi-test adjustment, significance level 0.05 using the AgriGo 2.0 website). The percentage of differentially expressed genes in drmy1-2 versus WT associated the GO term is shown with black bars. Genome (gray) reports the frequency of genes associated with that term in the Arabidopsis genome, which would be the frequency expected by chance for a randomly selected subset of genes. The percentage of genes was calculated as the number of genes associated with that term divided by the total number of genes. n = 3 biological replicates.

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Extended Data Fig. 4 | See next page for caption.

Extended Data Fig. 4 | Sepal cell growth is slower in the *drmy1-2* **mutant. a**, **b**, 24-hour late stage (from stage 6 to stage 9) cellular growth heatmap for both WT (a) and *drmy1-2* (b) outer sepals. Relative growth rate is defined as final cell size divided by initial cell size. Segmented cells outlined in yellow. Note the outer sepal base is at the bottom of the image and its tip points up. Scale bars: 20 µm. **c**, Growth curves of the late stage average cellular growth for both WT and *drmy1-2*. *: Flower stage 9 extends over multiple 24-hour intervals. Measure of centre: mean. Error bars represent standard error of the mean. n = 3 flowers. **d**, 36-hour cell division heatmap for both WT and *drmy1-2*. The total number of cells derived from one progenitor is represented in the heatmap with 1 meaning no divisions. Throughout sepal development, the *drmy1-2* sepal cells undergo fewer divisions than WT. Scale bars: 20 µm. **e**, Confocal images of sepals from individual flowers (shown in Fig. 3a,b) after 11 days of live imaging. Area variability was quantified by the coefficient of variation (CV). Two lateral primordia fused to form the left *drmy1-2* lateral sepal. Scale bar: 100 µm. Sepals from 1 flower are shown here, representing 3 live imaging series. **f**, The outer sepal area plotted as a function of the inner sepal area in individual flowers. Each color represents a pool of three plants and each point is for one flower (using the same dataset as Fig. 1d and Extended Data Fig. 1b). In addition to plotting the values, we use a Gaussian kernel to estimate their probability density function. We represent the probability density function with labelled contour lines shaping the density. We use a bandwith factor of 0.5, chosen to make the data distribution easier to read. n = 167 WT flowers and n = 148 *drmy1-2* flowers. **g**, The mean *drmy1-2* sepal area divided by WT mean sepal area ratio for each sepal type. Sepals from different flowers were pooled together (using flowers 10-25 from the same dataset as F

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Extended Data Fig. 5 | Spatiotemporal averaging is not affected in the *drmy1-2* **mutant at early stage. a**, The maximal principal direction of growth (PDG_{max} white line) of WT sepal cells calculated for 24-hour and 48-hour intervals. For 24-hour intervals, the PDG_{max} shows both spatial and temporal variations in WT. Cell outlines are shown in cyan. Over the whole 48-hour interval these variations average out such that the PDG_{max} are oriented vertically along the major growth axis of the sepal. One cell showing good temporal averaging is highlighted with blue boxes and magnified in insets. The PDG_{max} are visualized on the earlier time point. n = 3 live imaging series. Scale bar: 20 µm. **b**, The maximal principal direction of growth (PDG_{max}, white line) of the *drmy1-2* sepal cells calculated for 24-hour and 48-hour intervals. The PDG_{max} also shows similar spatial and temporal variations in the *drmy1-2* situation. One cell showing good temporal averaging is again highlighted with red boxes at different time points, indicating that temporal averaging of growth direction is not affected by *DMRY1* mutations. The PDG_{max} are visualized on the earlier time point. n = 3 live imaging series. Scale bar: $20 \,\mu$ m. **c**, Graph plotting the average spatial variability of the growth rates (V_{aree}) for sepal epidermal cells during sepal development. Blue curves are for WT sepals and red curves are for *drmy1-2* sepals. Measure of centre: mean. Error bars represent standard error. n = 3 flowers. **d**, Graph plotting the average temporal curves are for *drmy1-2* sepals. Measure of centre: mean. Error bars represent standard error. n = 3 flowers.

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Extended Data Fig. 6 | See next page for caption.

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Extended Data Fig. 6 | Auxin signaling is suppressed and more diffuse while cytokinin signaling expands and is enhanced in drmy1-2 mutants. a, Confocal imaging of the DR5 auxin response reporter (white) in the whole inflorescence of WT and the drmy1-2 mutant. p35S::mCherry-RCl2A: red, for plasma membrane. Scale bar: 20 µm. n = 10 inflorescences. **b**, Confocal imaging of the TCS cytokinin signaling reporter (gray) in whole inflorescences of WT and the drmy1-2 mutant. Chlorophyll autofluorescence: red. Scale bar: 20 µm. n = 10 inflorescences. c, Confocal imaging of PIN1 immunolocalization experiments to show PIN1 accumulation in inflorescences and flowers of WT and drmy1-2. PIN1 exhibits polar localization in drmy1-2 similar to wild type; however, it forms more convergence points in flowers. Blue/Red arrowheads: PIN1 convergence points. Inset: Same images with increased brightness to show the morphology of the flowers. Scale bars: 20 µm. n = 3 inflorescences. d, Confocal imaging of pPIN1::PIN1-GFP to show PIN1 accumulation in the inflorescences and flowers of WT and drmy1-2. Again, PIN1 forms abnormal convergence points in drmy1-2. Blue/Red arrowheads: PIN1 convergence points. Scale bars: 50 µm for IM and 20 µm for flowers. n = 3 inflorescences. e, Images of whole plants for WT and drmy1-2, showing the bushiness and short stature of drmy1-2. Scale bar: 2 cm. n = 3 plants. f, Confocal images of root meristems for WT and drmy1-2. The regions specified by yellow arrowheads indicate the meristematic zone. Scale bar: 50 µm. n = 3 root tips. g, Photograph of 10-day old seedlings for WT and drmy1-2, showing drmy1-2 has shorter roots and fewer lateral roots. Scale bar: 1 cm. n = 3 plates. h, Confocal images of inflorescence meristems for WT and drmy1-2 (Top view). Yellow dashed circles indicate how meristem sizes were measured in i. Scale bar: $50 \,\mu$ m. n = 10 inflorescence meristems. i, Quantification of inflorescence meristem sizes for WT and drmy1-2. Two-tailed Student's t test * p-value < 0.05 (p-value for the mean of inflorescence meristem sizes, WT versus drmy1-2: 2.01E-07). Measure of centre: mean. Error bars represent standard error of the mean. n = 10 inflorescence meristems. j, Histograms of divergence angles between siliques for WT and drmy1-2, showing the enhanced variability in phyllotaxy observed in drmy1-2 mutants. 137° is expected for spiral phyllotaxy observed in wild type.

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Extended Data Fig. 7 | See next page for caption.

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Extended Data Fig. 7 | Cytokinin treatment mimics the drmy1-2 mutant. a, b, Confocal imaging of the whole inflorescences of WT (a) and drmy1-2 (b) cultured in mock conditions or 5 µm BAP (synthetic cytokinin) for 6 days. p35S::mCitrine-RCI2A: gray, for plasma membrane. Phenotypes quantified in Extended Data Fig. 7c. Blue or red arrowheads: flowers with obvious delayed sepal initiation phenotypes. Scale bars: 50 µm. c, Graph characterizing the proportions of flower phenotypes observed after 5 µM BAP treatment for 6 days. Normal phenotype (N, black) is defined as similar to wild type. Mildly affected phenotype (M, grey) is similar to drmy1-2. Severely affected phenotype (S, silver) is more severe than drmy1-2. n = 23 (N: 23/23, M: 0/23, S: 0/23) flowers from 7 inflorescences for mock treated wild type; n = 42 (N: 4/42, M: 35/42, S: 3/42) flowers from 12 inflorescences for mock treated drmy1-2; n = 47 (N: 2/47, M: 14/47, S: 31/47) flowers from 16 inflorescences for BAP treated wild type; and n = 37 (N: 0/37, M: 3/37, S: 34/37) flowers from 18 inflorescences for BAP treated drmy1-2. A one-sided Kolmogorov-Smirnov test was used to compare the distributions of different situations. p-value for WT mock versus drmv1-2 mock: 7.73 E-14; p-value for WT mock versus WT BAP: 1.67 E-16; p-value for WT BAP versus drmv1-2 mock: 4.53 E-9; p-value for WT BAP versus drmy1-2 BAP: 7.55 E-3; p-value for drmy1-2 mock versus drmy1-2 BAP: 1.95E-15. d, Graph of flower radius to assess developmental stage based on flower size. The radii of flowers without sepal primordia (SP = 0) or with only the outer sepal primordium (SP = 1) were measured for wild-type and drmy1-2 inflorescences cultured in either mock or 5 µM BAP for 6 days. The critical size threshold for outer sepal initiation is specified with a yellow dashed line. Note this size is the same for wild type mock and wild type BAP samples, indicating the stage of outer sepal initiation is not affected by BAP treatment. In contrast, for BAP treated drmy1-2, this characteristic size is more variable, consistent with the strongly enhanced phenotype. In the violin plots, the black line represents the median and the individual data points are shown. WT Mock SP = 0: n = 5; WT Mock SP = 1: BAP SP = 1: n = 19. e, 5 µM BAP treatment on the DR5 auxin signaling reporter (white) for 3 days. p35S::mCherry-RCI2A: red, for plasma membrane; Red arrowhead: indicates the same flower before and after the BAP treatment. Scale bar: 50 µm. Note the DR5 signal becomes more diffuse after cytokinin treatment. n = 3 inflorescences. f, 5 µM BAP treatment on PIN1-GFP (cyan) auxin efflux carrier for 2 days. Red arrowhead: indicates the same flower before and after the BAP treatment; Scale bar: 50 µm. PIN1-GFP appears to form additional convergence points similar to drmy1-2. n = 3 inflorescences. g, Long-term treatment of flowers with 5 µM BAP causes severe sepal size defects. Wild-type inflorescences were cultured for 6 days on mock or BAP media, dissected to reveal flowers with initiating sepals, and further cultured for 14 days to examine the effects on sepal size. n = 3 flowers. **h**, The sepal area distribution for mature WT, drmy1-2, ahp6, drmy1-2 ahp6, and tir1-1 afb1-1 afb2-1 afb3-1 (tir1afb1-2-3 for short) sepals. The boxes extend from the lower to upper quartile values of the data and the whiskers extend past 1.5 of the interquartile range. Outliers are indicated with small dots. Sepals from different flowers were pooled together. n = 35 flowers. Wild-type and drmy1-2 data was subsampled from that shown in Extended Data Fig. 1b. Two-tailed Student's t test * p-value < 0.05 (p-value for the mean of sepal area, WT versus drmy1-2: 2.70E-33; WT versus ahp6: 1.42E-38; WT versus drmy1-2 ahp6: 9.91E-25; WT versus tir1 abf1-1 afb2-1 afb3-1: 1.22E-39). i, Average coefficient of variation (CV) calculated for the areas of all sepals in each single flower for WT, drmy1-2, ahp6, drmy1-2 ahp6, and tir1-1 afb1-1 afb2-1 afb3-1. n = 35 flowers. Two-tailed Student's t test * p-value < 0.05 (p-value for the mean of CV, WT versus drmy1-2: 4.99E-14; WT versus ahp6: 1.75E-13; WT versus drmy1-2 ahp6: 8.06E-16; WT versus tir1 abf1-1 afb2-1 afb3-1: 3.31E-32). Measure of centre: mean. Error bars represent standard error of the mean.

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Extended Data Fig. 8 | BAP treatment functions through cytokinin signaling. a, 5 μ M BAP treatment on the TCS cytokinin signaling reporter (gray) for 24 hours. Control showing that cytokinin treatment enhances TCS reporter expression. Chlorophyll autofluorescence: red. Scale bars: 50 μ m. n = 3 inflorescences for each treatment. **b**, 5 μ M BAP treatment on the cytokinin receptor mutant *wol-1* for 4 days. Control showing that mutation of the cytokinin receptor (*wol-1*) abrogates delayed sepal initiation in response to cytokinin. Lower left flower removed during imaging. Cell walls stained with PI: gray. Scale bar: 50 μ m. n = 3 inflorescences. **c**, NAA (auxin) treatment in a gradient of concentration on the TCS cytokinin signaling reporter (gray) for 24 hours. Auxin treatment did not enhance TCS reporter expression. Control showing that the induction of TCS reporter expression is specific to cytokinin treatment. Chlorophyll autofluorescence: red. Scale bars: 20 μ m. n = 3 inflorescences for each treatment.

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Extended Data Fig. 9 | See next page for caption.

Extended Data Fig. 9 | Cellular growth remains heterogeneous and randomly oriented for the *drmy1-2* inner sepals. **a**, **b**, Cumulative 18-hour cellular growth heatmap for both WT (a) and *drmy1-2* (b) floral meristems. White dashed boxes highlight the bands of cells with slower growth rate which specify the boundary. They are always adjacent to the fast growth regions at the periphery, where sepals initiate. Segmented cells outlined in yellow. Three replicates are shown. Note that the *drmy1-2* replicate 1 grows relatively normally. Scale bars: 10 µm. **c,d**, 18-hour cellular growth anisotropy heatmap for the same WT (c) and *drmy1-2* (d) floral meristems. Growth anisotropy was calculated by dividing the cell stretch at the maximum direction by the cell stretch at the minimum direction. Cyan indicates higher growth anisotropy while black indicates lower growth anisotropy. White lines within the cells shows the maximum principle directions of growth. The initiating regions have higher anisotropy with the periphery part showing longitudinal growth and the boundary parts showing latitudinal growth. Three replicates are shown. Scale bars: 10 µm. **e**, Side views of the floral meristems at the last time points with outer sepals on the right and inner sepals on the left. The morphology of outer sepals was used for staging and appears equivalent in all samples. Arrowheads: the initiation/bulging of sepals from floral meristems. Scale bars: 10 µm. **n** = 3 flowers shown. **R** = replicate. Heat map and anisotropy are visualized on the later time point.

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n/a	Cor	firmed
		The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	\square	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
		The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.
	\square	A description of all covariates tested
	\square	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
		A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
		For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted Give <i>P</i> values as exact values whenever suitable.
\boxtimes		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\boxtimes		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
\boxtimes		Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
		Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

Software and code

Policy information about availability of computer code Data collection For confocal imaging: ZEN 2010B SP1 for Zeiss 710 For AFM: JPK Nanowizard software 6.0 For RNA-seq data was collected with Illumina HISEQ 2500 rapid run and NextSeq 500 For SEM Leica 440 software For qRT-PCR Roch Lightcycler 480 software, version 1.5 Data analysis For image analysis: MorphoGraphX 2.0 r5a5d9b7 (https://www.mpipz.mpg.de/MorphoGraphX) Fiji 64-bit for Linux (ImageJ version 1.52n) (https://fiji.sc) For quantitative data analysis: Microsoft Excel 2016 Custom scripts for contour analysis, permutation test, and quantification of growth spatial variability and temporal variability is supplementary material Data S1 of Hong et al. 2016 Developmental Cell 38, 15–32. R version 3.4.4 (2018-03-15) scipy.stats library (https://docs.scipy.org/doc/scipy/reference/stats.html) For RNA-seq analysis: Custom perl scripts (https://github.com/SchwarzEM/ems_perl) Trimmomatic 0.36 (http://www.usadellab.org/cms/?page=trimmomatic) Salmon 0.14.1 (https://salmon.readthedocs.io/en/latest/salmon.html#) DESeq2 version 1.18.1(https://bioconductor.org/packages/release/bioc/html/DESeq2.html) agriGO version 2 (http://systemsbiology.cau.edu.cn/agriGOv2/) For AFM:

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a <u>data availability statement</u>. This statement should provide the following information, where applicable: - Accession codes, unique identifiers, or web links for publicly available datasets

- A list of figures that have associated raw data

- A description of any restrictions on data availability

The data that support the findings of this study are available within the paper, Supplementary Information, and Source Data or from the corresponding authors upon reasonable request. RNA-seq data is available at NCBI BioProject PRJNA564625. Materials are also available by request.

Field-specific reporting

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Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Behavioural & social sciences

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	We estimated replicate sizes based on similar prior publications. We conducted our experiments with at least three independent biological replicates across all of our studies. To analyze the variability in sepal sizes and shapes much larger sample sizes were used (n = 100). We determined the final sample sizes to be adequate based on the statistically significant differences in phenotype.			
Data exclusions	As mentioned in the text, we excluded flowers with more than four sepals from the size and shape variability analysis because altering the number of sepals definitely alters the size and shape. These flowers were excluded after data collection because before we did the analysis we did not know that we would find flowers with more than 4 sepals.			
Replication	Every experiment was subjected to at least three independent biological replicates and similar results were obtained.			
Randomization	Plants of different genotypes were used as study groups. When we grew the plants, the position within the chamber is varied to ensure positional effects within the chamber do not account for the phenotypic differences.			
Blinding	Experiments were not blinded. Data were always collected according to the genotypes of the plants.			

Reporting for specific materials, systems and methods

Methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study	n/a	Involved in the study
	Antibodies	\boxtimes	ChIP-seq
\boxtimes	Eukaryotic cell lines	\boxtimes	Flow cytometry
\boxtimes	Palaeontology	\boxtimes	MRI-based neuroimaging
\boxtimes	Animals and other organisms		
\boxtimes	Human research participants		
\boxtimes	Clinical data		

Antibodies

Antibodies used	PIN1 antibody: from Santa Cruz Biotechnology catalog number PIN AP20, Polyclonal, Host: goat, dilution 1:100.
Validation	Validation from the manufacturer (https://datasheets.scbt.com/sc-27163.pdf). Validation for immunofluorescence in the Arabidopsis vegetative shoot apex showing polar localization in Figure 2 of Wang, Ying,

October 2018

et al. "The stem cell niche in leaf axils is established by auxin and cytokinin in Arabidopsis." The Plant Cell 26.5 (2014): 2055-2067. Validation for immunofluorescence in the Arabidopsis root in Figure S5 of Hazak, Ora, et al. "A novel Ca2+-binding protein that can rapidly transduce auxin responses during root growth." PLoS biology 17.7 (2019): e3000085. Validation for immunofluorescence in the Arabidopsis inflorescense meristem showing polar localization in Figure 1 of Heisler, M.G., et al. "Alignment between PIN1 polarity and microtubule orientation in the shoot apical meristem reveals a tight coupling between morphogenesis and auxin transport." PLoS Biol 8 (2010): e1000516.