DELLA genes restrict inflorescence meristem function independently of plant height

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DELLA proteins associate with transcription factors to control plant growth in response to gibberellin1. Semi-dwarf DELLAs with improved harvest index and decreased lodging greatly improved global food security during the ‘green revolution’ in the 1960–1970s2. However, DELLAs are pleiotropic and the developmental basis for their effects on plant architecture remains poorly understood. Here, we show that DELLAs have genetically separable roles in controlling stem growth and the size of the inflorescence meristem, where flowers initiate. Quantitative three-dimensional image analysis, combined with a genome-wide screen for DELLA-bound loci in the inflorescence tip, revealed that DELLAs limit meristem size in Arabidopsis by directly upregulating the cell-cycle inhibitor KRP2 in the underlying rib meristem, without affecting the canonical WUSCHEL-CLAVATA meristem size regulators3. Mutation of KRP2 in a DELLA semi-dwarf background restored meristem size, but not stem growth, and accelerated flower production. In barley, secondary mutations in the DELLAs gain-of-function mutant Slt1d also uncoupled meristem and inflorescence size from plant height. Our work reveals an unexpected and conserved role for DELLAs in controlling shoot meristem function and suggests how dissection of pleiotropic DELLA functions could unlock further yield gains in semi-dwarf mutants.

Growth of the stem and of lateral organs, including leaves and flowers, is initiated at the shoot apical meristem (SAM), where a pool of stem cells continuously provides cells to form new tissues4. During reproductive development, the outer cell layers of the SAM (tunica) give rise to floral buds and contribute cells to form the outer tissues of the stem, whereas the inner stem tissues originate from the subapical region of the SAM, called the rib zone.

Stem growth is promoted by gibberellin, which binds to the GID1 receptor to promote ubiquitin-dependent degradation of DELLAs. Mutations that reduce gibberellic acid (GA) levels or disrupt the interaction between DELLAs and GID1 stabilize DELLAs and consequently inhibit stem growth1. Arabidopsis has five DELLAs with overlapping functions, of which two (GIBBERELLIC ACID INSENSITIVE, GAI and REPRESSOR of ga1-3, RGA) have a predominant role in regulating stem growth5 and are transcribed in the inflorescence apex, including the SAM (Supplementary Fig. 1). In accordance with their role in controlling cell proliferation6, localized activation of a GA-resistant version of the GAI protein7 using a Cre-loxP recombination system confirmed that most of the inhibitory effect on growth occurred within the apical 2 mm of the inflorescence stem (Supplementary Fig. 1), where cell division is most active6.

To understand in detail how GAI and RGA control stem growth, we looked for the earliest visible defects in the dwarf gain-of-function gai mutant and in dwarf transgenic plants expressing a GA-resistant version of RGA (RGAp:GFP-rgaΔ17). The CYCB1;1p:GFP reporter showed fewer mitoses in the developing internodes of gai, without obvious differences in cell size and before any visible change in internode length (Supplementary Fig. 2), in line with the role of DELLAs in regulating the supply of new cells for internode growth. In addition, gai and RGAp:GFP-rgaΔ17 appeared to reduce SAM size, including the tunica layers and the rib zone (Supplementary Fig. 2). Measurements of projected meristem area confirmed that the SAM was smaller not only in gai and RGAp:GFP-rgaΔ17, but also in the GA-deficient ga1-1 mutant8; conversely, global dwarf plants, with loss of function of all five Arabidopsis DELLAs genes9, had a notably enlarged SAM (Fig. 1a–c). The negative role of DELLAs in the inflorescence meristem was unexpected, because gibberellin has been shown to antagonize shoot meristem activity in seedlings9, in contrast to its positive role in the control of root meristem size9,10. The potentially different roles of DELLAs in the vegetative and reproductive SAM might reflect other changes in SAM function, such as activation of the rib meristem during flowering.

DELLA proteins interact with transcription factors bound to their target genes, which can be detected by chromatin immunoprecipitation (ChIP) using tagged DELLAs11. To reveal the molecular basis for DELLA control of stem growth and SAM size, we performed ChIP-seq to detect loci bound by RGAp:GFP-rgaΔ17 in inflorescence apices. As internal controls, we used ten genes previously shown to be DELLA-regulated (Supplementary Table 2 and Supplementary Fig. 3). Candidate target genes were selected if the region within 3 kb upstream and 1.5 kb downstream of their coding sequences contained ChIP-seq peaks consistently detected in three RGAp:GFP-rgaΔ17 replicates (false discovery rate <0.001) but not in the negative controls. From these genes, we selected 2327 high-confidence candidates with a peak enrichment at least as high as the internal control with the lowest enrichment (LEAFY (Supplementary Table 1 and Supplementary Fig. 3). In accordance with DELLA roles regulating growth in response to hormonal, developmental and environmental responses12, the set of high-confidence targets included numerous genes involved in responses to hormones (GA, auxin, brassinosteroid, abscisic acid and cytokinin), floral transition, organ patterning and growth, floral development and cell wall dynamics (Supplementary Table 2). The list was significantly enriched (P = 1.2 × 10−40, Fisher’s exact test) for genes bound by GFP–RGA in seedlings14, but the majority of genes were not
shared between the two sets, consistent with DELLA function being conditioned by interaction with tissue-specific transcription factors (Fig. 2a). Accordingly, the RGAp:GFP-rgaΔ17 ChIP-seq peaks were significantly enriched for motifs bound by DELLA-interacting transcription factors14, particularly of the bHLH, bZIP, DoF and SPL families (Supplementary Fig. 3).

The set of high-confidence targets revealed few direct links to the cell cycle machinery (Supplementary Table 2), supporting the idea that DELLA control of cell proliferation is mostly indirect. However, the list included three different members (KRP1, 2 and 4) of the Kip-related (KRP) family of cyclin-dependent kinase inhibitors, which play important roles in plant tissue growth6,17. To investigate direct links between DELLAs and cell cycle control, we focused on KRP2 because of its higher ChIP-seq peak enrichment relative to KRP1 and KRP4 (Supplementary Table 1) and because KRP2, but not KRP1 or KRP4, was upregulated in gai-1 mutant seedlings13. Binding of GFP-rgaΔ17 to KRP2 was independently confirmed by ChIP-quantitative polymerase chain reaction (qPCR) (Fig. 2b,c). A functional KRP2p-KRP2–GFP fusion (Supplementary Fig. 4) was expressed weakly below the inflorescence and floral meristems and at lateral organ boundaries (Fig. 2d, e), consistent with the repression of growth in boundary regions14. In gai mutants, KRP2p-KRP2–GFP was upregulated in the rib zone, in floral pedicels and in developing internodes (Fig. 2c, g). Thus, repression of cell proliferation in the inflorescence apex by DELLAs correlated with increased expression of the cell-cycle inhibitor KRP2.

We next tested the functional relevance of KRP2 activation by introducing the krp2-3 loss-of-function allele19 into genetic backgrounds with enhanced DELLA activity. To detect the effects of KRP2 in different meristem regions and to confirm size differences using a method that is not sensitive to meristem geometry, we also measured the number of cells in the tunica and rib zone regions. krp2-3 fully suppressed the reduction in SAM area seen in gai and restored cell numbers not only in the rib zone, but also in the overlying tunica layers (Fig. 3). However, the more severe defect of RGAp:GFP-rgaΔ17 and gai-1 meristems was only partially suppressed by krp2-3 (Supplementary Fig. 5), suggesting that RGA restricts SAM size through additional genes; plausible candidates would be KRP1 and KRP4. A more specialized role for RGA in the inflorescence meristem would be in its higher expression in the meristem relative to developing buds (Supplementary Fig. 1). Furthermore, the larger meristem of global della mutant than krp2-3 (Figs. 1a, b and 3c) and the binding of GFP-rgaΔ17 to known regulators of meristem size, such as CLV1 and cytokinin signalling genes (Supplementary Table 1 and Supplementary Fig. 3), suggested that DELLA proteins limit SAM size through additional, KRP2-independent mechanisms. In contrast to the effect on meristem size, krp2-3 did not significantly change stem elongation in wild-type or gai background (Fig. 3a, b), so activation of KRP2 in developing internodes (Fig. 2g) is not sufficient to explain the inhibition of stem growth by DELLA proteins. In summary, the effect of semi-dwarf DELLA alleles on SAM size is mediated in part by KRP2 and is genetically separable from their effect on stem growth.

The localized activation of KRP2 suggested that DELLA proteins function in the rib zone to regulate SAM growth. SAM size is controlled by the homeodomain protein WUSCHEL (WUS), which is expressed in the rib zone and moves to the overlying tunica layers to specify stem cells marked by CLAVATA3 (CLV3) expression20,21. Neither WUS nor CLV3 fulfilled our criteria for high-confidence ChIP-seq targets of GFP-rgaΔ17 (Supplementary Table 1); however, even if these loci are not directly targeted, their function could still be indirectly affected by DELLA proteins. To test this possibility, we compared expression of WUSp:GFP–WUS and CLV3p:GFP in gai and wild-type SAMs. The number of cells expressing either reporter was not significantly different, but the expression level per cell remained the same for CLV3p:GFP and was slightly increased in gai for WUSp:GFP–WUS (Supplementary Fig. 6), indicating that KRP2 activation in gai did not restrict meristem size by interfering with stem cell maintenance. The effect of rib zone–expressed KRP2 on the overlying tunica layers could result from mechanical constraints on tissue growth, as proposed to explain how DELLA function localized specifically in the endodermis limits growth of the whole root meristem22, in contrast with the hypothesis that plant organ growth is mechanically controlled by the outermost cell layers23.
Changes in SAM size can affect the number of floral buds formed and disrupt their arrangement around the meristem (phyllotaxis). gai, RGAp:GFP-rgaΔ17 or ga4-1 inflorescence meristems did not show obvious phyllotaxis defects, but the larger meristem of the global della mutant did (Fig. 1a). Assuming that successive buds develop at the same speed, we estimated the rate of bud initiation by comparing daily the number of flowers that reached maturity in the main inflorescence of wild-type, krp2-3, gai, gai krp2-3 and global della plants. Reflecting the differences in SAM size, the rate of bud production increased in the global della mutant and decreased in gai in a KRP2-dependent way (Fig. 3i). However, increased meristem longevity in gai resulted in a final number of flowers similar to the wild type (Fig. 3i). Meristem arrest correlates with seed development, although the molecular mechanism remains unknown, so the extended meristem activity in gai may result from the reduced fertility of DELLA gain-of-function mutants. We conclude that the KRP2-dependent decrease in meristem size in gai plants had a negative effect on meristem function, but the effect on final floral numbers depended also on meristem longevity.
To test whether the role of DELLA in SAM size is conserved in diverse angiosperms, we analysed mutants for the barley DELLA gene, SLENDER1 (SLN1). The barley inflorescence (spike) has a main axis (rachis) with nodes that support branches (spikelets), each containing a determined number of flowers (florets). Three-dimensional (3D) imaging of the inflorescence meristem when spikelets were initiated (double ridge stage) revealed that the SAM had significantly fewer cells in the gain-of-function mutant Slnd than the corresponding wild-type (Himalaya) (P = 6.54 × 10^{-3}, n = 7, Mann–Whitney test; Fig. 4f). To test whether control of meristem size is also separable from other DELLA functions in barley, we took advantage of an allelic series of Slnd. The Slnd.5 and Slnd.6 alleles, which contain second-site mutations in Slnd, have similar effects on a subset of the DELLA gain-of-function phenotypes, partially restoring leaf elongation and plant height (Fig. 4a). In spite of these similarities, Slnd.5 and Slnd.6 differentially affected meristem size (Fig. 4c–f). Furthermore, SAM size correlated with the number of rachis nodes produced by the SAM (Fig. 4h,f). Uncoupling of plant height from SAM size was also shown by the comparable meristems but distinct height of Slnd1 and Slnd5 (Fig. 4a,f). In conclusion, DELLA-induced restriction of meristem size led to reduced inflorescence size in a cereal crop. The smaller inflorescence of Slnd1 is reminiscent of the lower number of flowers seen in the rice semi-dwarf mutant sd1, which like gai-1 affects gibberellin biosynthesis, although it is not known whether sd1 also affects inflorescence meristem size.

Overall, our results show that DELLA genes have a conserved role in limiting inflorescence meristem size, in line with the role of DELLA in saving resources under environmental stress. This function involves a direct link to cell cycle regulation in the rib meristem and is genetically separable from the role of DELLA mutants in subsequent organ growth. Because the size of the inflorescence meristem limits yield potential in crop plants, separating the effects of DELLA on stem growth and meristem size could unlock further yield increases in the widely used semi-dwarf mutants.
Fig. 4 | DELLA control of meristem size is conserved and correlates with inflorescence size in barley. a, Wild-type barley (Himalaya) and delta mutants (Slnd1, Slnd1S, Slnd1d) at comparable stages after flowering. b, Mature spikes of genotypes shown in a, with alternating spikelets attached to nodes of the main axis (racchis) indicated for the wild type. c, 3D reconstruction of the confocal image of an MPS-P1-stained wild-type inflorescence meristem, fixed when the first spikelet primordium was initiated; a plane (red) fitted to landmarks placed on the boundaries of spikelet primordia was used to select SAM cells (details in Supplementary Information). d, e, 3D reconstructions of segmented images of wild-type (d) and Slnd1(e) apices; selected SAM cells are coloured. f, Correlation between meristem size (cell numbers) and inflorescence size (rachis nodes) in the wild type and slnd1 mutants; points and green lines show average and standard deviation per genotype, the red line shows the linear regression of averages, with the correlation coefficient r; for Himalaya, Slnd1, Slnd1S and Slnd1d, respectively, n = 18, 15, 18 and 18 spikes (for node numbers) and 8, 7, 3 and 4 apices (to image cell numbers). Scale bars, 10 cm (a), 1 cm (b), 100 μm (c–e).

Methods

Plant material. Arabidopsis thaliana Landsberg erecta (L-er) accession was used as the wild type unless otherwise specified. gai1, RGA:pGFP-rga17, pop4-1, global delta1, kerp2-3 (backcrossed three times to L-er), jag-1 kerp2-3, CYCB1;1p:GFP, MLA17p:GUS, RGA:pGFP-RGA, WUSp:GFP–WUS, CLV3p:GFP, 35SloxGUSlox–GFP and hop18.2Cre have been described. 35SloxGUSloxGUS, 35SloxGUSlox-GFP and RKRp2:pkRF2–GFP were constructed and transformed into Arabidopsis as described in the Supplementary Information. Arabidopsis plants were grown on JIC Arabidopsis Soil Mix (Leverington F2 compost with Intersect and 4 mm grit at a 6:1 ratio) at 16°C under continuous light. Dissection of inflorescence apices for imaging. ChIP and measurement of plant height were performed when the plants had produced the first three mature flowers. The barley (Hordeum vulgare) Himalaya (wild type), Slnd1 (M640), Slnd1.5 (TR9) and Slnd1.6 (TR13) mutants were grown on JIC Cereal Mix (containing 1.3 kg m–³ PG Mix 14-16-18 and 1 kg m–³ Osmocote Mini 16-8-11 2 mg) under long day photoperiod (16 light/8h dark) and 20°C/15°C day/night temperatures. Barley inflorescence apices were dissected for imaging at the double ridge stage. Cre-lox P recombination. hop18.2Cre, 35Sloxlox–GUSlox-lox–GFP and hop18.2Cre, 35Sloxlox–GUSlox–GFP plants were grown until they had three self-pollinated flowers. Open flowers were removed and ink marks were placed on the stem. For localized heat-shock, water at 38 °C wasstreamed for 5 minutes onto a 2 mm sponge clamped around the stem. Plants were photographed after 10 days of growth and distances between ink marks were measured with Fiji14. Similarly treated hop18.2Cre, 35Sloxlox–GFPlox–GUS were stained for GUS 30 h after heat shock.

Growth measurements. Arabidopsis stem height was measured from photographs using Fiji14. To determine the rate of floral initiation, plants were grown at 16°C under continuous light; starting on the day floral buds became visible, flowers at stage 13 and beyond11 were counted daily in the main inflorescence of eight to ten plants per genotype. The whole experiment was repeated three times with similar results. Rachis nodes in barley inflorescences were counted for the main stem and first tiller in two independent experiments.

Confocal imaging. Dissection, of shoot apices, live imaging and modified pseudo-Schiff propidium iodide (mPS-PI) staining were as described14, before imaging with a Zeiss LSM780 confocal microscope; resolution was 0.42 × 0.42 × 0.50 μm³ for Arabidopsis and 0.66 × 0.66 × 1.0 μm³ for barley. GFP was imaged in cleared apices by the ClearSee method16 before imaging with a Leica SP5 confocal microscope with a 20×/0.75 long-working distance objective (resolution 0.63 × 0.63 × 1 μm³). Vibratome sectioning is described in the Supplementary Information.

Image analysis. Custom Python scripts and Fiji macros based on a previous set of scripts21 were used to segment confocal image stacks, measure shoot meristem areas, define the position of cells within the shoot meristem and measure GFP signal within segmented cells. The function of each script is summarized in the Supplementary Information. Supplementary Table 3 lists the scripts used to produce the data for each manuscript figure and Supplementary Table 4 lists the filtering parameters (e.g. to select cells in meristem regions). The annotated source code with detailed instructions and folders containing the confocal images, files produced during image processing and final cell data tables are publicly available (see Data availability).

Statistics. Data were read and processed in a Python shell using the functions defined and annotated in script /3D_meristem_analysis/python_scripts/statistical_analysis.py (within Supplementary Software, DOI:10.6084/m9.figshare.4675801.v1), using Scientific Python (http://www.scipy.org) functions for linear regression, Shapiro–Wilk tests for normality, two-tailed Student's t-test and two-tailed Mann–Whitney U tests. For samples with n < 100, power analysis was performed with the pwr package in R (http://www.r-project.org/), using function pwr.2slnorm to calculate effect sizes, assuming a significance level of 0.05, power 0.8, and the alternative hypothesis that the mean was smaller for sample 2 than for sample 1. Raw values, descriptive statistics, P values and effect sizes for the data used in each figure are listed in Supplementary Table 4. In all figures, with boxplots, boxes extend from the lower to the upper quartile, with a line marking the median, and whiskers extend to 1.5 times the interquartile range.

ChIP-seq and data analysis. Dissected inflorescence apices of RGA:pGFP, pop4-1 and L-er (wt) plants were collected at the same stage used for imaging. Developing silique and open flowers were removed and the remaining apices was dissected, including 2–5 mm of stem below the SAM. Because DELLA proteins are thought to bind DNA indirectly through interactions with transcription factors, the tissue was fixed with 2% formaldehyde in PBS 1× supplemented with 1 μM dNTPs (N-succinimidyl) glutarate (DSG, Synchem) under vacuum for 20 min to improve protein–protein and protein–DNA crosslinks. Crosslinking was stopped with 100 μM glycerol and after two washes with water, the samples were blotted dry and frozen in liquid nitrogen. The subsequent ChIP-generation of ChIP-seq libraries and analysis were performed as described15. To detect enriched sequence motifs, MEME-ChIP was used in discriminative mode (http://meme-suite.org/tools/meme-chip16), comparing peak sequences with a tenfold larger control set of random peaks as described39. Raw and processed ChIP-seq data have been deposited at NCBI’s Gene Expression Omnibus32 (see Data availability).

ChIP-PCR. ChIP-qPCR was performed on RGA:pGFP-rga17 and L-er (wt) dissected inflorescence apices as described3, using the 2−ΔΔCt method27 to calculate the relative abundance of immunoprecipitated DNA, compared with controls containing a constant amount of input DNA. The first primers were used for each amplicon KRP2-1.6 5′-TGATGAGTGATGAGATGCG-3′ and 5′-AAAG CCGGTTGGTCGGTACG-3′; KRP2+1.3 5′-CCTTCGACAACCGAAATTTTA-3′ and 5′-GCTGGAGGAAATGAGATGCG-3′; MU like 5′-GTTTACCAAGGAA TCTGTGTTGGTG-3′ and 5′-CATAACATAGTTTTAGGACGGTC-3′; FB171, 5′-GAAGTACAGTGGAACCCCAAGCTC-3′ and 5′-GCTAATTGGAAGA ACGTGAGCTC-3′.

Data availability. Source data for all boxplots are listed in Supplementary Table 4; original ChIP-PCR data are found in Supplementary Tables 5 and processed ChIP-seq data have been deposited at NCBI’s Gene Expression Omnibus (accession GSE94926). The source code for image analysis and detailed instructions are available as Supplementary software (https://doi.org/10.6084/m9.figshare.4675801.v1). For each image folder listed on Supplementary Tables 3 and 4, the original confocal images, preprocessing data, processed cell data and metadata files data can be downloaded at https://doi.org/10.6084/m9.figshare.4675801.v1.

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Conceptualization, A.S.-M., S.Bo. and R.S.; investigation, A.S.-M., S.Be., M.B., S.Bo. and K.S.; software, R.S.; formal analysis and data curation, A.S.-M. and R.S.; writing — original draft, A.S.-M. and R.S.; review and editing, A.S.-M., S.Be., M.B., S.Bo. and K.S.; funding acquisition, R.S. and A.S.-M.; supervision, R.S.

Competing interests

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Additional information

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