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DOI:
10.1038/s41477-023-01372-6

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Document Version
Peer reviewed version

Citation for published version (Harvard):
Briones-Moreno, A, Hernández-García, J, Vargas-Chávez, C, Blanco-Touriñán, N, Phokas, A, Úrbez, C, Cerdán, PD, Coates, JC, Alabadí, D & Blázquez, MA 2023, 'DELLA functions evolved by rewiring of associated transcriptional networks', *Nature Plants*. https://doi.org/10.1038/s41477-023-01372-6

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Download date: 03. May. 2024

DELLA functions evolved by rewiring of associated transcriptional networks

Asier Briones-Moreno[1], Jorge Hernández-García[1,#], Carlos Vargas-Chávez[2], Noel Blanco-Touriñán[1], Alexandros Phokas[3], Cristina Úrbez[1], Pablo D. Cerdán[4], Juliet C. Coates[3], David Alabadí[1] and Miguel A. Blázquez[1,*]

1. Instituto de Biología Molecular y Celular de Plantas (CSIC-U Politécnica de Valencia), Valencia, Spain
2. Institut de Biologia Evolutiva (CSIC-U Pompeu Fabra), Barcelona, Spain
3. School of Biosciences, University of Birmingham, Birmingham, UK
4. Fundación Instituto Leloir, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina

* Corresponding author: mblazquez@ibmcp.upv.es

Present address: Laboratory of Biochemistry, Wageningen University, 6708WE Wageningen, The Netherlands

The Version of Record of this article is published in Nature Plants, and is available online at https://doi.org/10.1038/s41477-023-01372-6

DELLA proteins are land-plant specific transcriptional regulators that transduce environmental information to multiple processes throughout a plant's life[1–3]. The molecular basis for this critical function in angiosperms has been linked to the regulation of DELLA stability by gibberellins and to the capacity of DELLA proteins to interact with hundreds of transcription factors (TFs)[4,5]. Although bryophyte orthologs can partially fulfill functions attributed to angiosperm DELLA[6,7], it is not clear whether the capacity to establish interaction networks is an ancestral property of DELLA proteins or it is associated with their role in gibberellin signaling[8–10]. Here we show that representative DELLAs from the main plant lineages display a conserved ability to interact with multiple TFs. We propose that promiscuity was encoded in the ancestral DELLA protein, and that this property has been largely maintained, while the lineage-dependent diversification of DELLA-dependent functions mostly reflects the functional evolution of their interacting partners.

To gain insight into the conservation of the DELLA interactome in plants, we selected a core set of 42 proteins (covering all major families of TFs and transcriptional regulators) known to be DELLA partners in *Arabidopsis thaliana*. We examined the ability of DELLAs from another angiosperm (*Solanum lycopersicum,* SlPRO), a lycophyte (*Selaginella moellendorfii,* SmDELLA1), and a liverwort (*Marchantia polymorpha*, MpDELLA) (Extended Data Fig. 1) to interact in a yeast two-hybrid assay with selected orthologs of these 42 AtDELLA partners in each species, according to the literature and phylogenetic analyses (Fig. 1a; Supplementary Table 1). Given that all the interactions occur through the C-terminal GRAS domain, a truncated version of each DELLA without the N-terminal domain was used. As expected, all of the AtTFs interacted with AtRGA, and the interactions were conserved at very high level in the other three species: 74% for SlPRO, 71% SmDELLA1, and 85% for MpDELLA (Fig. 1b; Supplementary Table 2). Moreover, 98% of the interactions were detected in at least two species, suggesting that the ability of DELLAs to interact with multiple TFs has been extensively conserved during land plant evolution.

To investigate to what extent the conservation of these protein-protein interactions depends on the DELLA protein itself, or it is the result of DELLA-TF coevolution, we tested the capacity of DELLAs from several lineages to establish heterologous interactions with the set of *A. thaliana* TFs (AtTFs). All the DELLA proteins analyzed in the previous experiment, as well as the two moss DELLAs (from *Physcomitrium patens*), the two gymnosperm DELLAs (from *Picea abies*) and a second SmDELLA2 were able to interact with at least 86% of the AtTFs (Fig. 1c). This result suggests that promiscuity

is a property encoded in the ancestral GRAS domain of DELLA proteins, an idea further supported by two observations: (i) a resurrected GRAS domain based on the predicted ancestral-most sequence of DELLAs[11] displayed a ratio of 81% of positive interactions with this set of AtTFs (Fig. 1c); and (ii) reciprocal heterologous interactions were also conserved, as the AtRGA protein interacted with 73% of the MpTF set (Fig. 1d). Although other non-DELLA GRAS proteins also showed a significant capacity of interaction with TFs, the highest ratio observed was only 20% for the closest GRAS paralogs of AtDELLA proteins, SCARECROW-LIKE3 (SCL3) *vs* the AtTF set (Fig. 1c), and 50% for MpGRAS7 *vs* the MpTF set (Fig. 1d). This is in tune with the reported low interacting capacity of other GRAS proteins[5]. Considering this and the higher conservation level of the GRAS domain within the DELLA clade, compared with eight other clades in the GRAS family (Extended Data Fig. 2), we propose that DELLAs' promiscuity is an advantageous property actively maintained during evolution, rather than a characteristic achieved by convergent evolution in different lineages or an intrinsic feature of the GRAS domain fold.

Despite the conservation of a high interactive capacity in DELLA proteins during plant evolution deduced from the qualitative assays shown above (Fig. 1b-d), there are indications that DELLA-TF coevolution has contributed to the specificity of the interactions in different lineages. By comparing yeast growth in the absence and in the presence of 3-amino-1,2,4-triazole (3-AT) to titrate homologous and heterologous interactions between DELLAs and TFs from *A. thaliana* and *M. polymorpha* (Fig. 1e), we found that in 6/22 cases the strength of the interaction was equivalent for homologous and heterologous interactions; in 7/22, the strength was determined by the TF species; and in 9/22 cases the strength was determined by the combination of DELLA and TF, suggesting a relatively high level of fine-tuning of the DELLA-TF affinity in a species-dependent manner.

Angiosperm DELLA proteins have been shown to undergo different post-translational modifications in various environmental contexts which modulate their activity[12]. A reasonable scenario emerges in which species-specific regulatory mechanisms and differences in DELLA-TF relative affinities would have contributed to the optimization of DELLA function during evolution. To obtain an accurate picture of the relevance of such mechanisms *in vivo*, we decided to examine the ability of different DELLAs to complement the At*dellaKO* mutant. We introduced five DELLA proteins, each one from a different species (*A. thaliana,* AtRGA; *S. lycopersicum*, SlPRO; *P. abies*, PaDELLA2; *S. moellendorfii*, SmDELLA1; and *M. polymorpha*, MpDELLA) fused at their C-termini to YFP, under the control of a 4 kb promoter fragment and 3 kb terminator fragment of *AtRGA* to obtain native expression patterns. We selected lines with

comparable DELLA levels (Extended Data Fig. 3) and, to avoid the interference of possible species-specific differences in the sensitivity towards GAs, all the experiments were performed in the presence of paclobutrazol (PAC), a GA synthesis inhibitor. Among the processes affected by DELLA proteins in *A. thaliana*, we evaluated the degree of heterologous complementation of At*dellaKO* in the control of plant size[13–15], seed germination[16], skotomorphogenic development[17], and salt stress resistance[18]. While all DELLAs conveyed certain degree of complementation, there were marked differences between species: the complementation achieved by AtRGA was almost matched by the angiosperm SlPRO and gymnosperm PaDELLA2, but the lycophyte SmDELLA1 and the liverwort MpDELLA were less efficient in the correction of the defects caused by DELLA loss of function (Fig. 2a-e; Extended Data Fig. 4). The capacity to substitute endogenous DELLAs may be correlated with the evolutionary distance between *A. thaliana* and the corresponding species, as supported by the reduced effect of AtRGA expression compared to MpDELLA in *M. polymorpha* in a reciprocal heterologous expression test (Fig. 2f,g).

Given that DELLA function is mostly exerted through transcriptional regulation via the interaction with TFs, we further investigated the complementation capacity in terms of transcriptomic changes, for which we performed RNA-seq analyses of the uncomplemented At*dellaKO* mutant, and one of each of the complemented lines (Supplementary Fig. 1). All DELLAs were associated with a substantial number of differentially expressed genes (DEGs) (Fig. 3a, Supplementary Table 3). We observed that 86% of the AtRGA-dependent DEGs were also under the regulation of the DELLA of at least one other species, and the sense of the transcriptional regulation was the same in over 95% of the cases (Fig. 3a,b). The highest overlap was detected with the evolutionarily closer species *S. lycopersicum* and *P. abies* and, consistently, an overlap was also found among the biological functions of the DEGs regulated by each DELLA (Fig. 3c; Supplementary Table 4). Functions related to the response to pathogen infections are among the ones regulated by all DELLAs in *A. thaliana*, while other functions, like the response to water deprivation, are regulated only by DELLAs from Spermatophyta (Fig. 3c). Particularly interesting is the overlap between the five species in the regulation of a set of 211 DEGs which define a set of functions that had not been previously attributed to DELLA regulation, like the response to hypoxia, heat, antibiotics, or to unfolded proteins in the endoplasmic reticulum (Supplementary Table 4). According to our TF enrichment analysis, a small set of TFs –all of which interact with AtDELLA– would be enough to explain the coincident regulation of the 211 target genes by all DELLAs (Fig. 3d; Supplementary Table 5). On the other hand, we observed that heterologous expression of SlPRO caused the exclusive alteration of over 2300 genes

(Fig. 3b). To test whether this feature results from the ability of SlPRO to interact with particular AtTFs that are not natural AtDELLA partners, we performed a hierarchical analysis of TF-target regulations among the SlPRO-exclusive targets. This analysis predicted that the expression of 20% of these genes could be regulated by a small set of TFs (Fig. 3e; Supplementary Table 6), none of which interacted with AtRGA, while at least two of them (AT1G74840 [SANT/Myb family] and AT5G01380 [GT3a family]) interacted with SlPRO (Fig. 3f). Curiously, only the tomato ortholog of AT1G74840 showed interaction with SlPRO (Extended Data Fig. 5), indicating that heterologous interactions not always reflect biologically relevant interactions.

In summary, the partial complementation at the phenotypic and transcriptomic level of the At*dellaKO* mutant by DELLAs from other species suggests that, despite the general conservation of promiscuity among DELLAs, additional factors modulate DELLA-TF interactions in a species-specific manner.

Although all previous results point to an intrinsic capacity of the ancestral DELLA protein to act as a transcriptional hub, they do not demonstrate that this function is indeed conserved across land plant evolution. To investigate the capacity of DELLAs to act as hubs in species other than *A. thaliana*, we compared the DELLA-dependent transcriptomes in the dicots *A. thaliana* and *S. lycopersicum*, the monocot *O. sativa*, the lycophyte *S. kraussiana*, the moss *P. patens* and the liverwort *M. polymorpha* –spanning an evolutionary distance of no less than 470 M years[19]. For the first three species and the moss, a DELLA loss-of-function mutant is available[6,20–22], so the comparison between At*dellaKO*, Os*slr1*, Sl*pro* and and Pp*dellaab* and their respective wild types would define the transcriptome mobilized by DELLAs in each species. Although there are no *della* mutants available in *S. kraussiana*, a GA treatment is an efficient way to remove DELLAs in *Selaginella* spp[6,23]. Furthermore, the DELLA-dependent transcriptome in *M. polymorpha* is available through the comparison between wild-type and MpDELLA overexpressing lines[7]. RNAseq analyses were performed (see Methods for details) and DEGs were determined between the conditions with high and low DELLA levels for each species (Supplementary Table 7). For inter-species comparison of the transcriptomes, orthogroups (OGs) were first defined in all species (Supplementary Table 8). Relatively large numbers of genes were mobilized by DELLAs in each species, ranging from 1553 OGs in *P. patens* to 4592 in *S. lycopersicum* (Fig. 4a). Interestingly, around 20% of the OGs were unique for a single species, while a larger set of the OGs were common to at least three species (54% in *A. thaliana*, 46% in *S. lycopersicum*, 56% in *O. sativa*, 64% in *S. kraussiana*, 48% in *M. polymorpha* and 64% in *P. patens*) (Fig. 4a). Together with the conservation of enriched GO categories among DELLA-dependent DEGs common to at least 4 species (Fig. 4b; Supplementary Table 9), these results are a strong

indication of extensive conservation in functions and molecular targets for DELLAs across evolution, possibly caused by the largely conserved interactome. However, the identification of genes regulated in a species-specific manner highlights the existence of alternative mechanisms that have operated during evolution to optimize DELLA functions in extant plants. The differences in DELLA transcriptional targets between species may have emerged from the loss or gain of particular DELLA-TF interactions, but also from the loss or gain of an interacting TF's capacity to regulate downstream targets[24]. To explore these two possibilities, we first searched for enriched regulatory elements in the promoters of exclusive DELLA targets for each of the species, and then established the conservation of these elements in the orthologs of the rest of the species (Fig. 4c). We observed two contrasting behaviors: on one hand, only a marginal number of the TF-target regulations of species such as *A. thaliana* or *P. patens* were conserved in the other species, supporting a mechanism by which DELLA functional evolution would also depend on the evolution of its interacting TFs with respect to target recognition; on the other hand, most of the TF-target regulations in *S. lycopersicum* and *O. sativa* were conserved in the orthologous targets of *A. thaliana*, pointing to the alternative mechanism by which DELLA functions would evolve through the gain or loss of TF partners. In agreement with this prediction, we found that SlPRO was able to interact with SlDOF14 (one major regulator of *S. lycopersicum*-exclusive DELLA targets [Supplementary Table 10]) in a yeast two-hybrid assay, while AtRGA did not interact with its ortholog AtDOF1 (Fig. 4d). The observation that a reconstructed ancestral GRAS domain was also unable to interact with either one of the DOF orthologs leads to speculate that this DELLA-DOF interaction was gained, rather than lost, after the separation between Rosids and Asterids.

In summary, our work indicates that (i) DELLAs have conserved their role as transcriptional hubs based on a remarkable capacity to establish physical interactions with TFs, and (ii) that the actual biological functions controlled by DELLAs in different plant lineages relies on two mechanisms: the gain and loss of specific DELLA-TF interactions in certain clades, and also evolutionary changes attributable to the TFs themselves[25]. This work provides experimental evidence that supports previous observations using *in silico* network analyses that pointed to stress responses as a likely ancestral function of DELLAs[26].

Our study reveals an evolutionary model in which the ancestral DELLA soon displayed an extensive capacity to interact with multiple TFs, which is now maintained in extant plants of vascular and non-vascular clades, irrespective of the presence of a GA perception module. Such a model –contrary to a model suggesting the gradual development of the high degree of connectivity– has multiple implications, both from

basic and applied perspectives. For instance, it becomes evident that the diversity of functions regulated by GAs in vascular plants (many of which have profound impact in cultivated species) is a direct consequence of DELLA's conserved promiscuity. The fact that this property has been conserved for over 470 M years is a faithful measure of its physiological relevance and highlights the constraints under which this type of 'hub' protein evolves.

Methods

Plant materials and growth conditions. Used *della* mutants and their parental lines are the following: *Arabidopsis thaliana* Landsberg *erecta* (L*er*) wild type, and the pentuple *dellaKO* mutant (stock nr. N16298 in the Nottingham Arabidopsis Stock Center); *Solanum lycopersicum* cv. M82, and the severe loss-of-function *della* mutant $pro^{\Delta GRAS}$ [27]; *Oryza sativa* subsp. *japonica* cv. Nipponbare and the *slr1-1* mutant[21]. These three species were grown and propagated from seeds in the greenhouse. Tomato and rice *della* mutations were maintained in heterozygosity due to sterility, and homozygous mutants selected by phenotyping and genotyping by Sanger sequencing when necessary (primers in Supplementary Table 11). The *Physcomitrium* (*Physcomitrella*) *patens Ppdellaab* mutant[6] and its parental strain Gransden (Gd UK) were maintained *in vitro* as previously described[28]. An undetermined cultivar of creeping *Selaginella kraussiana* was obtained from the University of Valencia botanical collection, maintained in soil, and propagated by cuttings. *Marchantia polymorpha* subsp. *ruderalis* Tak-1 accession was maintained *in vitro* asexually from gemma[29].

All species were cultivated in growth chambers at 22ºC under long-day conditions unless otherwise stated. For *in vitro* culture, half-strength MS medium[30] (*A. thaliana*, *S. lycopersicum* and *O. sativa*), half-strength Gamborg's B5 medium[31] (*M. polymorpha*) or BCD medium[32] (*P. patens*) were used. Transformation of *A. thaliana* and *M. polymorpha* were performed by *Agrobacterium* floral dipping and thallus cuttings respectively, as previously described[33,34].

Phylogenetic analysis. For DELLA phylogenetic analysis, DELLA protein sequences from different species were collected based on previously published phylogenetic trees (Supplementary Table 1)[11], with the addition of *Anthoceros agrestis* DELLA (Sc2ySwM_228: 2934964..2932688 (-))[35]. Protein sequence alignments were performed with M-Coffee using a combination of the multiple alignment methods MAFFT, T-Coffee, MUSCLE, and POA2)[36]. Trimming was performed in unambiguously aligned regions, and deleting non-GRAS domain parts. The LG model of amino acid replacement was selected as best-fit using the AIC model for ranking, and used to construct a rooted

maximum-likelihood tree with PhyML (v3.1)[37], using empirically estimated amino acid frequencies when indicated (+F). Statistical significance of branches was evaluated by the SH-like approximate likelihood ratio test. The graphical representation of the phylogenetic tree was generated using FigTree (version 1.4.3) software (http://tree.bio.ed.ac.uk/software/figtree/), and the final figure edited manually.

For transcription factor gene family phylogenetic trees, OrthoFinder-implemented pipeline was utilized, including MAFFT-based multiple sequence alignment and FastTree-based phylogenetic tree construction under default settings. The trees are accessible at Mendeley Data v1 (http://dx.doi.org/10.17632/prfnj59kbs.1).

Species-specific DELLA interactome studies. To assess the conservation of the DELLA interactome through yeast two-hybrid screening, collections of DELLA putative interactors expressed in yeast were created for four different species. To select the members of the *A. thaliana* core collection, an exhaustive literature search was conducted on DELLA reported interactions; this information was compiled, and representative members of each protein family were chosen (Supplementary Table 1). Gateway entry clones were obtained for these DELLA known interactors in *Arabidopsis*, by resorting to existing transcription factor collections, personal donations, and *de novo* cloning when needed. The unavailable genes were amplified from *A. thaliana* wild-type cDNA using *att*B-PCR primers and introduced in entry vectors through BP Clonase II (Invitrogen) reaction. Expression clones were created by transferring these genes to the destination vector pGADT7-GW through LR Clonase II (Invitrogen) reaction. This process results in the fusion of the CDS with the Gal-4 activation domain contained in the pGADT7-GW vector.

For the collections of DELLA putative interactors in *S. lycopersicum*, *S. moellendorfii* and *M. polymorpha*, a search for orthologs was conducted using PLAZA Integrative Orthology Viewer[38], BAR expressolog identification[39], Phytozome[40], MarpolBase (http://marchantia.info), OrthoMCL-DB[41], OneKP[42] and extensive manual curation. The retrieved gene sequences were synthesized (Genscript) and introduced in pGADT7 for direct transformation in yeast, with exceptions from *M. polymorpha* that were obtained by either cloning from cDNA as described above, or by donations (Supplementary Table 1).

Additional Gateway-based entry clones for DELLA interactors included in other analyses (Fig. 3, Fig. 4, and Extended Data Fig. 5) were constructed by either PCR-based cloning, or gBlock synthesis, or directly ordered from clone collections (Supplementary Table 1). In all cases, these clones were transferred into pGADT7-GW as indicated above.

As baits, truncated versions of DELLAs from different species were used: *A. thaliana RGA*, *M. polymorpha* Mp*DELLA, P. patens* Pp*DELLAa* and Pp*DELLAb*, and *S. lycopersicum PRO* GRAS domains were amplified from cDNA; full length *DELLA* CDS from *S. moellendorfii* (Sm*DELLA1* and Sm*DELLA2*) and *P. abies* (Pa*DELLA1* and Pa*DELLA2*) were synthesized as gBlocks (I.D.T.) with *att*B overhangs. GRAS domain truncations were obtained by PCR amplification from these gBlocks. The sequence of the GRAS domain from the ancestral *DELLA* gene was obtained from a previous report[11] and synthesized as a gBlock (I.D.T.) with *att*B overhangs. Non-DELLA *GRAS* genes were also amplified from cDNA: *SCARECROW* (At*SCR*) and *SCARECROW-LIKE 3* (At*SCL3*) from *A. thaliana*, and GRAS7 (Mp*GRAS7*, Mp8g01770) from *M. polymorpha*. gBlocks and PCR products containing *att*B overhangs were introduced in Gateway entry vectors as previously described and transferred by LR Clonase II (Invitrogen) reaction into pGBKT7-GW to produce bait vectors. Sequences of all truncated versions can be found in Supplementary Table 12. Putative DELLA interactors in pGADT7 and truncated DELLAs in pGBKT7 were transformed in the yeast haploid strains Y187 and Y2H-Gold (Clontech) respectively, by subjecting yeast cells to a 42ºC heat shock in the presence of polyethylene glycol and Lithium acetate. Transformants were grown in SD selective medium without leucine or tryptophan depending on the transformed vector (-L for pGADT7 and -W for pGBKT7). Diploid yeast containing both types of plasmids were obtained by yeast mating, induced by co-culture of both strains in liquid YPD medium. After selecting diploids in SD -L/W, they were grown in liquid until saturation and dropped in SD plates (-L/W as a growth control and -L/W/H) (Supplementary Table 2; Supplementary Fig. 2). All Y2H screenings were performed in the same conditions, and the strength of the interactions was assessed by using SD -L/W/H plates supplemented with 2.5 mM 3-Amino-1,2,4-triazole (3-AT), a competitive inhibitor of HIS3 that reduces histidine production by the yeast. Interactions were considered strong when diploids grew in the presence of 3-AT, and weak when they did not.

Heterologous complementation in *A. thaliana* and *M. polymorpha*. Plasmids were generated using a combination of Gateway™ (Invitrogen) and GoldenBraid (GB)[43] systems. A pRGA::GW:YFP:tRGA Destination Vector was built containing a 3.7-Kb fragment upstream of the RGA start codon, the Gateway recombination cassette, the gene encoding Yellow Fluorescent Protein (YFP), and the 2.8-Kb sequence downstream the *RGA* stop codon. As selection marker, we used the fluorescent protein DsRED under the control of the seed-specific At2*S3* promoter and the 35S terminator[44]. All full-length sequences used in this study were cloned into the Gateway cassette.

For *M. polymorpha*, we used a previously described pMpGWB106-MpDELLA[7] plasmid harboring a *35S:*Mp*DELLA-Citrine* cassette, and built an analogous plasmid for At*RGA* overexpression in *M. polymorpha* transferring the *RGA* gene from a Gateway entry plasmid into the pMpGWB106 destination vector[45] by LR Clonase II (Invitrogen) reaction as described above.

Transgenic *A. thaliana* plants were examined for DELLA subcellular localization using confocal microscopy, and for DELLA levels by western blotting with anti-GFP (JL-8) and anti-DET3 as control (Clontech) (Extended Data Fig. 3). Two independent lines were chosen from each set for further phenotypical analysis. All tests were performed in the presence of PAC to maximize DELLA accumulation. For all *in vitro* assays, seeds were sown in half strength MS medium supplemented with PAC (1 μM for the germination tests and 0.5 μM for the rest). Hypocotyl length and apical hook angle were measured for 20-40 seedlings of each line using ImageJ[46]. Fresh weight was determined for 28-32 seedlings of each line, in sets of 4. Germination ratios were examined using 75 seeds from each line after 24h at 22ºC in darkness. Seeds were considered germinated if emerging radicles were detected under binocular microscope. Tolerance to salt stress was assessed by transferring 7-day-old seedlings (50 seedlings per line) grown in light, to plates supplemented with 250 mM NaCl, and counting the number of surviving seedlings after 6 days. Seedlings were considered alive when green areas were observed. For size measurement in adult plants, seeds were sown in individual pots containing soaked soil mix (2:1:1 peat, vermiculite and perlite), and grown under a long-day photoperiod. After 7 days, plants were watered once a week with 10 μM PAC dissolved in water. The length of the main stem, from the rosette to the tip, was measured in 30-day-old plants (18 plants per line). One-way ANOVA with post-hoc Tukey HSD test was employed to find statistically significant differences between phenotypes of different complemented lines.

Quantitative RT-PCR and transcript copy count analysis. For quantification of Mp*DELLA* and At*RGA* transcript abundance, 14-days-old *M. polymorpha* plants were flash-frozen and homogenized in liquid nitrogen. Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen) following manufacturer's instructions. cDNA was prepared from 1 μg total RNA using NZY First-Strand cDNA Synthesis Kit (Nzytech). Quantitative PCR was performed using 1 μl of cDNA per reaction in a 10 μl total volume with SYBR Premix ExTaq (TliRNaseH Plus) Rox Plus (Takara Bio Inc). For transcript copy count, the full length CDS of Mp*DELLA*, At*RGA* and Mp*EF1α* (Mp3g23400) were amplified, purified, and quantified in a Qubit 4 Fluorometer using the Qubit dsDNA BR Assay Kit (ThermoFisher). After quantification, serial dilutions were made to construct

calibration standard curves for each gene. The number of gene transcripts was determined for each sample and normalized using Mp*EF1α* transcript number. Quantitative PCR in plant samples and for standard curve construction were performed in a QuantStudio 3 real-time PCR system (ThermoFisher).

RNA-seq analyses. For the transcriptomic analysis of At*dellaKO* complementation with DELLAs from different species, seven-day-old seedlings grown in long-days with 0.5 μM PAC were collected. For the comparative analysis of DELLA function in *A. thaliana*, *S. lycopersicum*, *O. sativa*, *S. kraussiana* and *P. patens*, wild-type and *della* mutant plants of the first three species were grown in the presence of PAC at the minimal concentration that caused maximal reduction of hypocotyl or coleoptile growth (Supplementary Fig. 3), which was 0.5 μM for *A. thaliana* and 5 μM for *S. lycopersicum* and *O. sativa*. Soil-grown *S. kraussiana* plants were submerged in water with either 10 μM PAC or 100 μM GA_{4-7} up to the bottom half of the pots, and sprayed 24 and 72 h after watering, with the same solutions (this time including 0.02% Tween 20), similarly to previously described[23]. Tissue samples were collected 1 h after the second spray. *P. patens* wild-type and Pp*dellaab* mutants were grown as described above. The transcriptomic analysis of MpDELLAoex plants has been previously described[7].

Total RNA was extracted from triplicate samples with the RNeasy Plant Mini Kit (Qiagen), and the RNA concentration and integrity (RIN) were measured in an RNA nanochip (Bioanalyzer, Agilent Technologies 2100). The preparation of libraries and subsequent sequencing in an Illumina NextSeq 500 platform was carried out at Beijing Genomics Institute (BGI) yielding at least 20M 100-bp paired-end reads per sample. The read qualities were explored using FastQC version 0.11.9. The adaptors were removed from the reads processing the paired-end files together using bbduk version 38.42 with the default adapters file and the following parameters: “ktrim=r k=23 mink=11 hdist=1”. Next, the reads were quality filtered using Trimmomatic[47] version 0.39 with the following parameters: “-phred33 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:35” and the quality of the filtered files was assessed with FastQC.

For the *de novo* assembly of the full *S. kraussiana* transcriptome, all the available filtered reads were included, and Trinity[48] version 2.9.1was used with default parameters.

For the differential expression analysis, the full genome and transcriptome were downloaded from NCBI for *A. thaliana, O. sativa, P. patens,* and *S. lycopersicum*. A decoys file was created for each species using the genome and next the index was created using the index command from *Salmon* [49] version 1.1.0. The full transcriptome of *S. kraussiana* was indexed without a decoys file given the lack of a genome assembly. The number of reads per transcript was determined with *salmon quant* using the –

validateMappings parameter and the filtered reads file. Both paired-end files while processing each replicate when available. Using the accessory scripts abundance_estimates_to_matrix.pl from Trinity a matrix with counts per transcript in all the replicates was obtained. Finally, the differential expression analysis using DESeq2 was performed using the run_DE_analysis.pl accessory script from Trinity. All the detected DEGs for the complementation of At*dellaKO* can be found in Supplementary Table 2, and DEGs for the five species can be found in Supplementary Table 7.

For the definition of orthologous genes, the full proteome was downloaded for the aforementioned species in NCBI and for *S. kraussiana*, the proteome was obtained from TransDecoder (https://github.com/TransDecoder/) version 5.5.0 output of our full transcriptome assembly. Next, the longest isoform was selected for each gene and the proteins were written on a single file per species in the same folder. Finally, OrthoFinder[50] version 2.3.11 was run on the folder containing the proteomes with default parameters. The obtained orthogroups are listed in Supplementary Table 8.

GO enrichment and TF enrichment analyses were performed using the corresponding tools available at PlantRegMap[51], selecting the corresponding species for each dataset and “all” in the Method options. A TF is considered enriched if the number of possible targets for it on the input list of genes is higher than expected; and a gene is considered a target if there is experimental evidence or it has *cis* regulatory elements or binding motifs for the TF. The obtained data were represented using Cytoscape, and they can be found in Supplementary Tables 5, 6 and 10.

Analysis of gene regulatory networks. To identify *A. thaliana* TFs putatively recognized by all heterologous DELLA proteins when expressed in *A. thaliana*, we run the TF-enrichment tool provided by PlantRegMap[51] on the list of 211 DEGs common to all DELLA (Fig. 3a) and selected the subset of those known to interact with AtDELLAs[5]. The result was graphed using Cytoscape. To identify TFs that could putatively mediate the DELLA-dependent transcriptional regulation exclusively exerted by Sl*PRO* in the At*dellaKO* mutant (Fig. 3e), we followed this pipeline: (1) Run the TF enrichment tool provided by PlantRegMap[51] on the Arabidopsis genes misregulated only in the line expressing Sl*PRO*; (2) Focus on the TFs over-represented with a p-value<0.001. Most of them belong to the ERF family and are known to interact with AtDELLAs[5], so they are not the candidates to mediate SlPRO-exclusive regulation. However, some of them are also misregulated in the complemented line, so we hypothesize that SlPRO might regulate an upstream regulator of one of those ERFs; (3) Extract all the possible TF-*cis* element regulations for *ERF2*, eliminate those that interact with AtDELLA, select 4 at

random (RTV1, AT1G74840, AT1G76870, AT5G01380), and test the interaction with A. thaliana and S. lycopersicum DELLAs by Y2H (Fig. 3f).

To identify TFs that could putatively mediate the species-specific DELLA-dependent transcriptional regulation and compare between species, we did the following: (1) For each species, select the set of genes under DELLA regulation exclusively in that species; (2) Run the TF enrichment tool provided by PlantRegMap[51]; (3) Extract the list of TF-target regulations for those enriched TFs; (4) Search for the occurrence of those TF-target regulations among the orthologous targets in the other species. Two situations may occur: (a) the occurrence is low, which implies that the reason why DELLA does not regulate those genes in the other species may be the diversification of the cis elements in those promoters; (b) the occurrence is high, which means that those genes are conserved targets for the same TFs in the other species, and if they are not DELLA-dependent targets it may be because DELLA does not interact with those TFs in the second species.

Data availability

All materials generated in this study are freely available upon request from the corresponding author. All data are available in the main text or the supplementary materials. The RNA sequencing data generated in this study have been submitted to the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/) under accession numbers PRJNA695247 (“Complementation of an Arabidopsis thaliana dellaKO with DELLAs from different plant species”) and PRJNA695244 (“DELLA-dependent transcriptomes in different plant species”).

Acknowledgements

We thank David Weiss and Sivan Livne (The Hebrew University of Jerusalem) for the tomato *pro* mutant and advice on the selection of homozygous seedlings, and Ko Hirano (Nagoya University) for the rice seeds. The Pp*dellaab* mutant was obtained from Yuki Yasumura, Eric Belfield and Nicholas P. Harberd (University of Oxford). We also thank Javier Agustí, Bruno Catarino and Maite Sanmartín (IBMCP, Valencia) for excellent input on the manuscript. Work was performed with grants BFU2016-80621-P and PID2019-110717-GB funded by Spanish MCIN/AEI /10.13039/501100011033/ and by “ERDF, A way of making Europe”.

Author contributions

A.B-M., J.H-G., and M.A.B. conceptualized the project. A.B-M., J.H-G., C.V-C., N.B-T., C.U., and A.P. conducted the investigation. A.B-M., J.H-G., C.V-C., N.B-T. and M.A.B.

conducted the formal analysis. P.D.C., J.C.C., D.A. and M.A.B. supervised the project. J.C.C. and M.A.B. acquired the funding. A.B-M. and M.A.B. wrote the original draft of the paper. All authors revised and edited the paper.

Competing interests statement

The authors declare no competing interests.

Figure Legends

Fig. 1 | Capacity of interaction of DELLAs from different plant lineages. **a**, Scheme of plant phylogeny depicting representative species of the lineages selected for this study. **b**, Interaction between each DELLA and the corresponding orthologs in each species of a core set of AtTFs known to interact with AtDELLAs. Green squares represent positive interaction results in a yeast 2-hybrid assay. Dark grey squares indicate that no orthologous TF could be found in that species. BD- and AD- denote in-frame fusions to the GAL4 DNA Binding- and Activation Domains, respectively. Negative controls include either the empty BD- or AD- vectors, indicated with Ø. **c**, Heterologous interaction between DELLAs from different species and the selected AtTFs. AncDELLA is the reconstructed most likely GRAS domain of DELLA in the last common ancestor of land plants. AtSCR, AtSCL3 and MpGRAS7 are the *A. thaliana* and *M. polymorpha* proteins representative of the GRAS clade most closely related to the DELLA clade. **d**, Heterologous interaction between AtRGA and the set of orthologous MpTFs used in a. **e**, Scheme of the strength of interaction between homologous and heterologous DELLA-TF pairs of *A. thaliana* and *M. polymorpha*. Each diamond represents the interaction between AtRGA or MpDELLA with either an AtTF or its *M. polymorpha* ortholog in a yeast 2-hybrid test. The color indicates the strength of the interaction; if growth is visible in the presence of 2.5 mM 3-AT, it is classified as “strong”; if it is visible only in the absence of 3-AT, it is “weak”. The number in the center of each diamond represents how many DELLA-TF combinations show that particular behaviour.

Fig. 2 | Heterologous complementation of DELLA function. Wild-type *A. thaliana* plants (WT), plants mutant for the five *DELLA* genes (At*dellaKO*) and At*dellaKO* plants transformed with DELLAs from the species indicated (At, Sl, Pa, Sm, or Mp) under the control of the AtRGA promoter and terminator, had their phenotypes examined in the presence of PAC (a-e). **a**, Hypocotyl length of 3-day-old seedlings grown in darkness (n=28 seedlings; black bar is the mean). **b**, Fresh weight of 7-day-old seedlings (n=6 individuals per genotype; black bar is the mean). **c**, Images of representative 30-day-old

plants showing the effect on inflorescence length. Scale bar=5 cm. **d**, Hook angle of 3-day-old seedlings grown in darkness (n=18 seedlings; black bar is the mean). **e**, Percentage of seedlings surviving the presence of 250 mM NaCl (n=3 independent biological replicates with 150 seeds per test). **f**, Thallus size of 14-day-old *M. polymorpha* plants overexpressing Mp*DELLA* or At*RGA* under the control of the 35S promoter (n=26 gemmalings; black bar is the mean). **g**, Images of representative *M. polymorpha* plants used in f. Letters in a, b, d, e and f indicate statistical differences between groups after one-way ANOVA followed by Tukey's HSD post hoc test (p<0.01).

Fig. 3 | Behaviour of DELLAs from different plant lineages as transcriptional regulators in *A. thaliana*. **a**, UpSet plot representing the overlap of DEGs regulated by the different DELLAs (according to the RNAseq analysis using line #1 of each DELLA transgenic construct). Colours indicate the proportion of genes up- and down-regulated in each case. The 211 genes regulated by all DELLAs have been highlighted in pale blue. **b**, Heatmap of fold-change values among DELLA targets common for at least three species. The largest overlap is observed between the DELLAs of the three spermatophytes (*A. thaliana*, *S. lycopersicum* and *P. abies*). **c**, GO categories (Biological Process) enriched among the targets of DELLAs common to at least two species. **d**, Network representation of the 211 DEGs common to DELLAs from all species, showing the most likely regulators according to TF enrichment analysis. **e**, Scheme representing the most likely small gene regulatory network that would explain the regulation of SlPRO's exclusive downstream targets in *A. thaliana*, highlighting the three most-upstream regulators (in green). **f**, Yeast 2-hybrid assay showing the interaction of two of the most-upstream *A. thaliana* regulators with SlPRO, but not with AtRGA. H, Histidine; 3AT, 3-amino-1,2,4-triazole.

Fig. 4 | Comparison of the transcriptional regulatory activity of DELLAs across the plant lineage. **a**, UpSet plot representing the overlapping and unique OGs regulated by DELLA in the different species. **b**, ReviGO representation of the GOs overrepresented in at least four of the species examined. **c**, Degree of conservation of enriched TF-promoter regulatory interactions that mediate putative DELLA regulation exclusive in one species, when compared with the corresponding orthologous sets in the other four species. Smaller circles indicate that the original TF-promoter interaction pairs do not happen in the other species. Larger circles indicate that the same TF-promoter interaction pairs in one species are present as orthologous TF-promoter in the other species –suggesting that the reason for the orthologous genes not being DELLA targets is because of lack of the necessary DELLA-TF interactions in that species. An example

is the conservation between SlDOF14-promoter and AtDOF1-promoter interactions. **d**, Yeast 2-hybrid assay showing that protein-protein interaction between SlPRO and SlDOF14 is not conserved between the orthologous pair in *A. thaliana*: AtRGA and AtDOF1. H, Histidine; 3AT, 3-amino-1,2,4-triazole.

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a

Rosids
Asterids
Angiosperms
Monocots
Gymnosperms
Ferns
Lycophytes
Mosses
Liverworts
Hornworts
Green algae
GA-GID1
DELLA

b

TF family
bHLH
TCP
MYB
G2-I
Zn finger
NAC
AP2
GRAS
Homeobox
MADS
Chromatin /Transcription
AD-TFs (Sp.)
FIT ORG3 MYC2 PIF5 UNE10 SACL1 TCP15 TCP2 MYBL2 MYB61 ARR1 KAN1 IDD4 ZFP8 STH CO CUC2 NAC2 RAP2.12 ERF3 BZR1 SCL3 HAM1 GAI SAW2 KNAT1 STIMPY HAT1 bZIP63 Dof4.2 AGL86 FLC JAZ9 EIL2 GI SWI3C HUB1 NF-YA2 MED31 ELF7 PFD6 Lsm2 Ø
BD-DELLA^GRAS
AtRGA
SlPRO
SmDELLA1
MpDELLA

c

AD-AtTFs
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e

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Figure 1

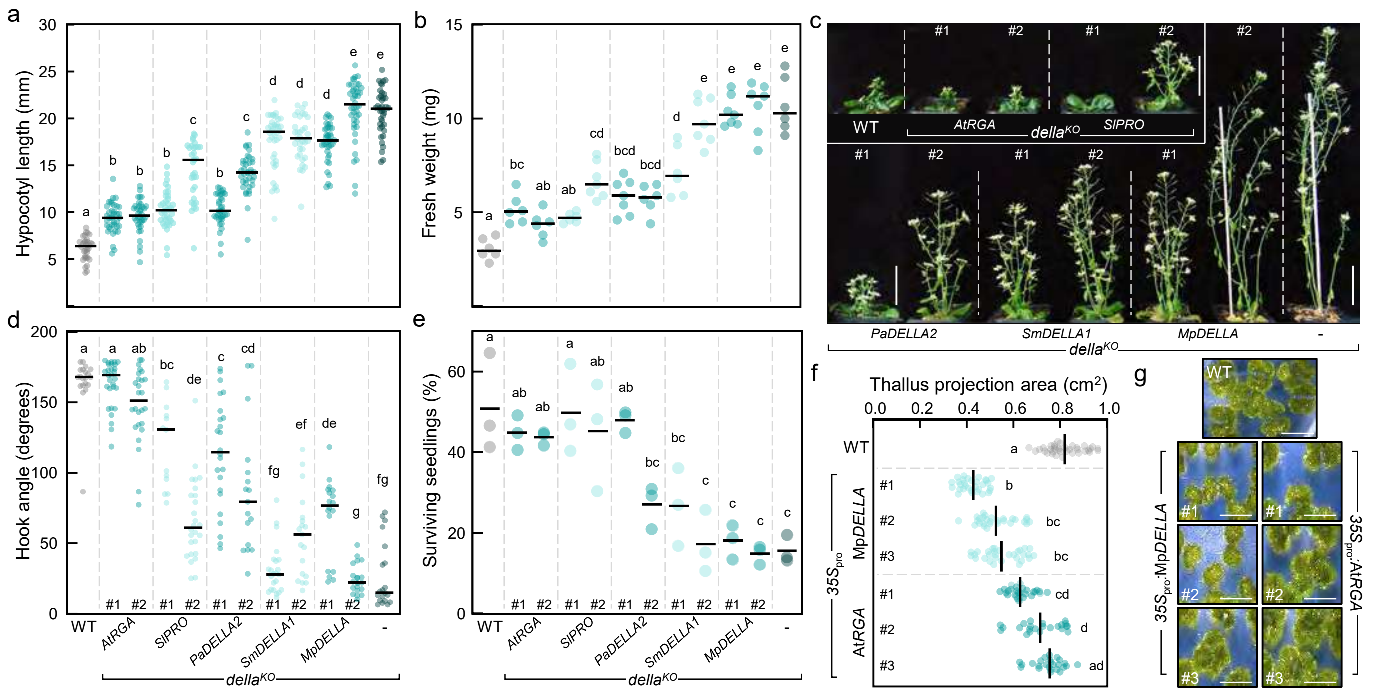

Figure 2

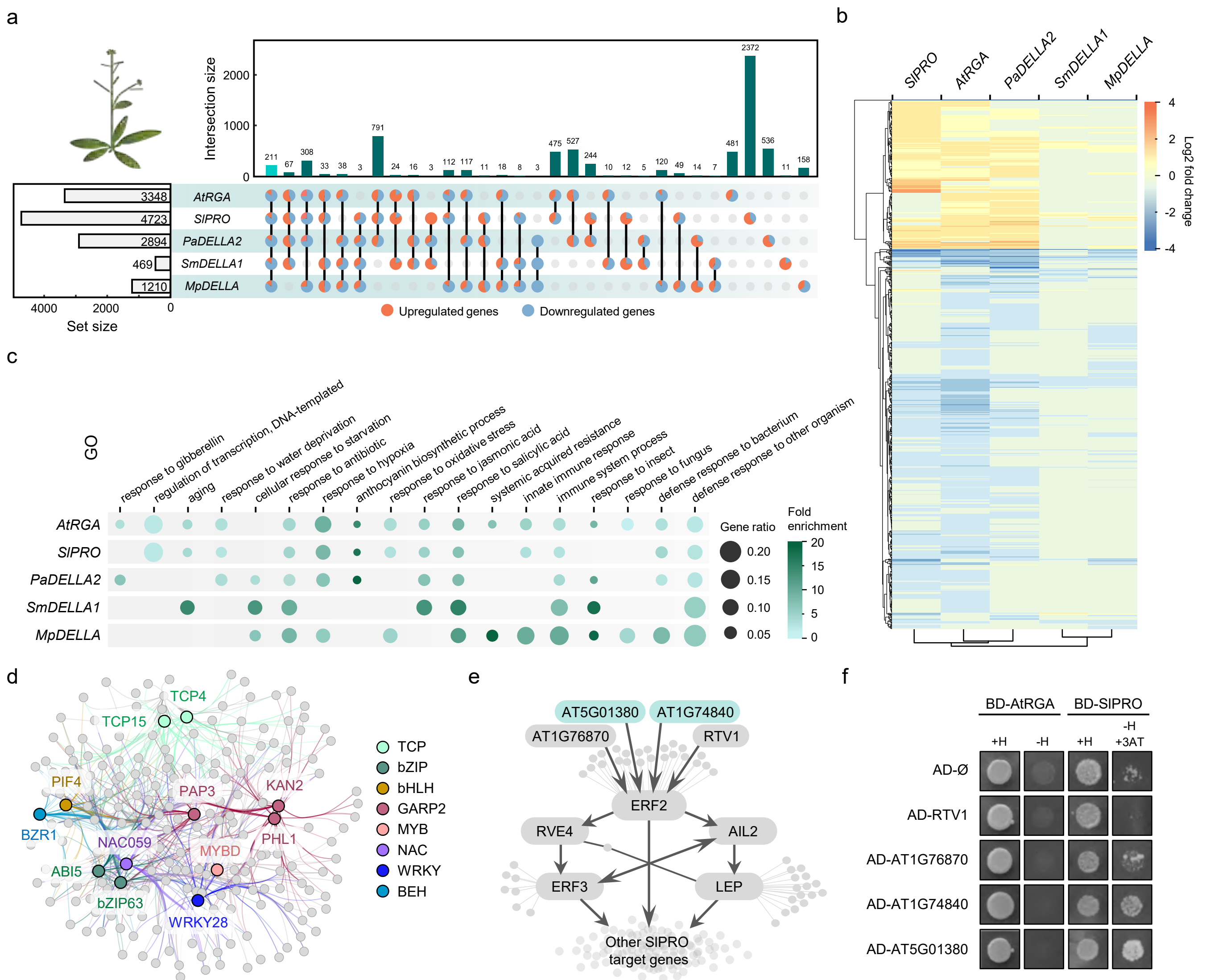

Figure 3

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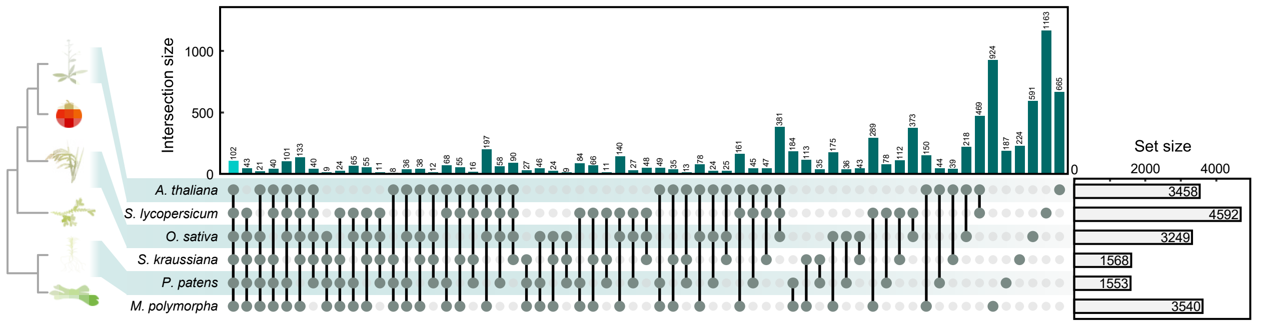

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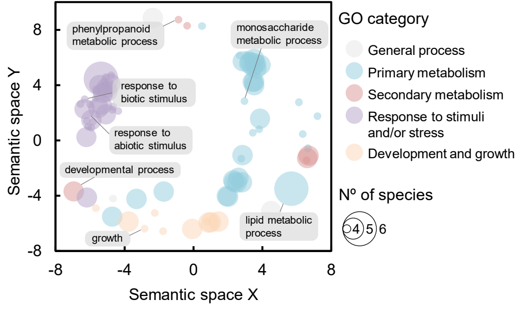

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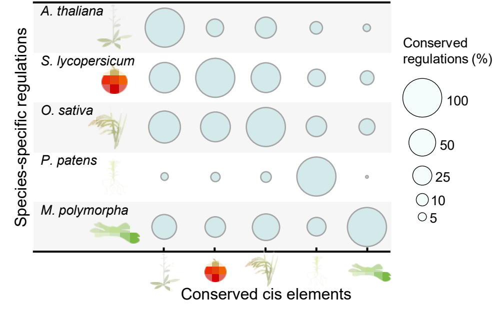

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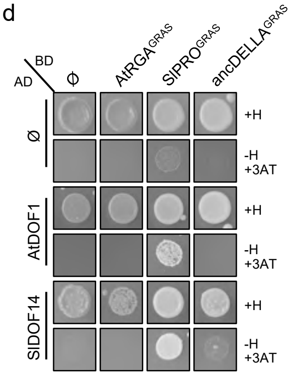
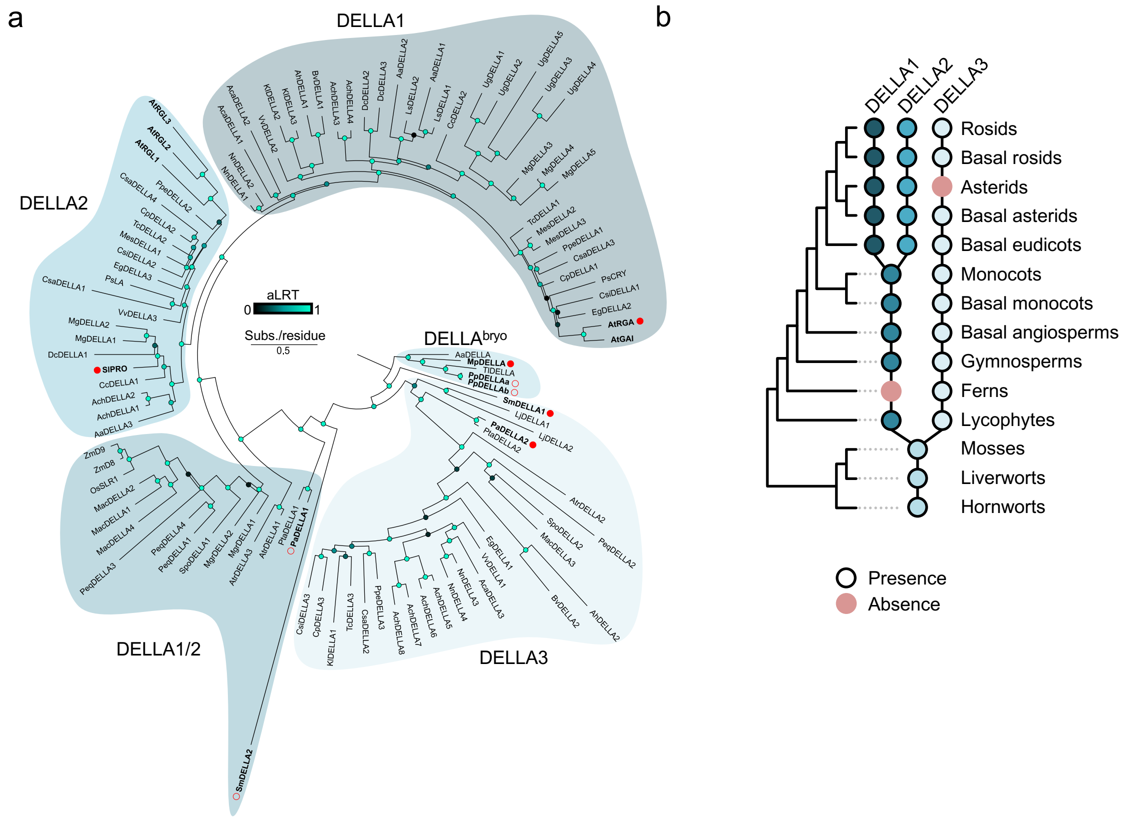


Figure 4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Mosses
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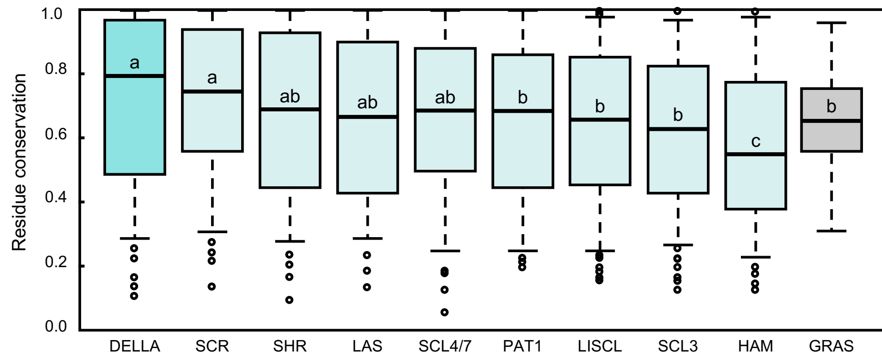
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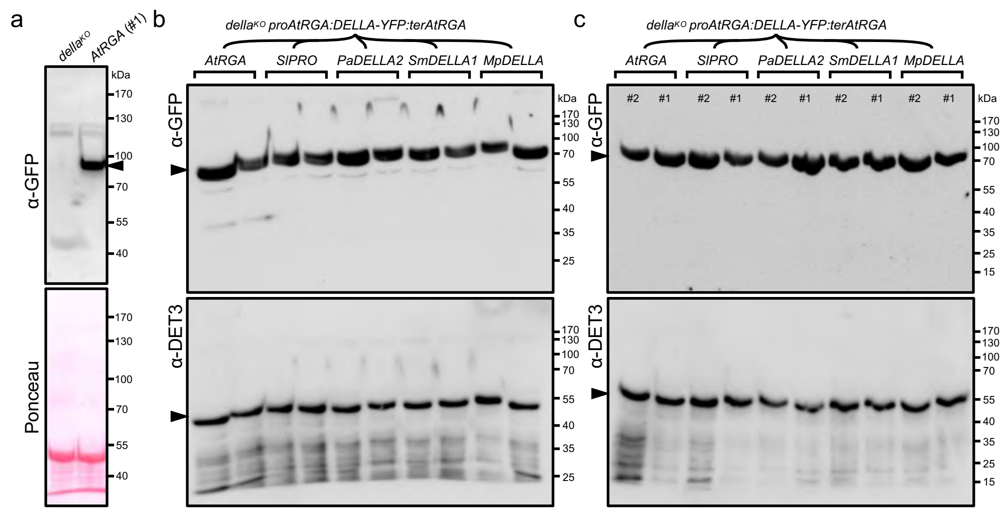
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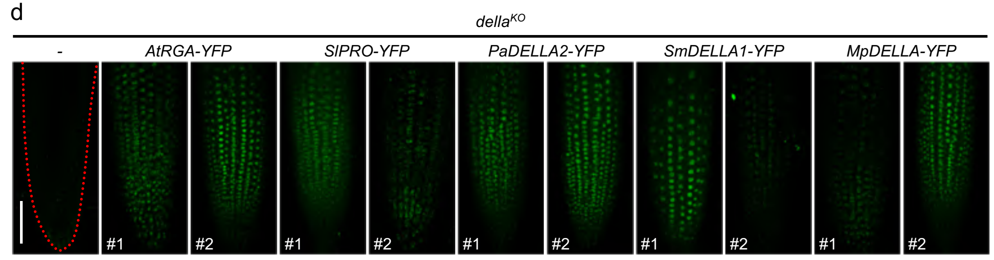
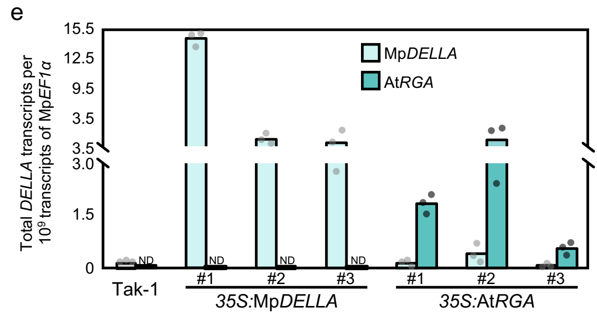
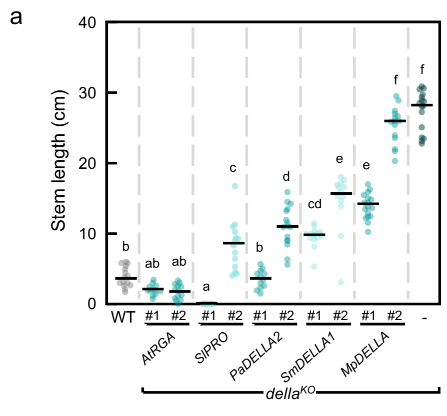
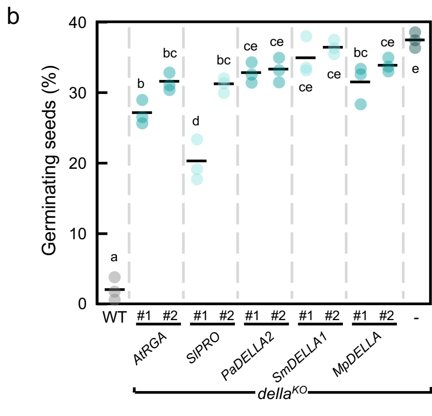
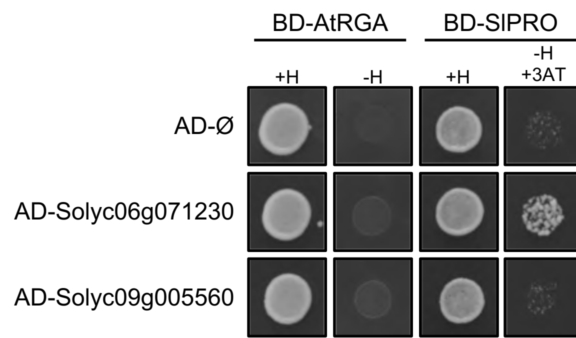
SlPRO
PaDELLA2
SmDELLA1
MpDELLA
#2
#1


d
dellaKO
-
AtRGA-YFP
SlPRO-YFP
PaDELLA2-YFP
SmDELLA1-YFP
MpDELLA-YFP
#1
#2


e
Total DELLA transcripts per 10⁹ transcripts of MpEF1α
MpDELLA
AtRGA
ND
Tak-1
35S:MpDELLA
35S:AtRGA
#1
#2
#3


a
Stem length (cm)
40
30
20
10
0
b
ab
ab
a
c
b
d
cd
e
e
f
f
WT
#1
#2
#1
#2
#1
#2
#1
#2
#1
#2
-
AtRGA
SlPRO
PaDELLA2
SmDELLA1
MpDELLA
della^KO


b
Germinating seeds (%)
40
30
20
10
0
a
b
bc
d
bc
ce
ce
ce
ce
bc
ce
e
WT
#1
#2
#1
#2
#1
#2
#1
#2
#1
#2
-
AtRGA
SlPRO
PaDELLA2
SmDELLA1
MpDELLA
della^KO


BD-AtRGA
BD-SlPRO
+H
-H
+H
-H
+3AT
AD-Ø
AD-Solyc06g071230
AD-Solyc09g005560