

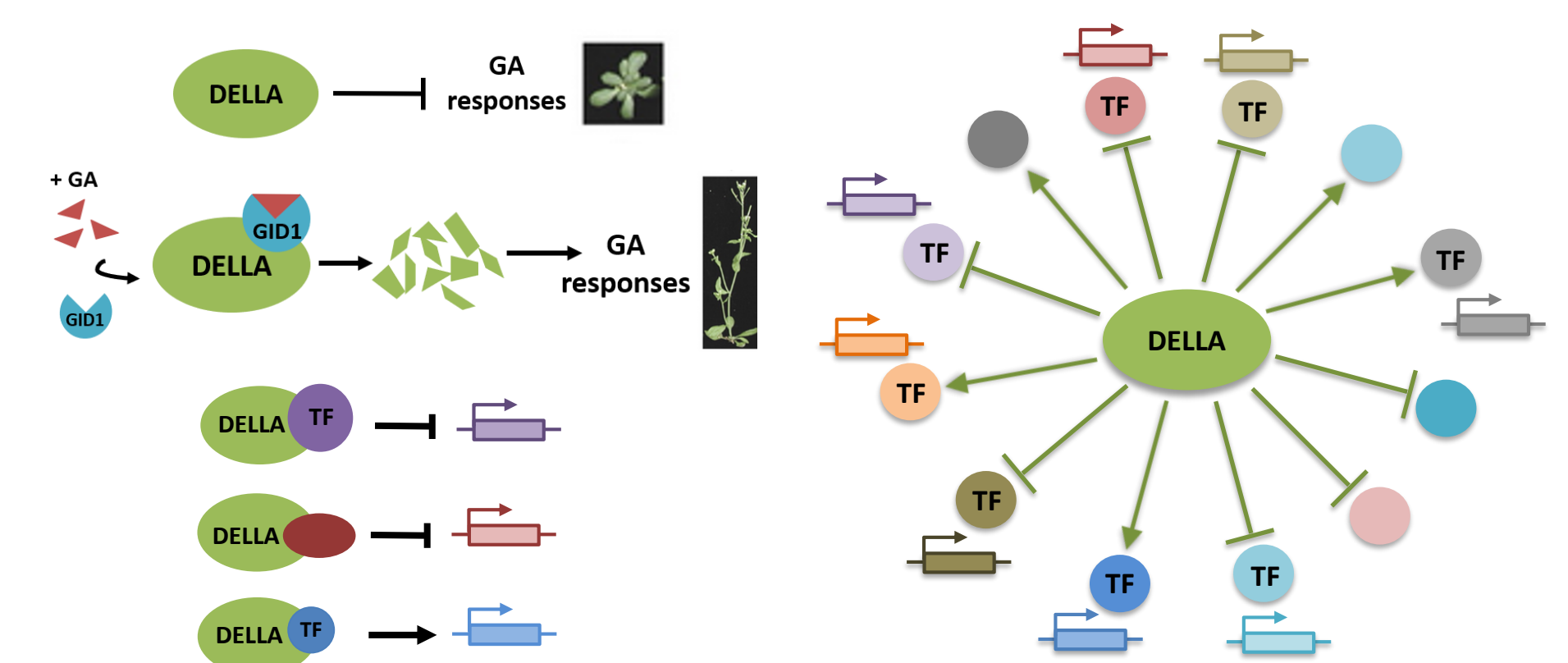
DELLA proteins acted as transcriptional hubs in the common ancestor of all land plants

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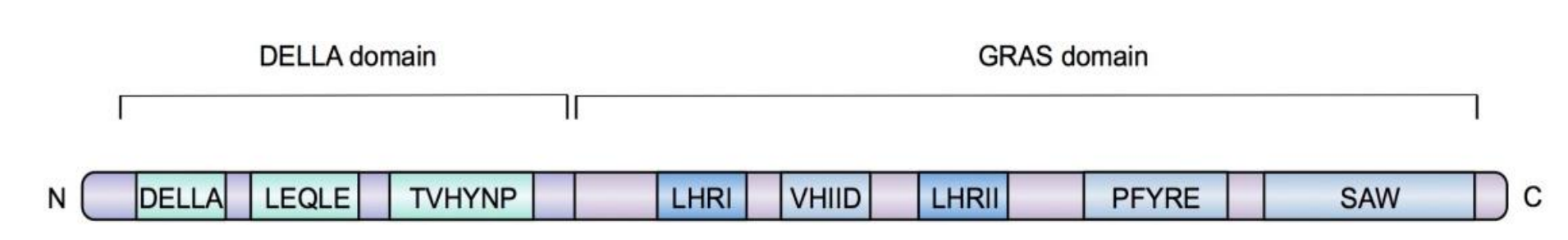


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1 DELLA proteins regulate multiple processes through interactions with TFs



DELLAs regulate a variety of plant developmental processes and responses to the environment. They are growth repressors that can be degraded in response to high gibberellin (GA) levels. These proteins modulate the transcription of multiple genes by interacting through their GRAS domain with more than 150 TFs and other regulators. They are able to trans activate gene expression, or reduce it by sequestration.

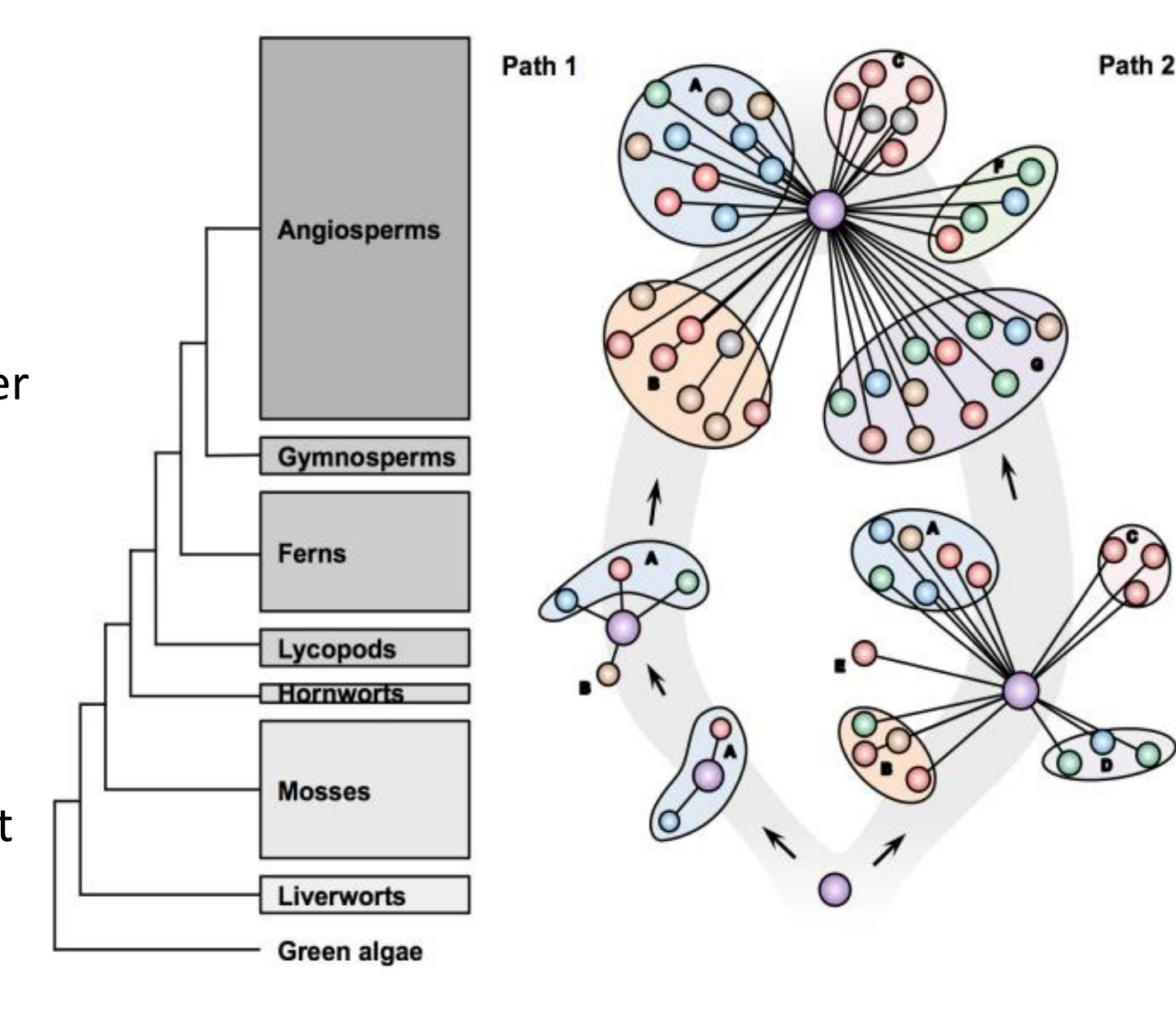


2 DELLA regulation appeared and developed throughout evolution

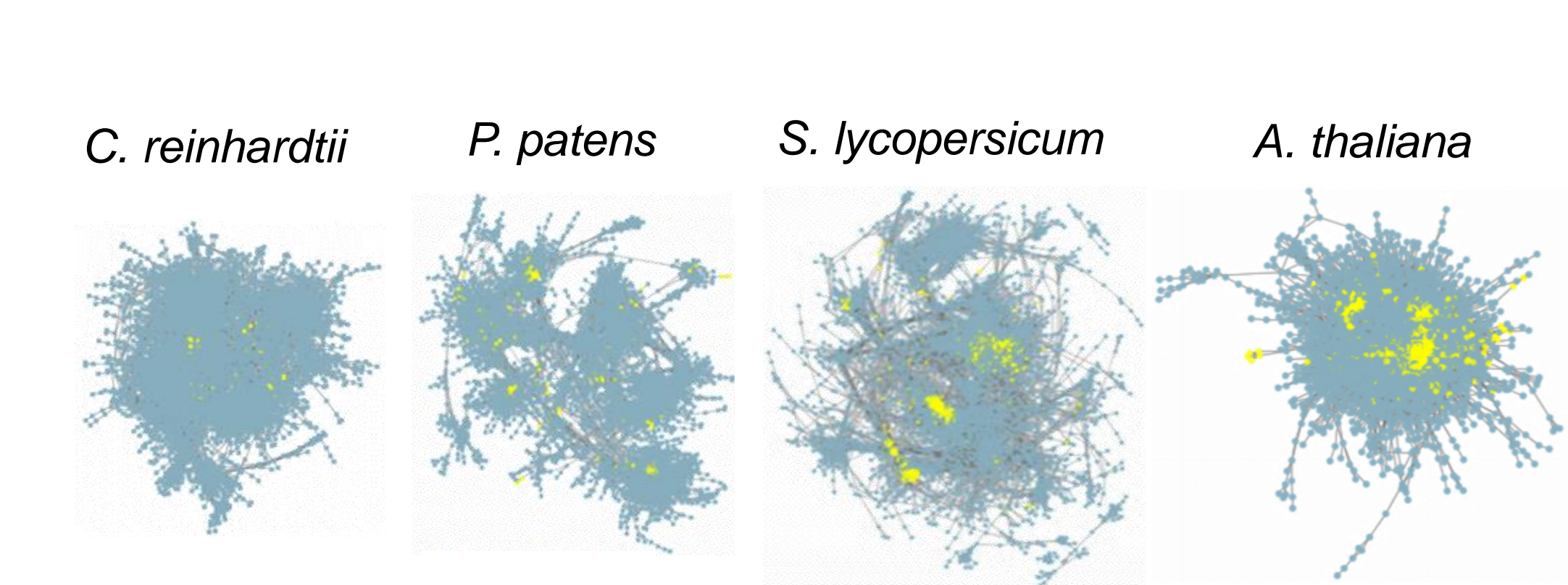
DELLA proteins belong to the GRAS family, which is exclusively present in plants. They can be found in most land plants, but only since the emergence of vascular plants are they part of GA signaling. Their influence in regulation of gene expression has been widely studied in higher plants, but the role of ancestral DELLAs and how they became hubs still remains unknown.

There are two main possible evolutionary paths:

1. The physical interactions and thus the control over transcriptional programs appeared gradually.
2. DELLAs suddenly gained promiscuity and became hubs at a certain point of evolution.

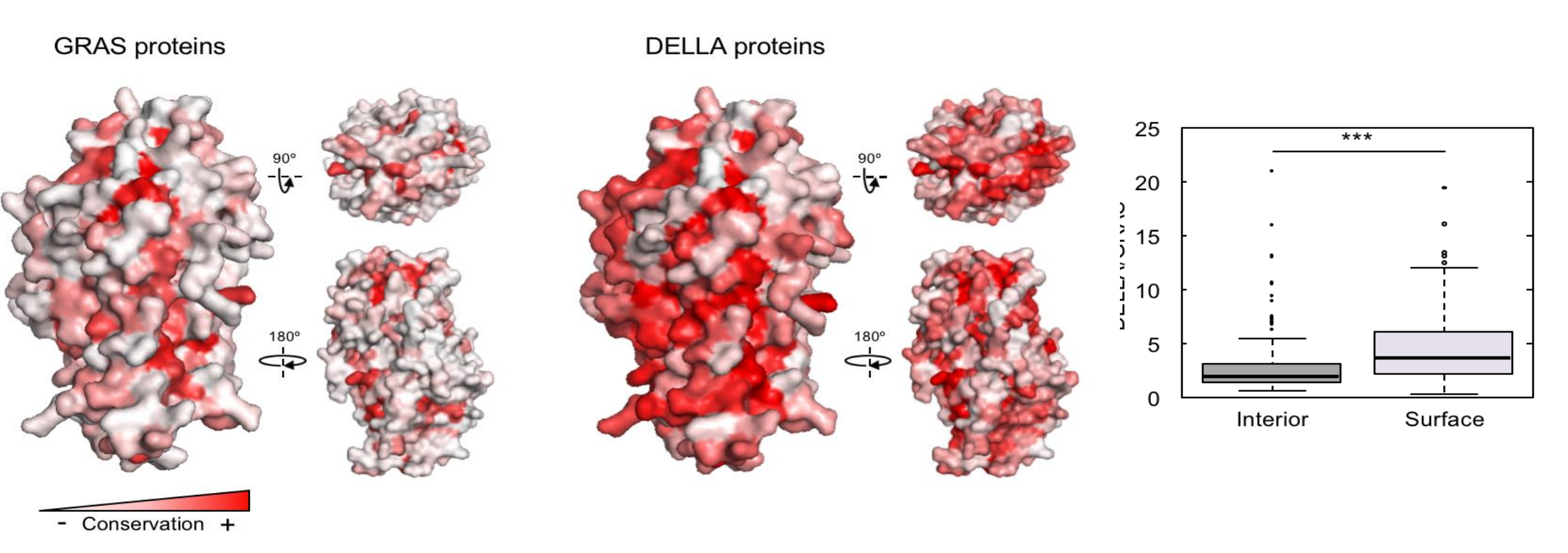


3 Gene co-expression networks show the importance of DELLAs' role on transcriptional landscapes



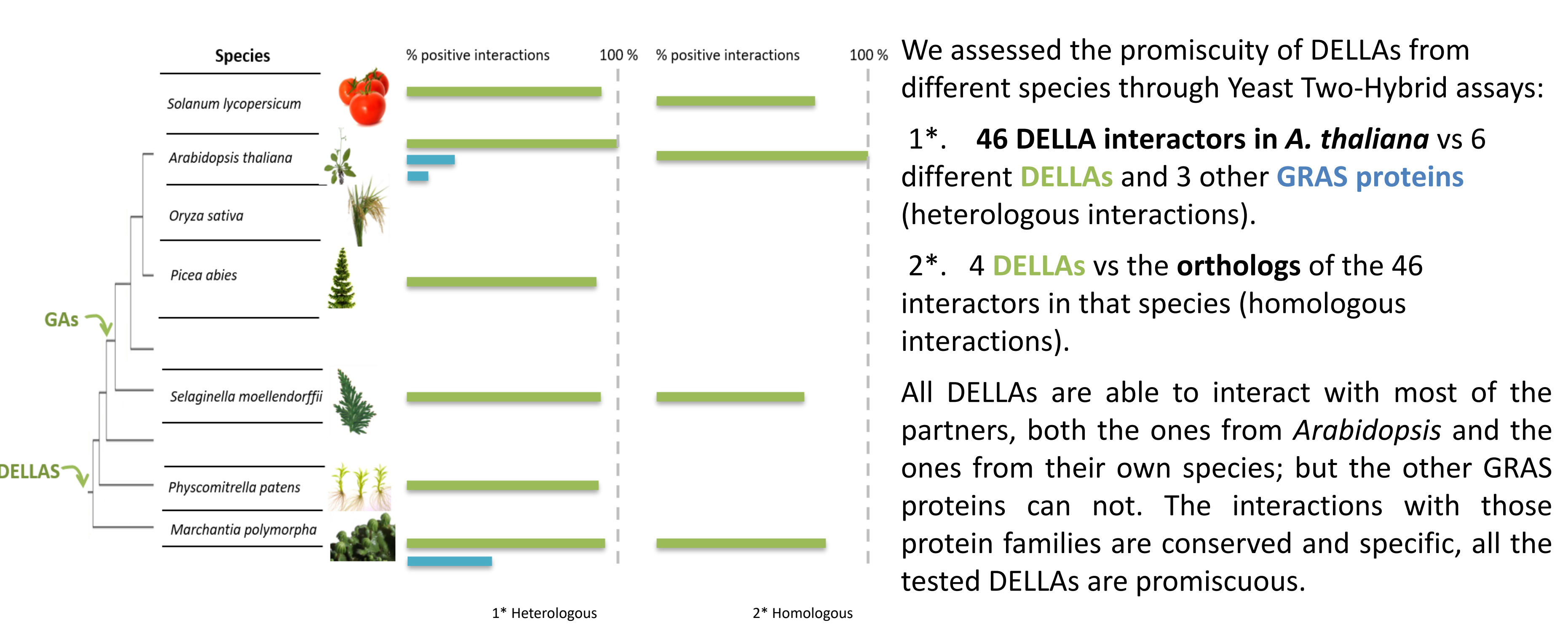
The analysis of gene co-expression networks and DELLA-related subnetworks (in yellow) of 4 different species across the green lineage reveals an increase in the coordination between otherwise independent processes in plants with DELLAs, which is even more apparent in plants displaying GA-dependent DELLA regulation; and a possible ancestral role for these proteins: stress response.

4 The surface of the DELLA GRAS domain is highly conserved



The surface of the GRAS domain of DELLA proteins (C-terminal) displays higher conservation than the rest of GRAS proteins. This domain is responsible for the interaction with other proteins, and the high conservation of its surface may be the key for maintaining the DELLA interactome through evolution.

5 Evolution of the DELLA interactome: has the promiscuity of DELLAs increased during evolution?



We assessed the promiscuity of DELLAs from different species through Yeast Two-Hybrid assays:

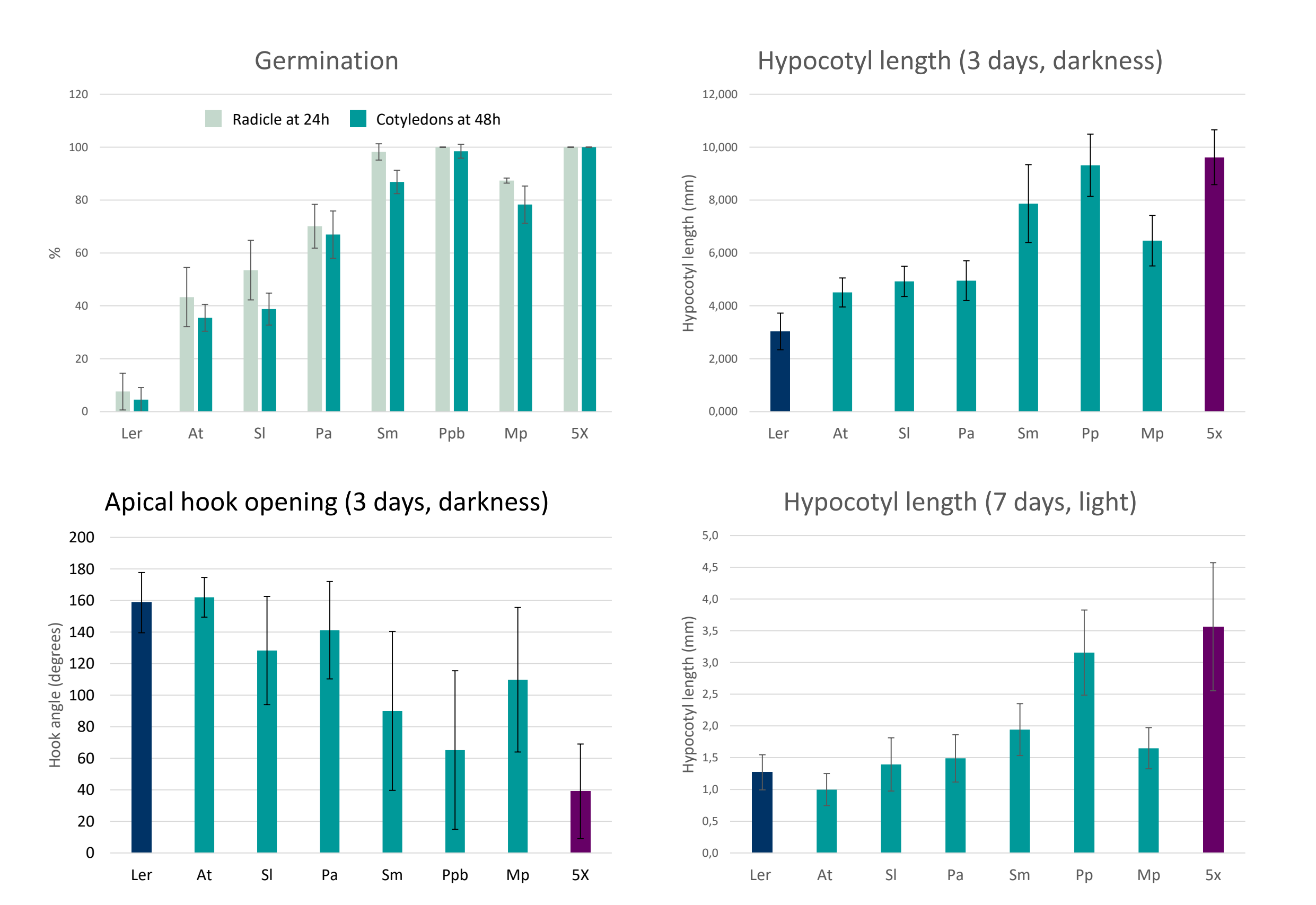
1*. **46 DELLA interactors in *A. thaliana*** vs 6 different **DELLAs** and 3 other **GRAS proteins** (heterologous interactions).

2*. **4 DELLAs** vs the **orthologs** of the 46 interactors in that species (homologous interactions).

All DELLAs are able to interact with most of the partners, both the ones from *Arabidopsis* and the ones from their own species; but the other GRAS proteins can not. The interactions with those protein families are conserved and specific, all the tested DELLAs are promiscuous.

6 Do DELLAs have the intrinsic ability to act as hubs? Assessing the functional conservation of DELLAs across species through complementation assays

Heterologous expression of DELLA proteins from six different species in *A. thaliana* *della* pentuple mutant (5x) shows clear differences in their performances. DELLAs from *A. thaliana* (At), *S. lycopersicum* (Sl), *P. abies* (Pa), *S. moellendorffii* (Sm) and *M. polymorpha* (Mp) recover the wild type (Landsberg, Ler) phenotype in different degrees. DELLA activity *in vivo* seems to be directly correlated with evolutionary distance to *A. thaliana*, except for Mp. Although all these proteins have similar interactomes, they do not exhibit the same ability to restore DELLA-associated phenotypes. Current protein affinity assays may uncover a correlation between interaction strength and complementation degree, and future transcriptomic comparisons may give insight into each DELLA's regulated genes.



7 Conclusions

- Since DELLAs appeared, they acquired a great influence in the transcriptional landscape, acting as hubs and clustering the expression of certain genes, and their effect was increased since they began to participate in GA signaling.
- All the studied DELLAs are highly promiscuous, and they share the ability of interacting with certain protein families.
- This interactome is specific for DELLAs, and not for all GRAS proteins. This fact may be due to their higher surface conservation.
- The interactions are conserved, most of them occur independently of the species to which the DELLA and the partner belong.
- When expressed in the *Arabidopsis 5xdellaKO* mutant, DELLAs from different species show diverse performances that do not correlate perfectly with their interactome.
- DELLAs are probably hubs in all land plants, and they may control stress response in the most basal species.