
# Evolution and Function of a Retrograde Signal in Plant Abiotic Stress Response

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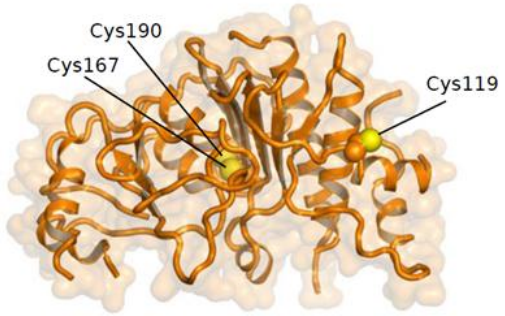
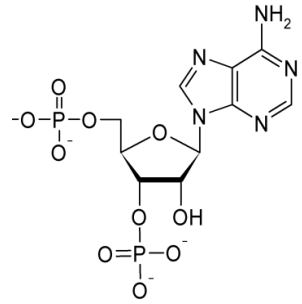
Adelaide University




# Function of SAL1/PAP retrograde signaling in Arabidopsis




- AtSAL1 (ALTERED EXPRESSION OF APX2 8, ALX8, FRY1), a chloroplast phosphatase, encodes a bifunctional protein that has 3‘(2’),5‘-bisphosphate nucleotidase （二磷酸核苷酸酶） and inositol polyphosphate 1-phosphatase （多磷酸肌醇磷酸酶）activities.

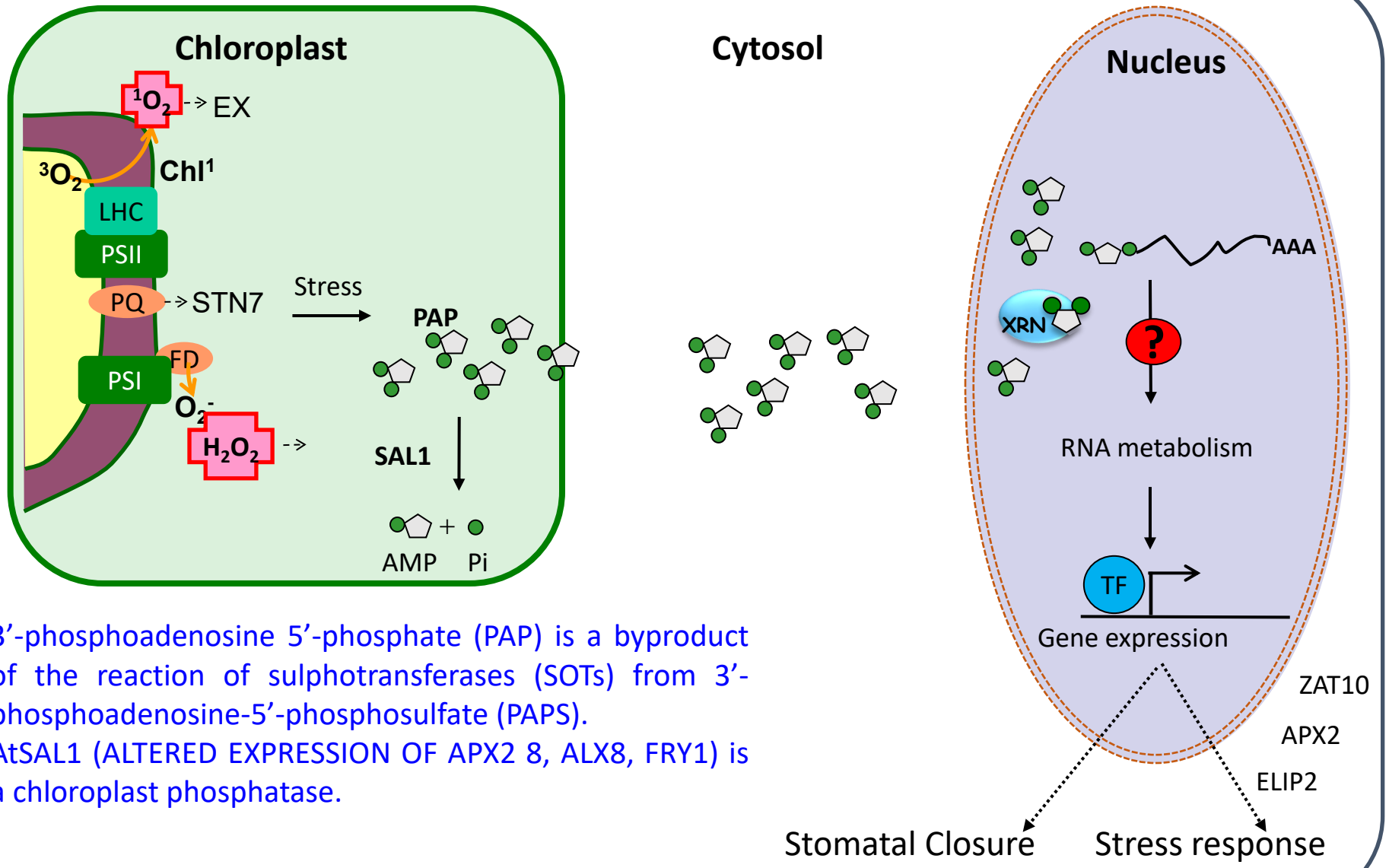
- AtSAL1 is involved in the response to cold, salinity and drought (negative regulator of drought tolerance).

- 3’-phosphoadenosine 5’-phosphate (PAP 磷酸腺苷磷酸) is a byproduct of the reaction of sulphotransferases (SOTs, 磺基转移酶) from 3’-phosphoadenosine-5’-phosphosulfate (PAPS).

- AtSAL1 regulates the signal PAP via dephosphorylation. PAP can move between the chloroplast and nucleus, accumulates in response to chloroplast oxidative stress, including high light and drought.

Is SAL1/PAP retrograde signal conserved for abiotic stress tolerance in green plants?

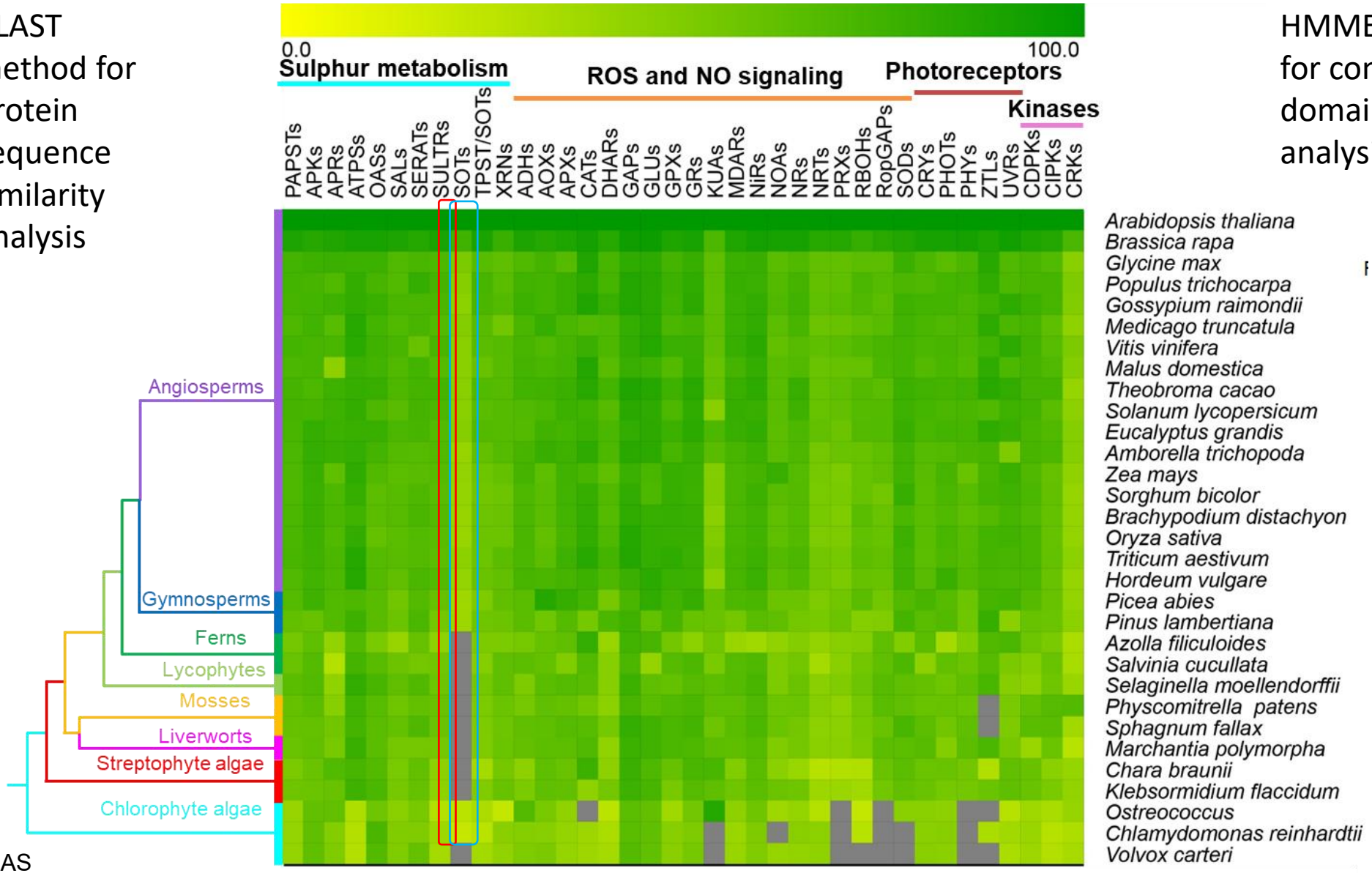
# PAP is a plant retrograde signal




3’-phosphoadenosine 5’-phosphate (PAP) is a byproduct of the reaction of sulphotransferases (SOTs) from 3’-phosphoadenosine-5’-phosphosulfate (PAPS).
AtSAL1 (ALTERED EXPRESSION OF APX2 8, ALX8, FRY1) is a chloroplast phosphatase.

# Similarity heatmap for the evolution of key families in plant and algal species

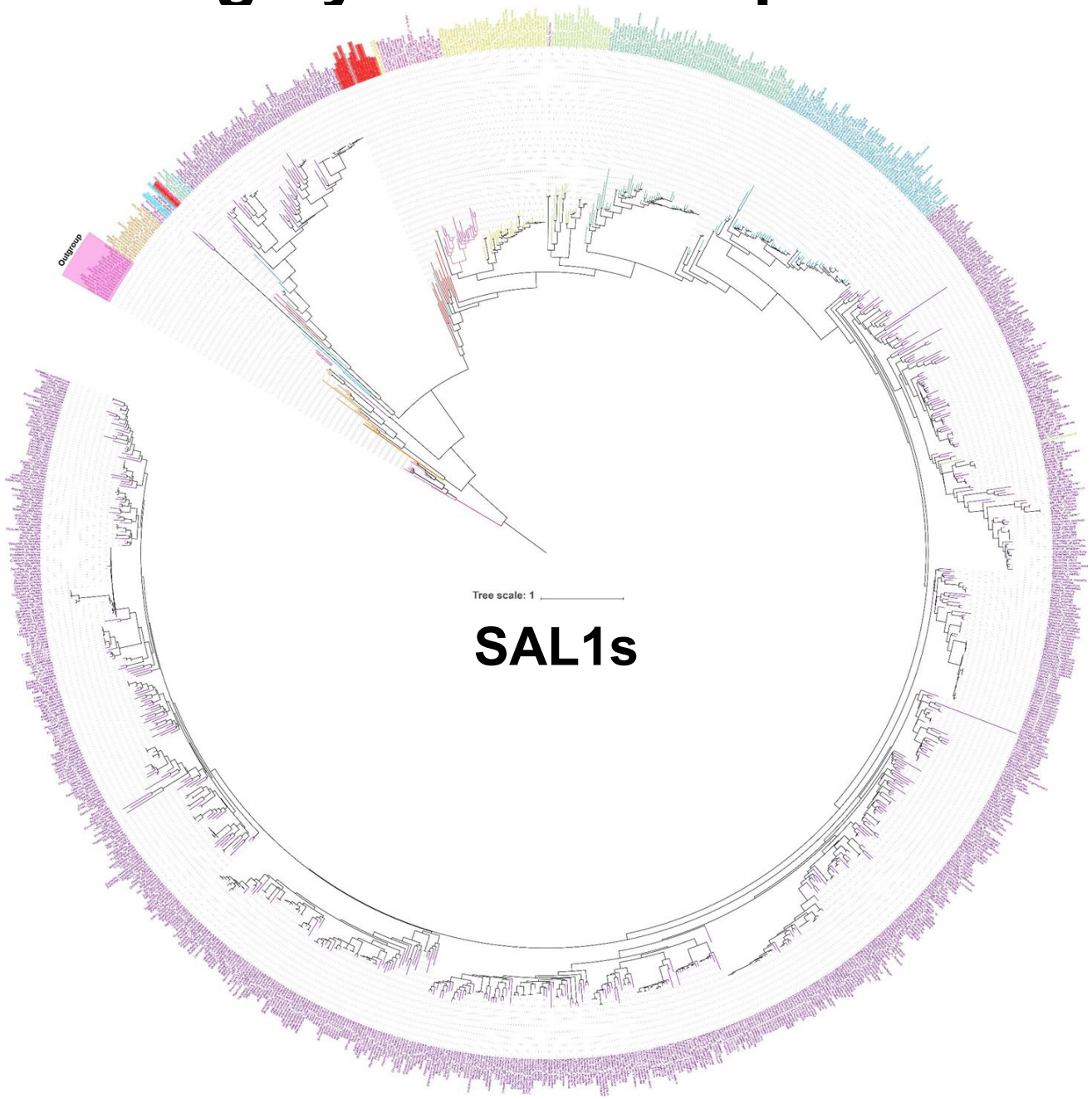
BLAST method for protein sequence similarity analysis




HMMER method for conserved domain similarity analysis

Zhao et al., 2019 PNAS

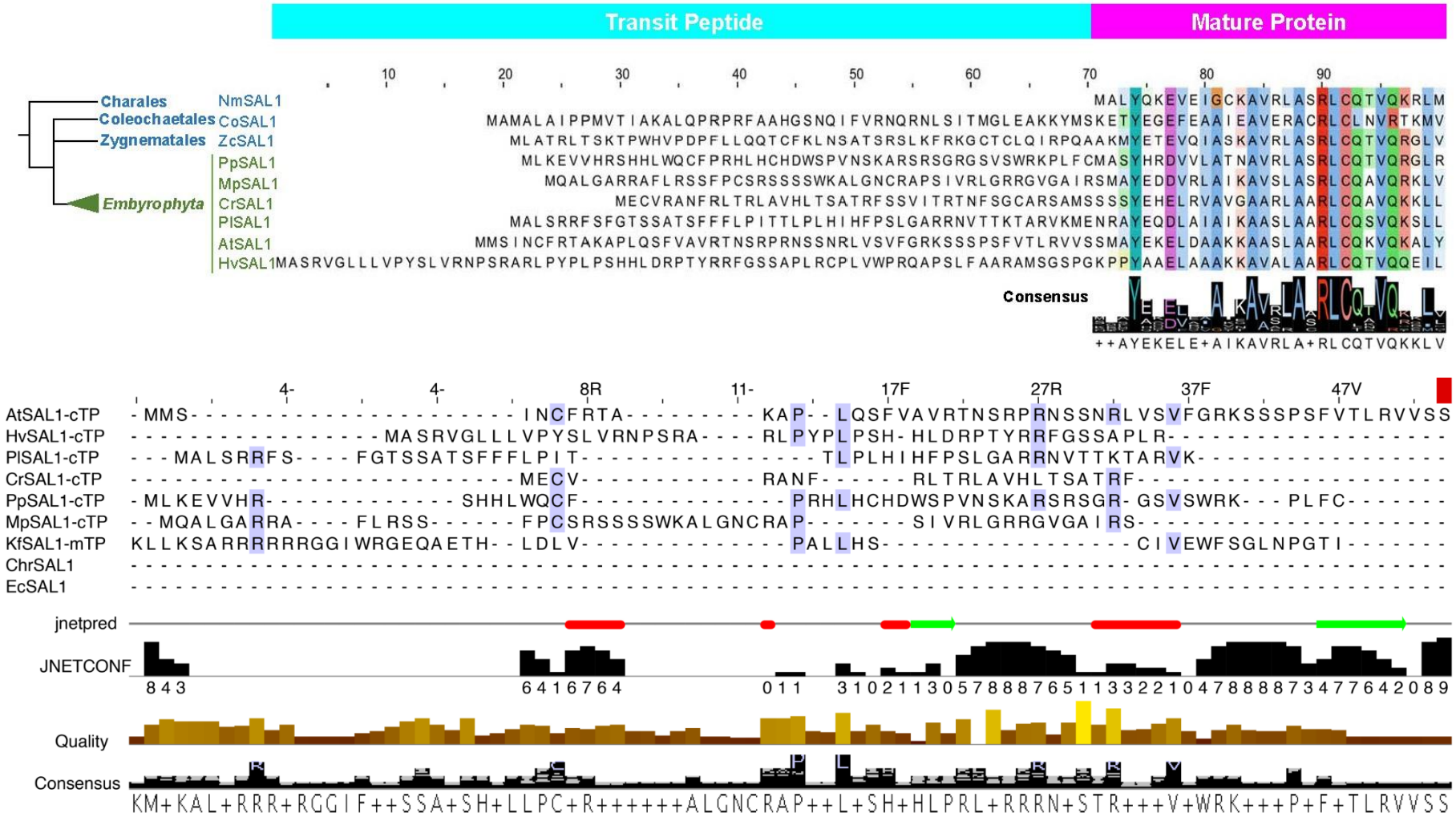
# SAL1 is a highly conserved protein in plants and algae



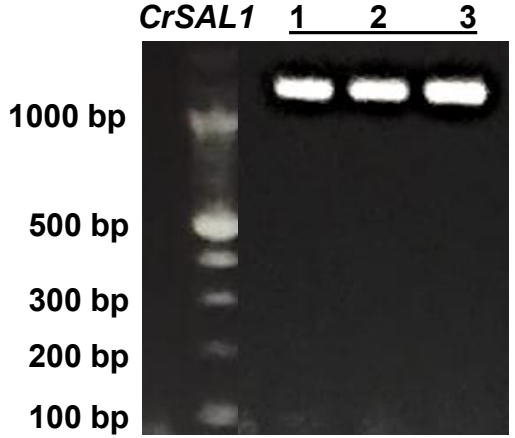

- Angiosperms
- Gymnosperms
- Ferns
- Lycophytes
- Mosses
- Liverworts
- Streptophyte algae
- Chlorophyte algae
- Rhodophyta
- Chromista

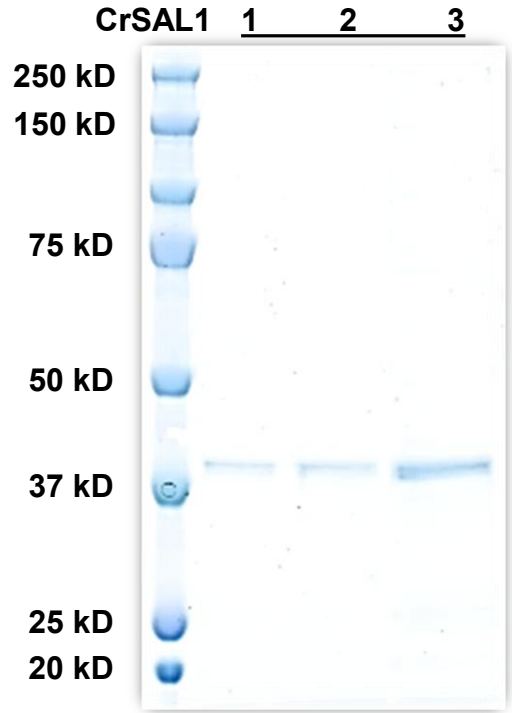
**1KP database**

# SAL1 transit peptides for targeting chloroplasts are originated from Streptophyte algae and conserved in land plants



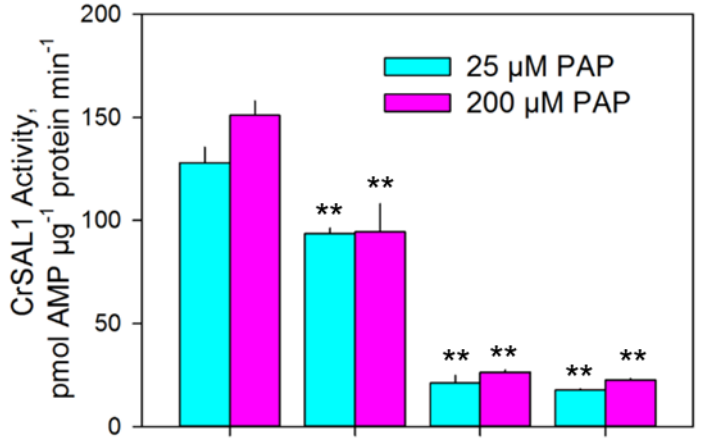
# SAL1 biochemical function is conserved in fern and moss

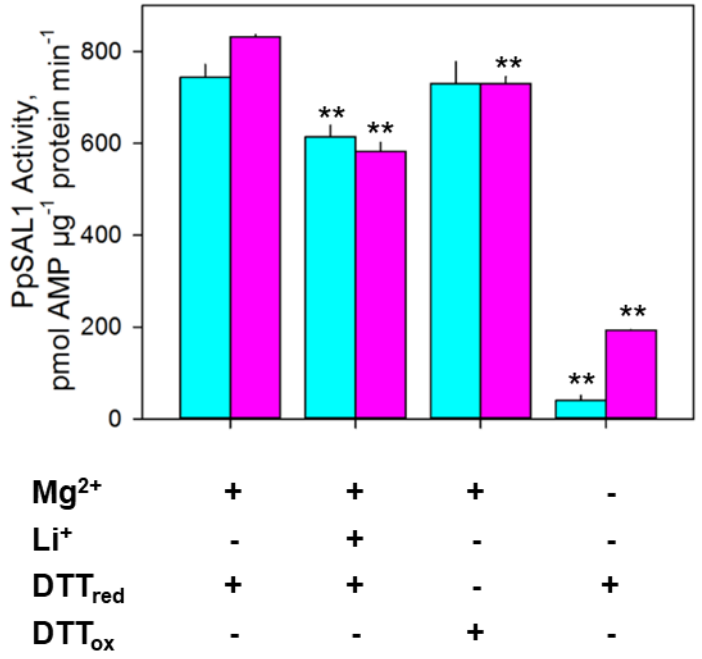


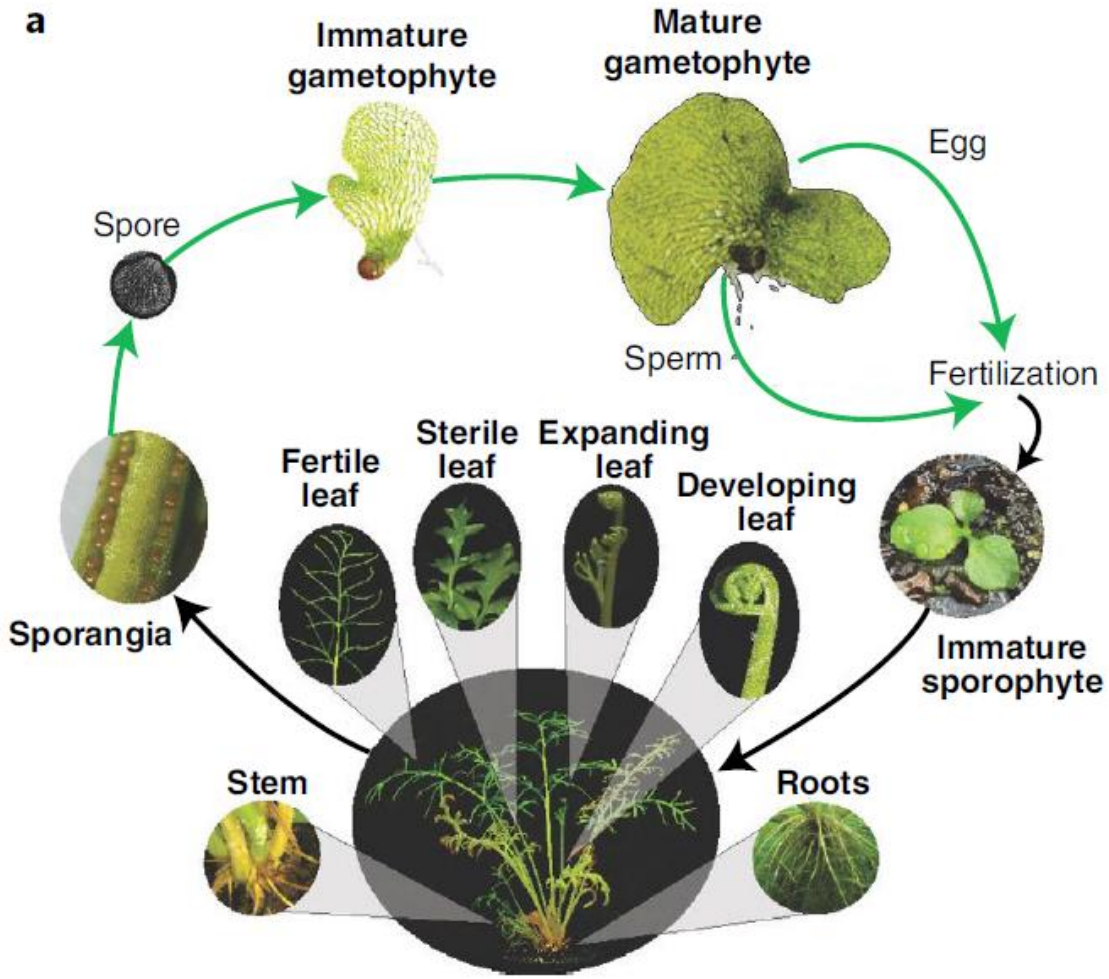

***Ceratopteris richardii***
水蕨

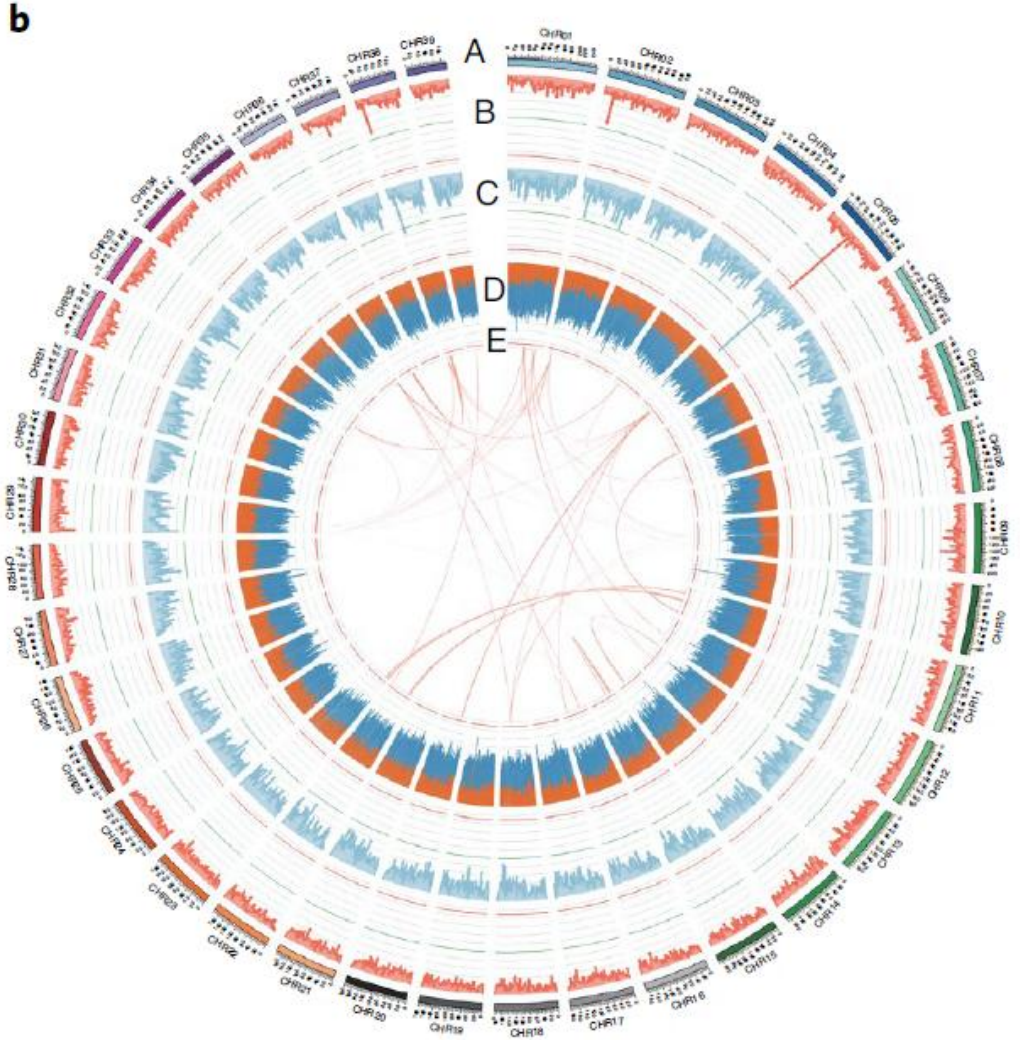
***Physcomitrella patens***
*小立碗藓*



# *Ceratopteris richardii* life cycle and genome assembly

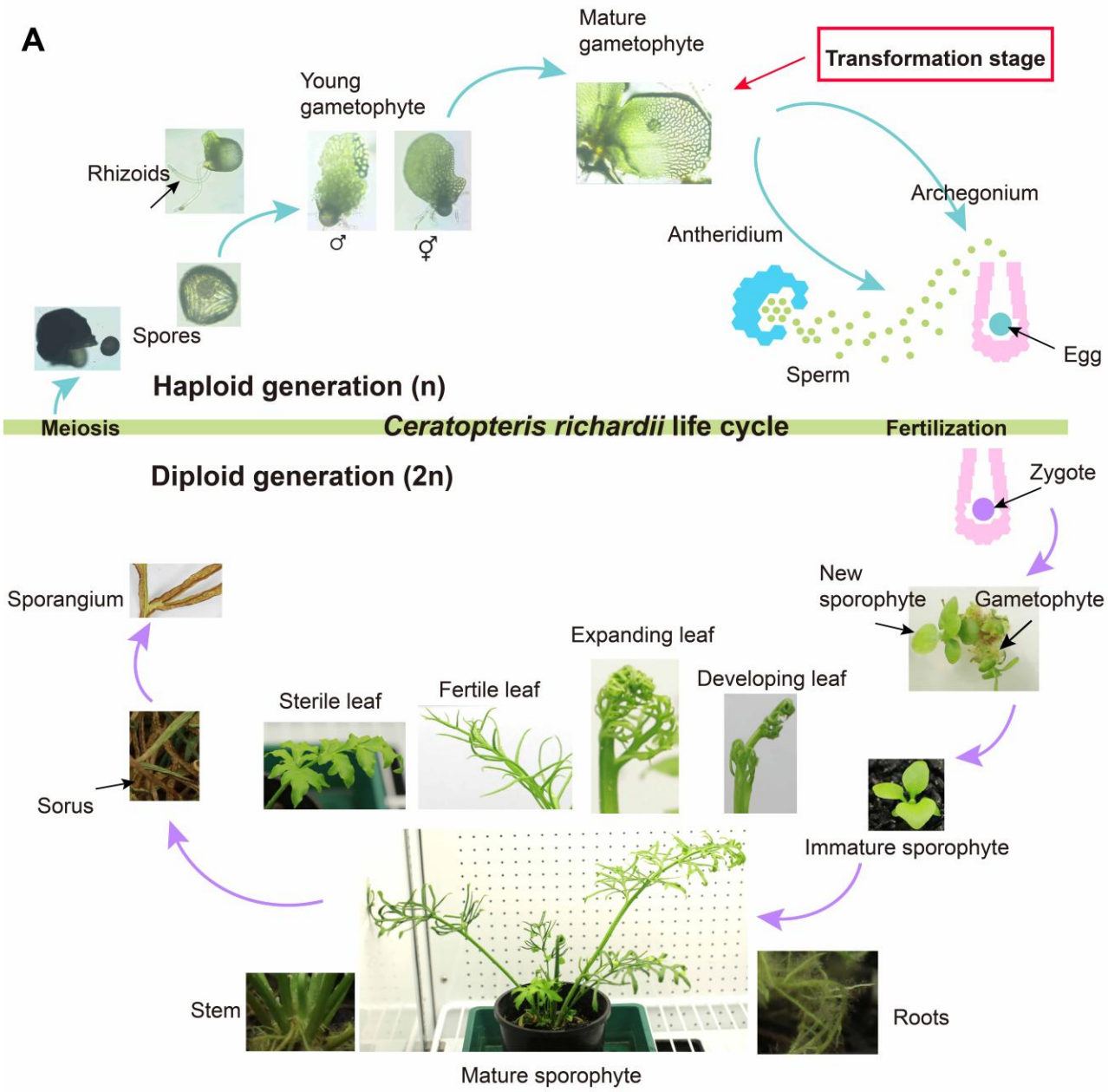


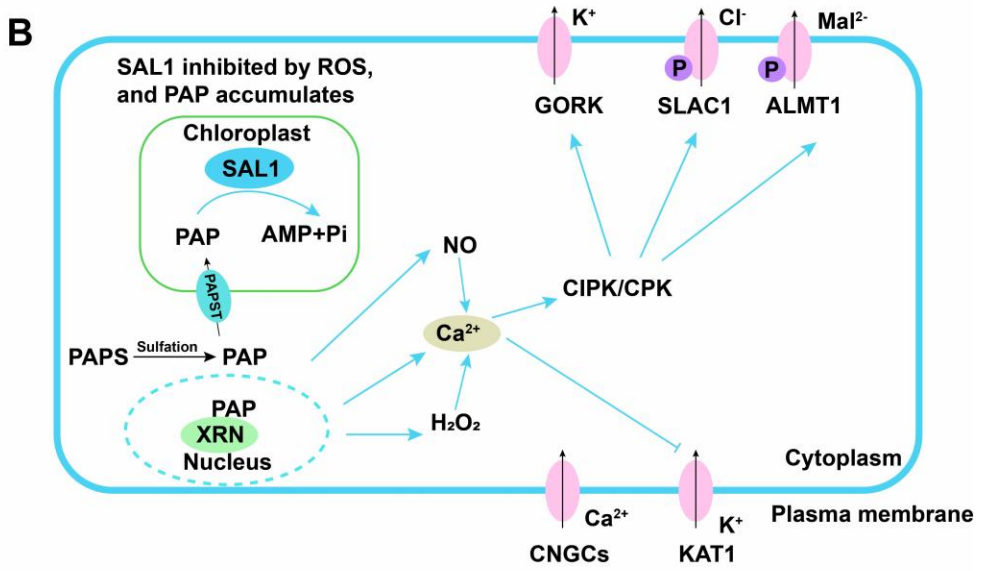



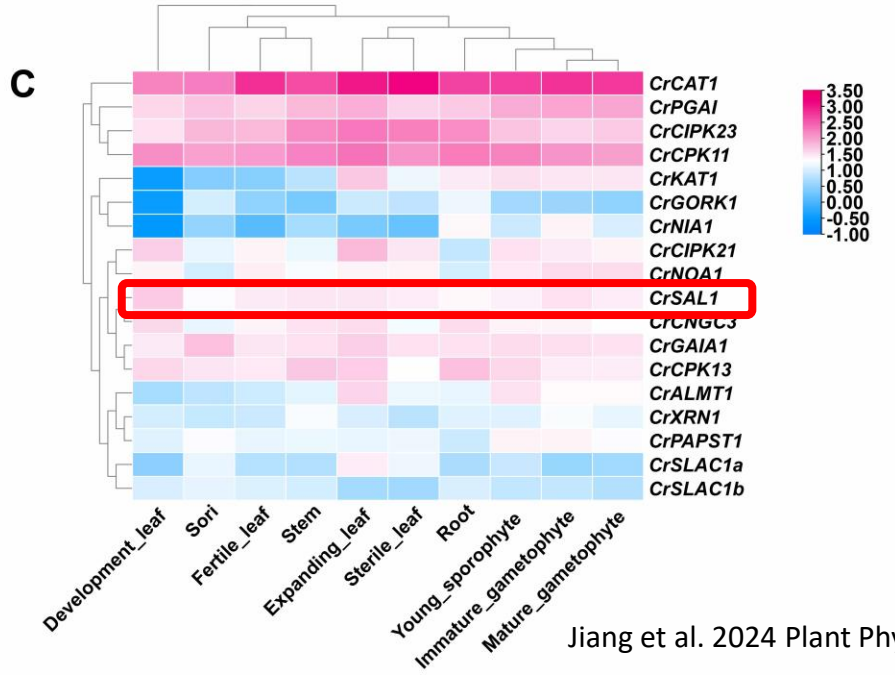

**a**, Life cycle of *Ceratopteris*. **b**, Genome assembly of *Ceratopteris* with: (A) chromosomes, (B) gene density in a 3-Mb sliding window, maximum value of 139; (C) mRNA expression density in a 3-Mb sliding window, maximum value of 170; (D) long terminal repeat retrotransposon density in a 3-Mb sliding window, orange and blue bands represent Ty3 and Ty1 LTRs, respectively, maximum value of 970; and (E) intragenomic syntenic regions of ten or more genes.

Marchant et al. 2022 Nature Plants

# Key stage for *Ceratopteris* transformation – *CrSAL1*

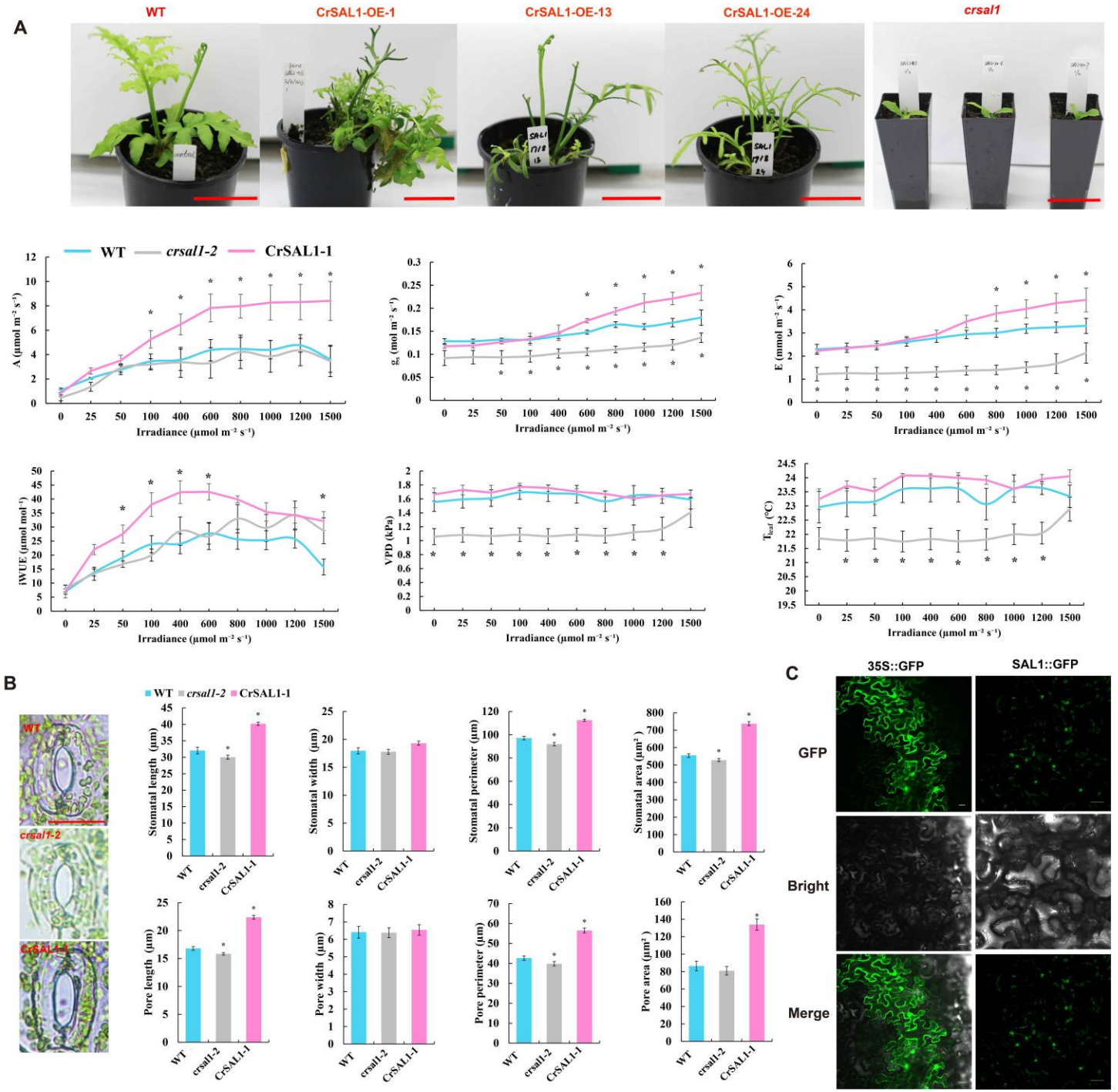


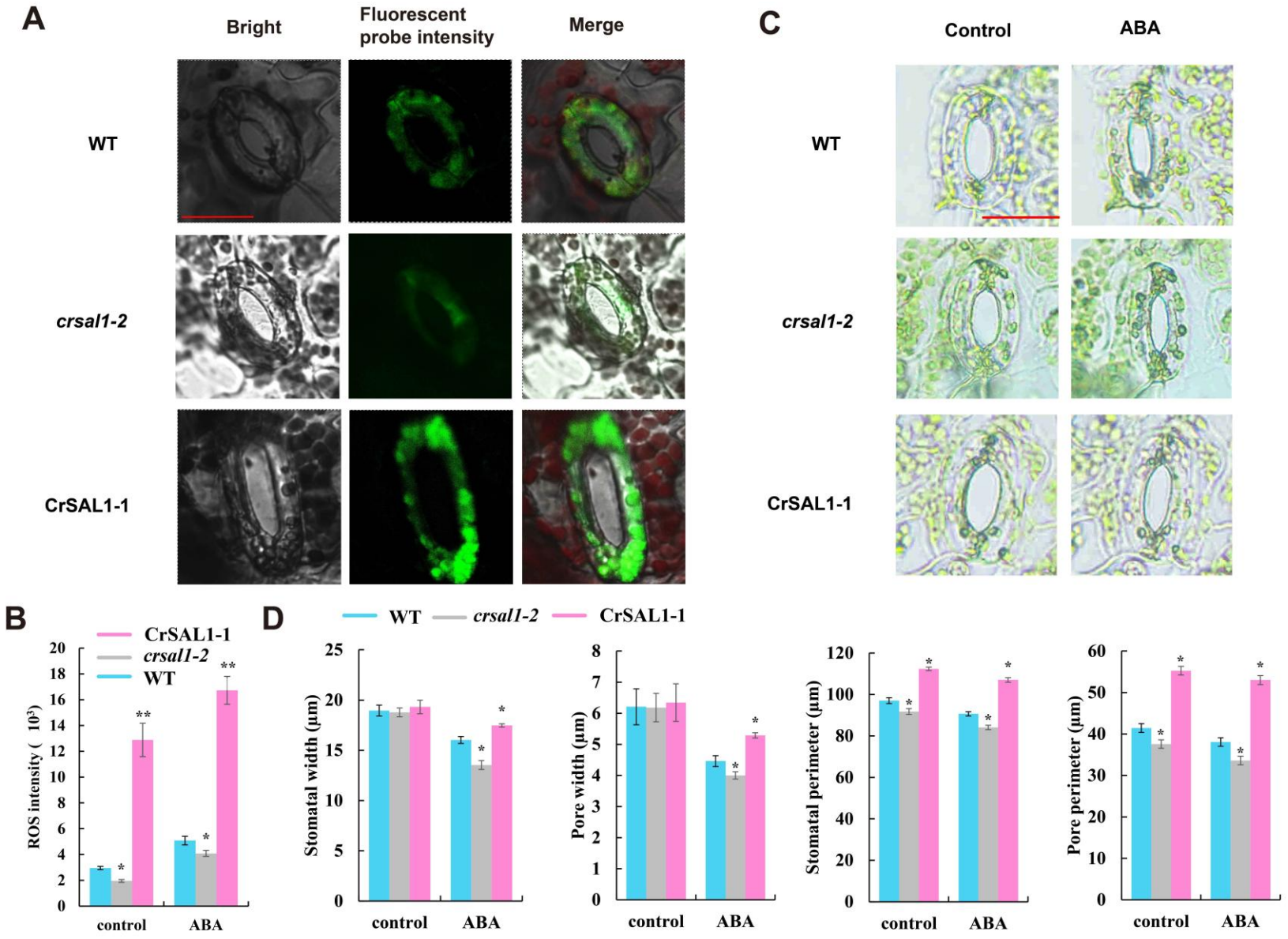




Jiang et al. 2024 Plant Physiology

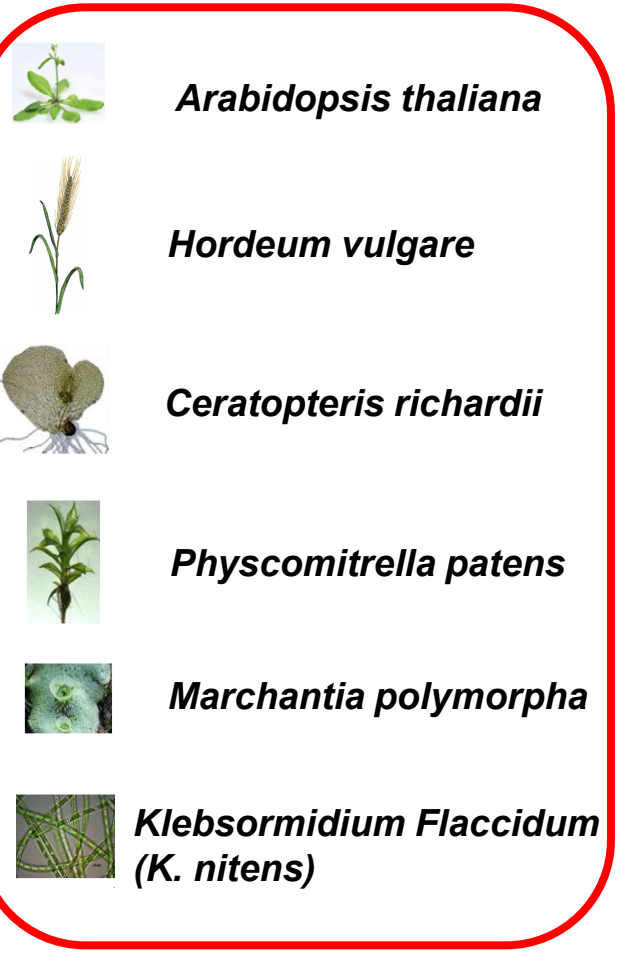
# Effects of gene editing and overexpression *CrSAL1* on photosynthesis and stomata of fern plants

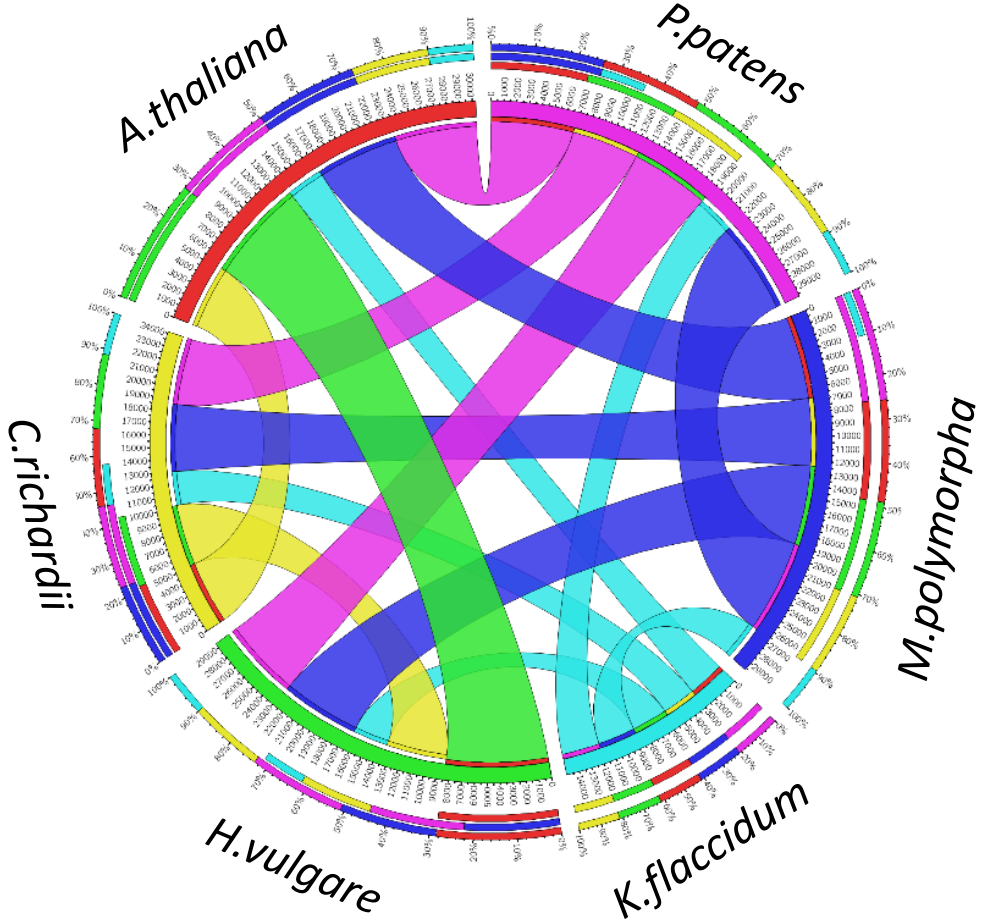


# Effects of gene editing and overexpression *CrSAL1* on ROS and ABA response of fern plants



# Are retrograde signals conserved at omics levels?

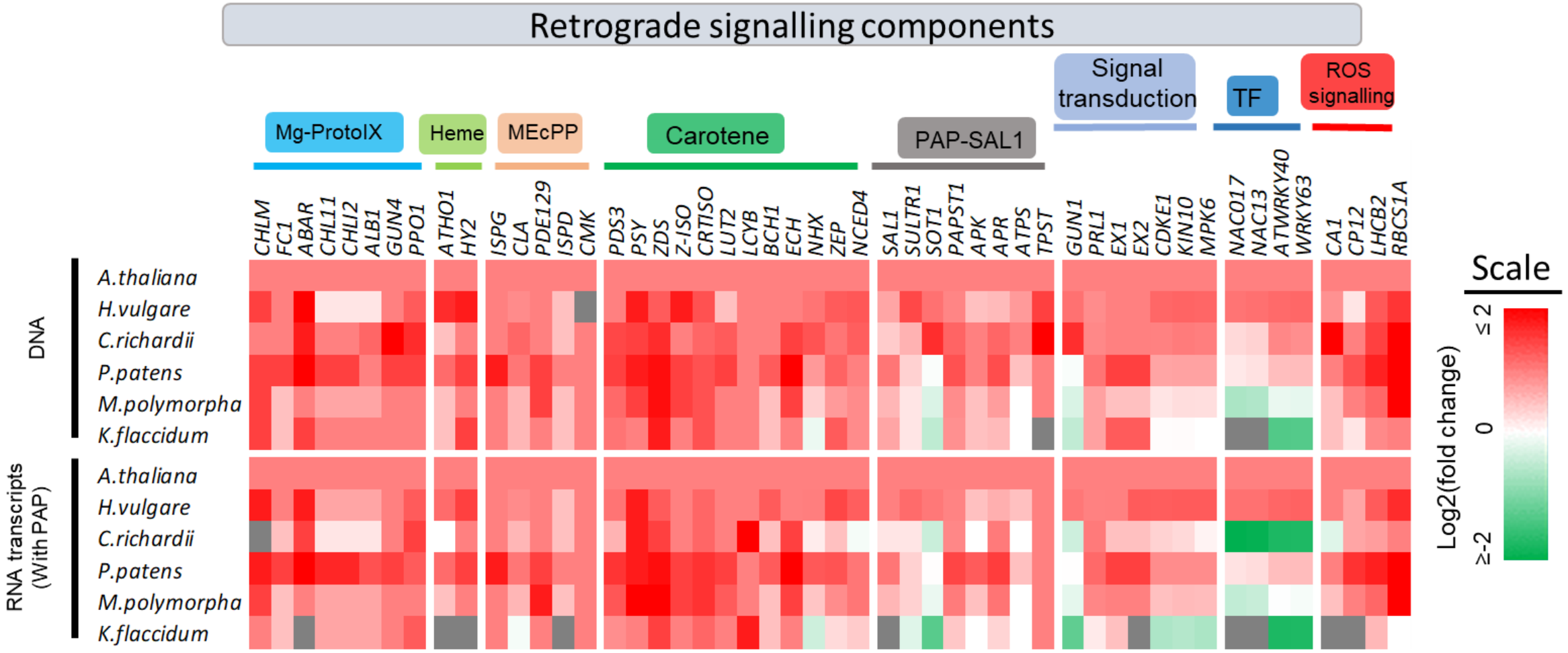



Cirsos plot indicates *K. flaccidum* shares the least number of genes with other investigated plant species, while angiosperm species Arabidopsis and Barley are overlapped with a large group of genes.

Wang, Zhao, et al. unpublished

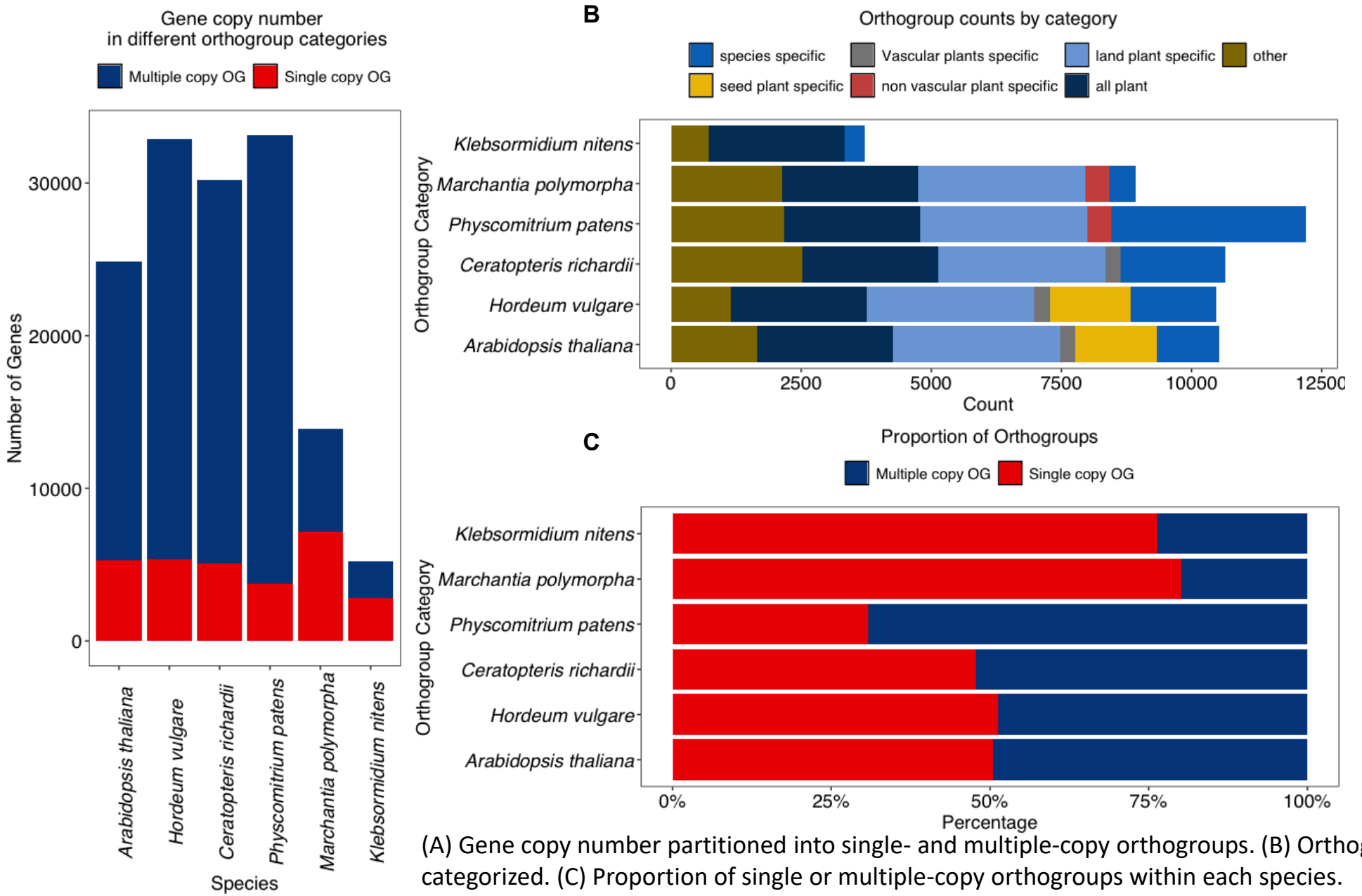
# Conserved retrograde signalling genes maintain responses to exogenously applied PAP




Representative retrograde signalling pathway elements were detectable in the RNA transcripts while some representative genes were still missing during the evolution

Wang, Zhao et al. unpublished

# DEGs of orthogroup distribution in 6 green plant species after PAP treatment

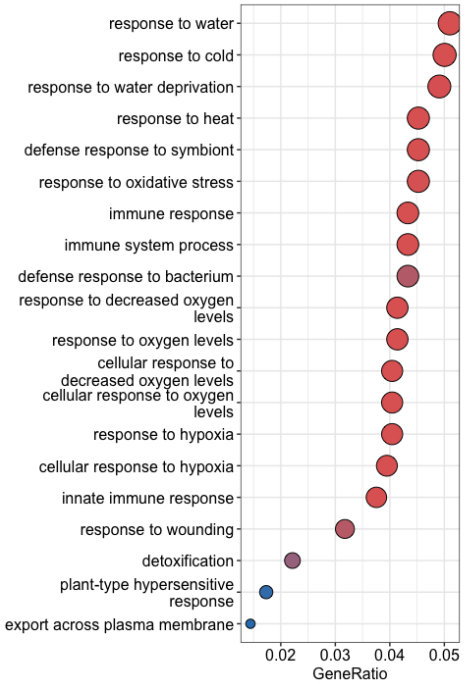


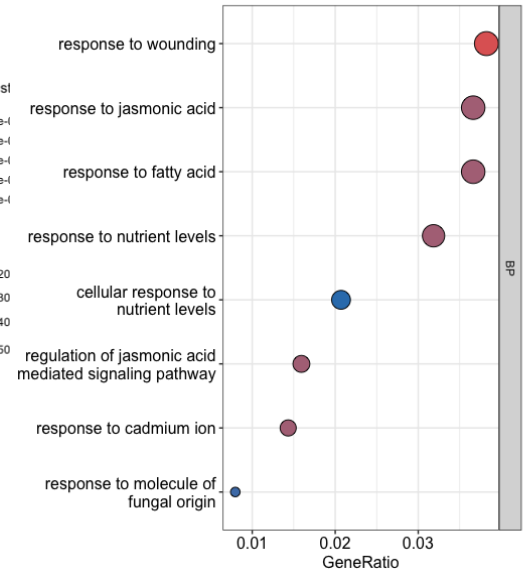

(A) Gene copy number partitioned into single- and multiple-copy orthogroups. (B) Orthogroup categorized. (C) Proportion of single or multiple-copy orthogroups within each species.

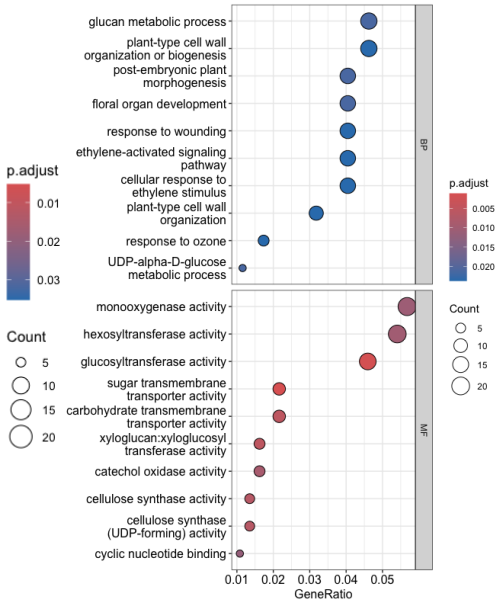
# GO enrichment of PAP-responsive DEGs in land plants

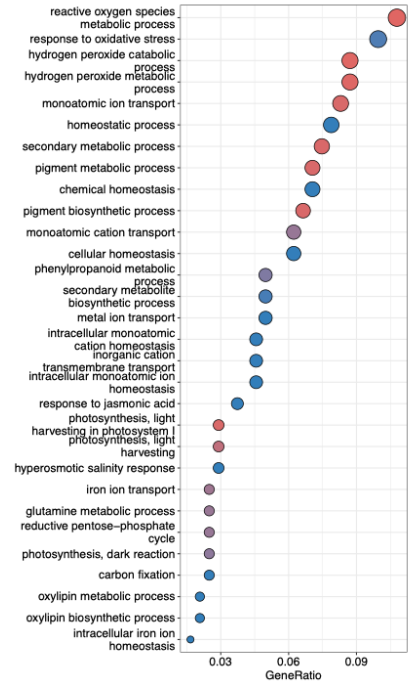


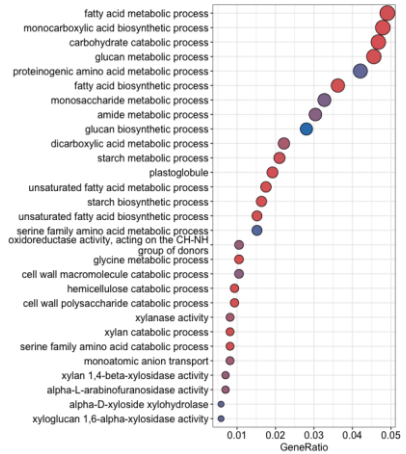




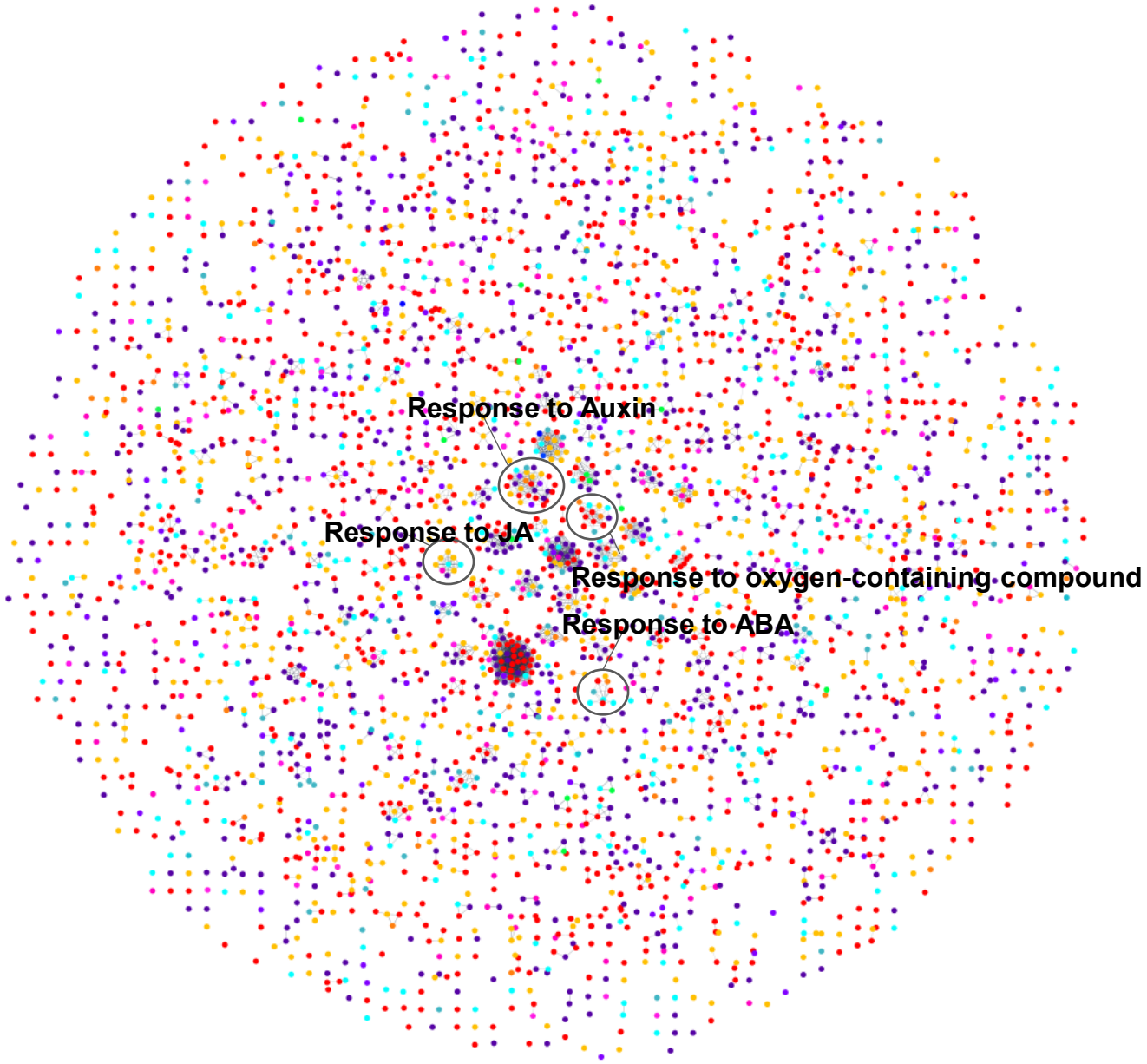

**Shared GO terms:**

Ion transport

Response to wounding

Plant hormones

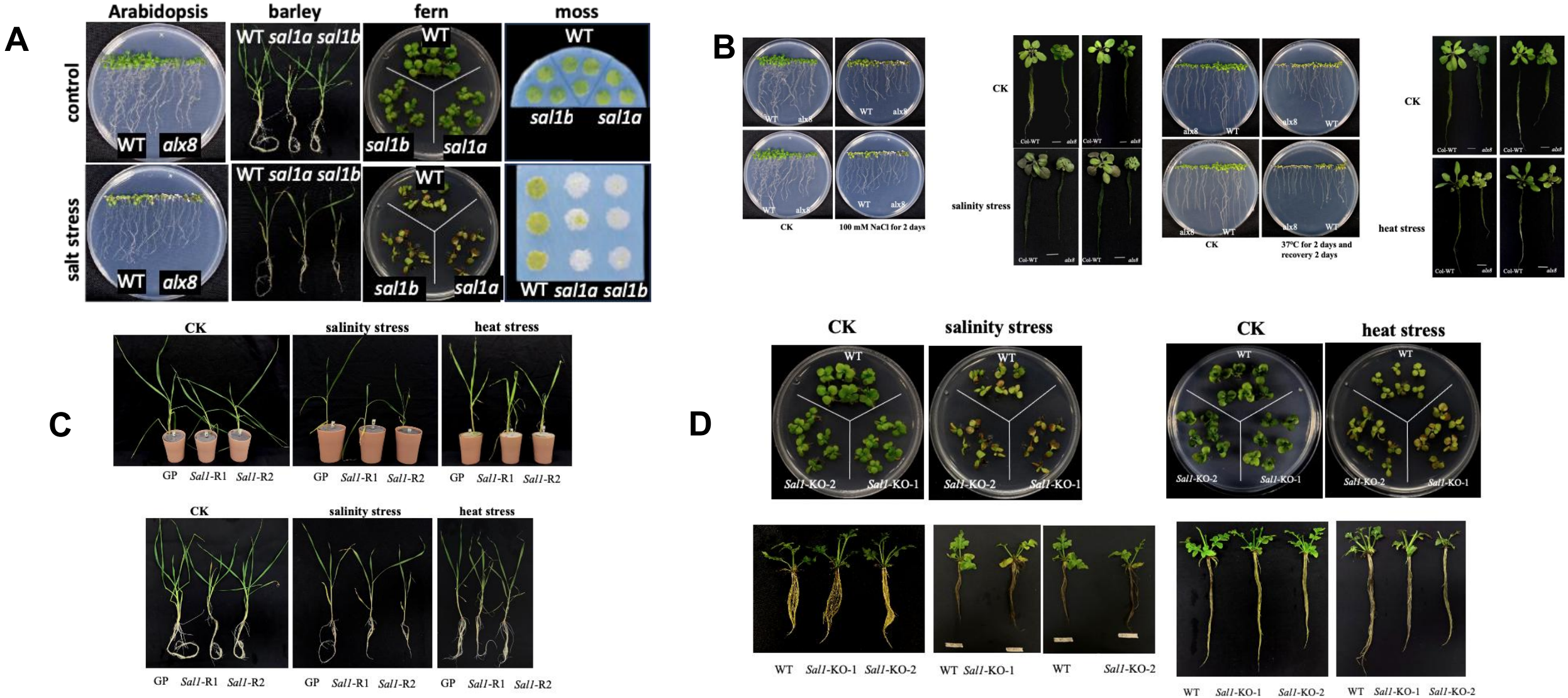
# STRING network of PAP-responsive genes in 6 species




- Shared in Embryophyta
- Angiosperms
- Non-angiosperms
- *Arabidopsis thaliana*
- *Ceratopteris richardii*
- *Physcomitrium patens*
- *Marchantia polymorpha*
- *Klebsormidium flaccidum*

Protein–protein association network built in Cytoscape via the STRING app using *Arabidopsis thaliana* identifiers. Differentially expressed genes (DEGs) from six species were first mapped to *A. thaliana* by BLASTP best hits (only one best hit reserved per query); the resulting *A. thaliana* gene list was submitted to STRING to retrieve protein functional associations. Edges denote STRING functional links and edge thickness/opacities reflect the STRING confidence score (medium-to-high confidence). The network was clustered with the STRING “Cluster” (MCL) routine. Node colors indicate in which lineage the DEG was detected. Example modules highlighted in the center are enriched for hormone-response terms (e.g., auxin, ABA, JA) and “response to oxygen-containing compound”.

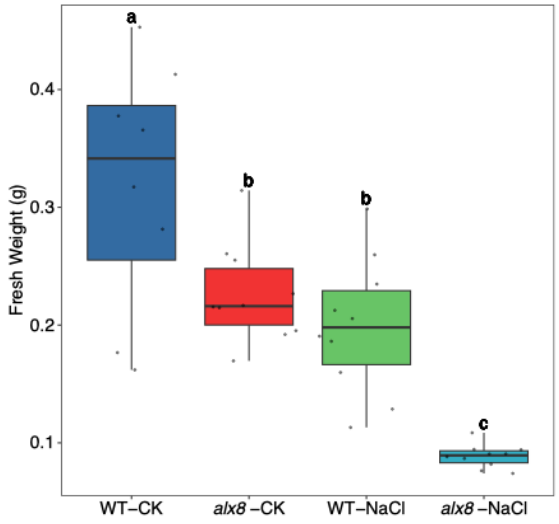
# *sal1* knockout mutants are sensitive to heat and salinity stresses

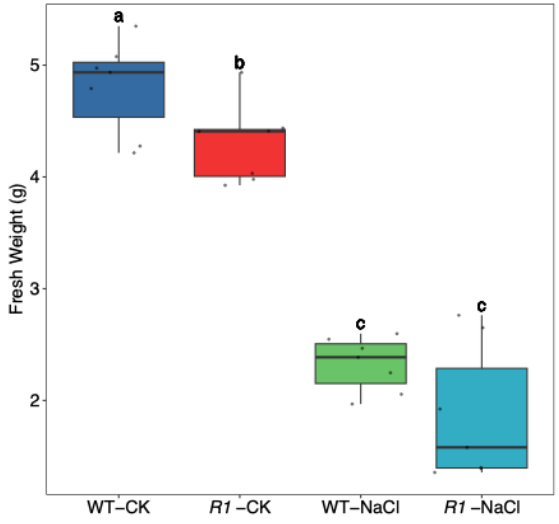



Phenotypic responses of *sal1* knockout or RNAi lines under salinity and heat stress in (A) four species in Salinity stress (B) *Arabidopsis thaliana,* (C) *Hordeum vulgare,* (D) *Ceratopteris richardii.*

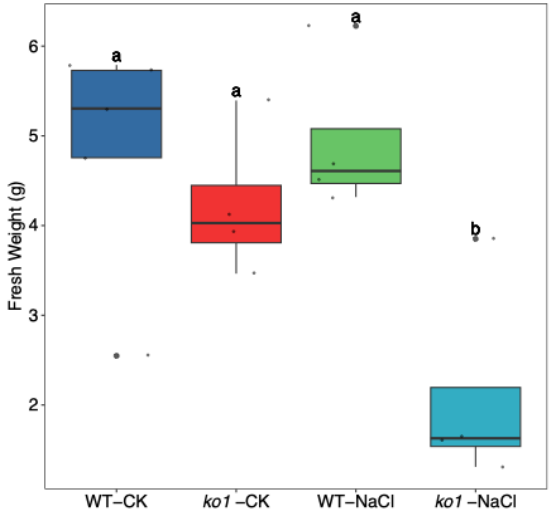
# Knockout mutants are sensitive to salinity and heat stress

Salinity

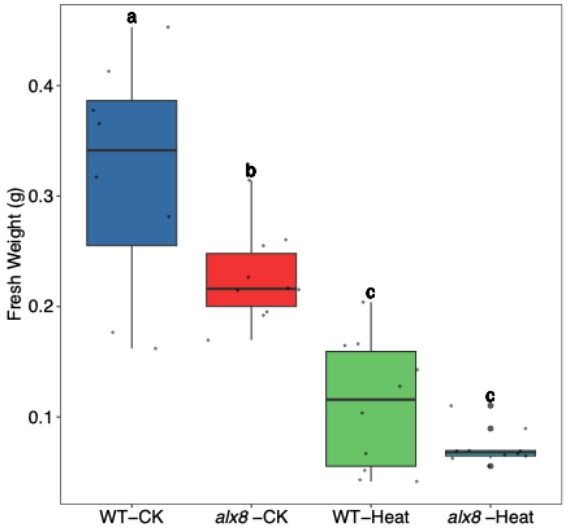


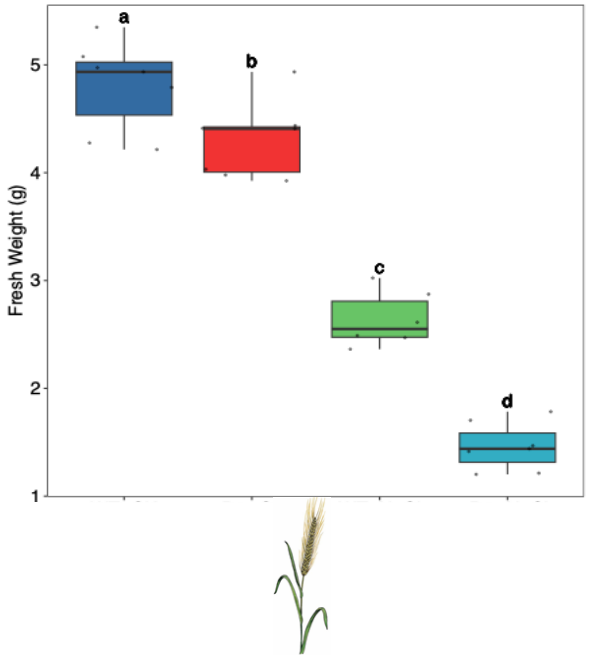



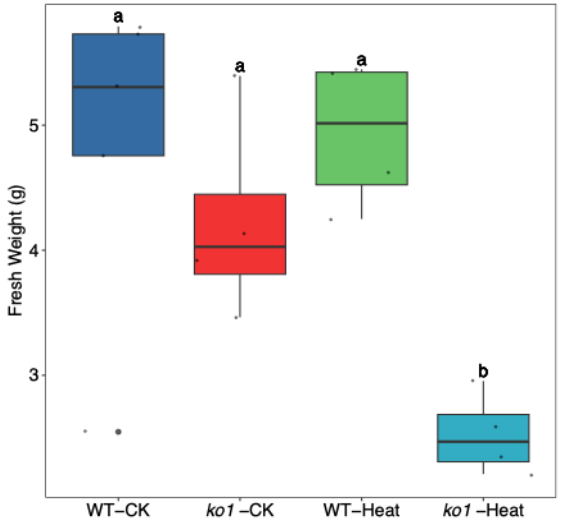

F




Heat



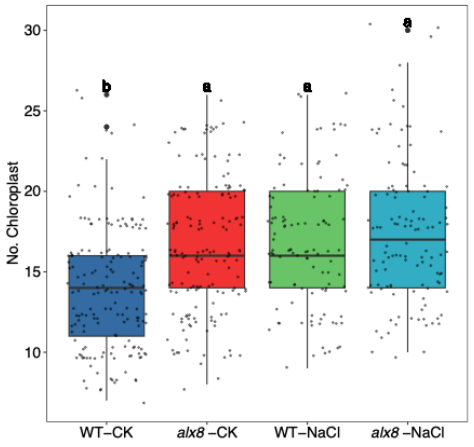



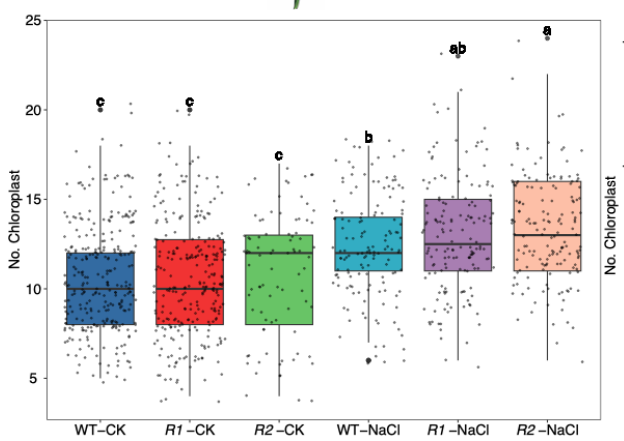


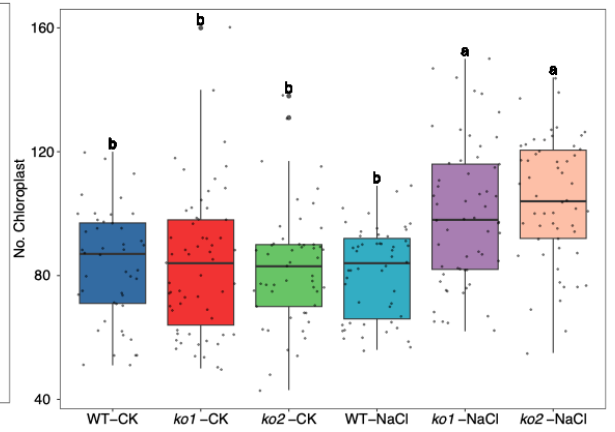

# Chloroplast number of wild type and *sal1* mutants under salinity and heat stress

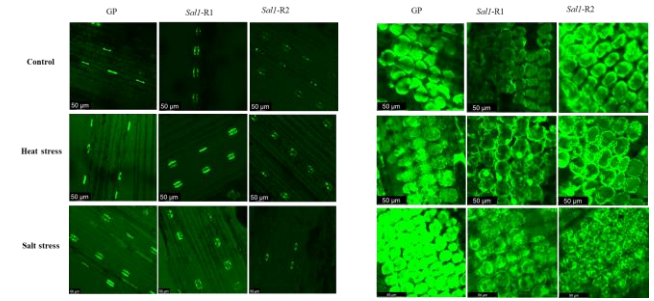
Salinity

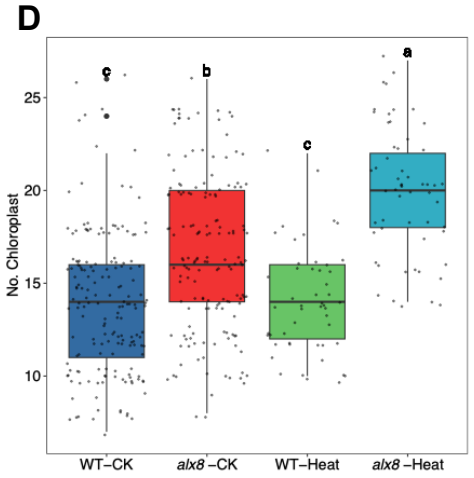
Heat

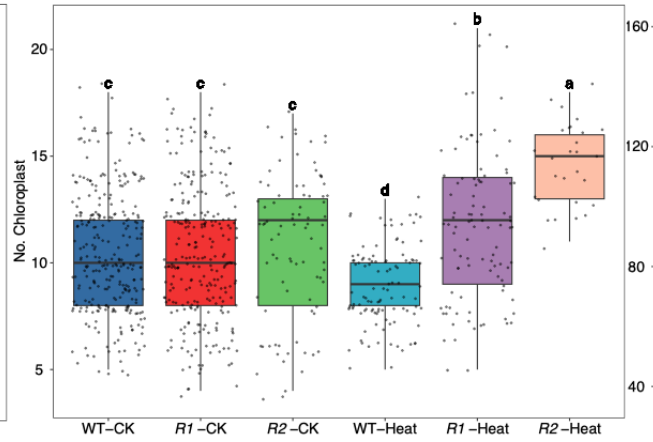


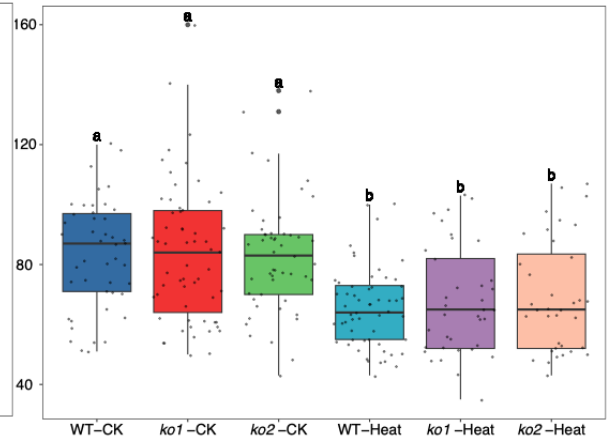



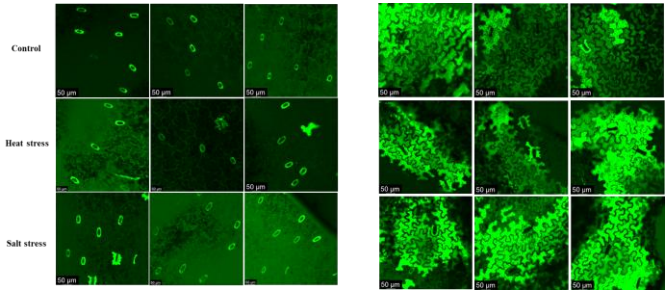



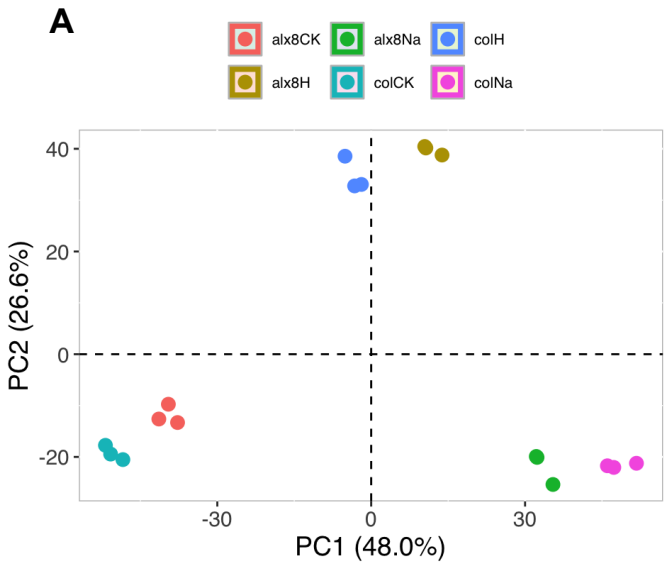



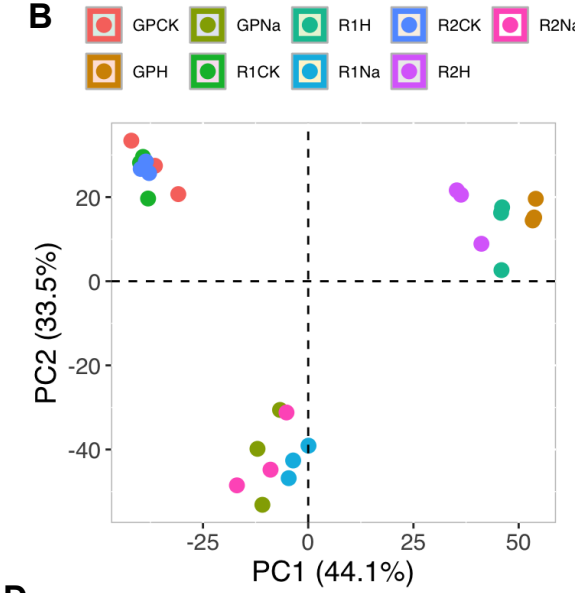



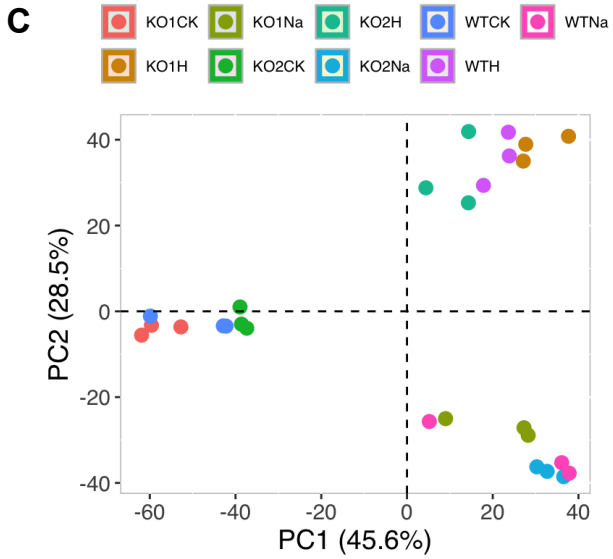



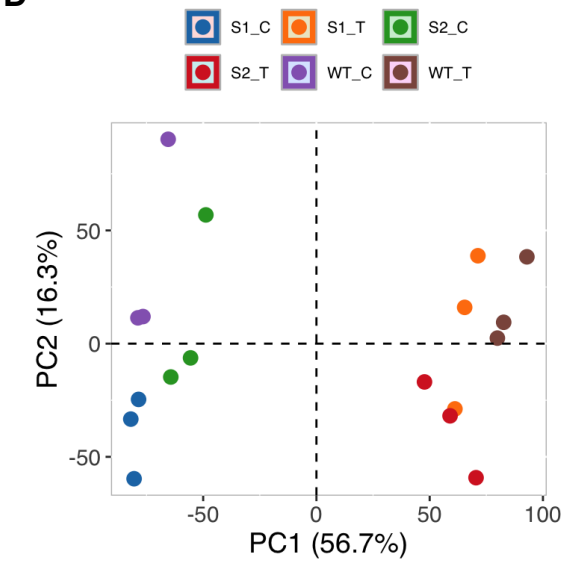

Boxplots show chloroplast counts per mesophyll cell measured in wild type and *sal1* mutant or RNAi lines of representative species. Each dot represents the measurement from a single mesophyll cell. Different letters denote statistically significant differences among treatments based on one-way ANOVA followed by Tukey's HSD test ($P < 0.05$).

# *sal1* knockout mutants show differential expression pattern to heat and salinity stresses



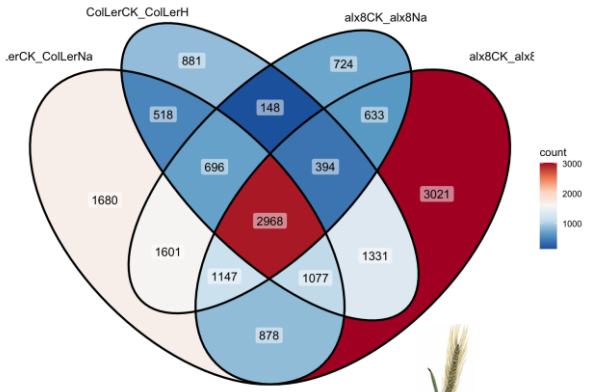



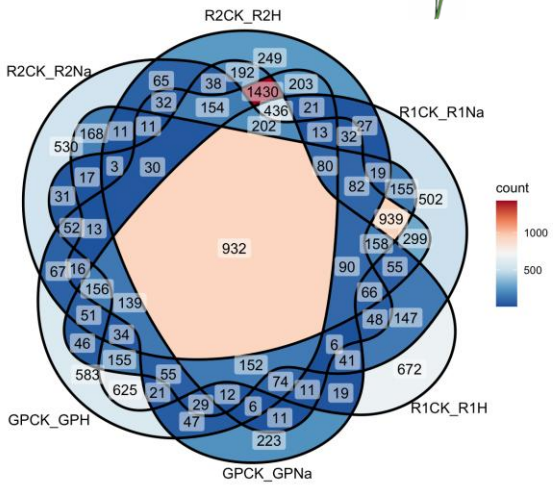

**Principal component analysis (PCA) of transcriptomes.** PCA plots based on TPM values show transcriptional variation in (A) *Ceratopteris richardii*, (B) *Hordeum vulgare*, (C) *Arabidopsis thaliana*, and (D) *Physcomitrium patens*. PC1 and PC2 represent the major principal components, with axis percentages indicating the proportion of variance explained.

# *sal1* knockout mutants show differential expression pattern to heat and salinity stresses

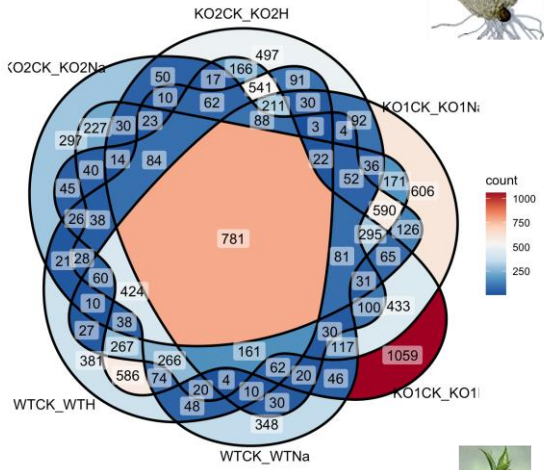
***Arabidopsis thaliana***



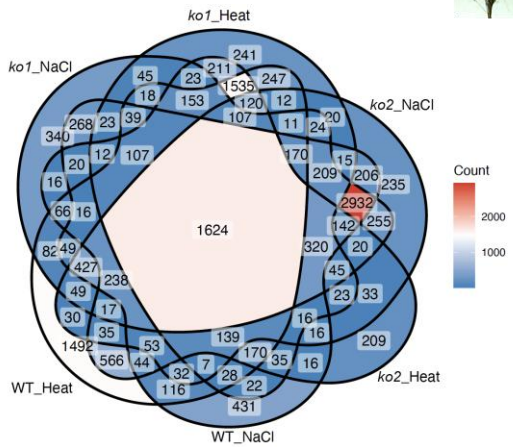

***Hordeum vulgare***

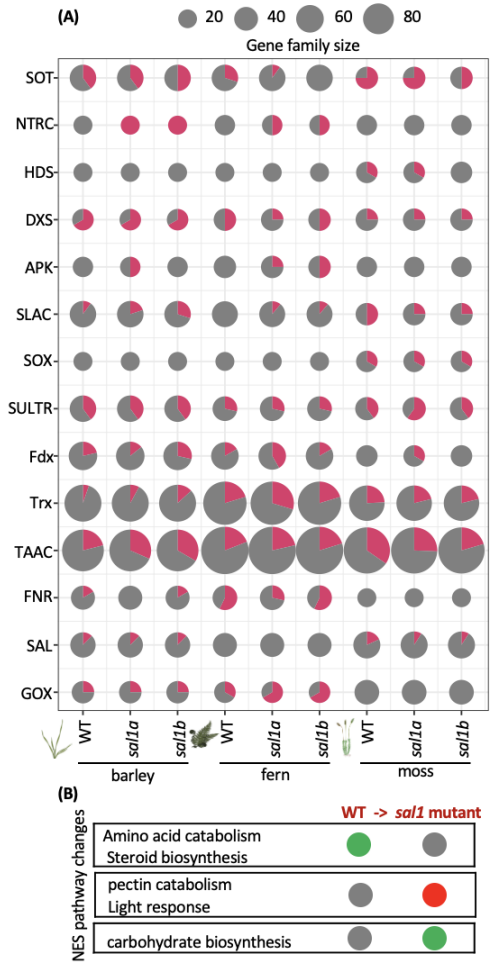



***Ceratopteris richardii***




***Physcomitrella patens***




Transcriptomic data identifying major gene families with differentially expressed genes (DEGs; in pink) under control and saline conditions. Abbreviations: SOT, sulphotransferase; DXS, 1-deoxy-D-xylulose 5-phosphate (DXP) synthase; SULTR, sulfate transporter; Fdx, ferredoxin; Trx, plastidial thioredoxin; TAAC, thylakoid ATP/ADP carrier;

(B) Normalised enrichment score (NES) pathway changes between wild type and sal1 mutants under salinity stress. Selected pathways are shown. Green, red and grey circles represent pathway activation, repression and no significant change, respectively.

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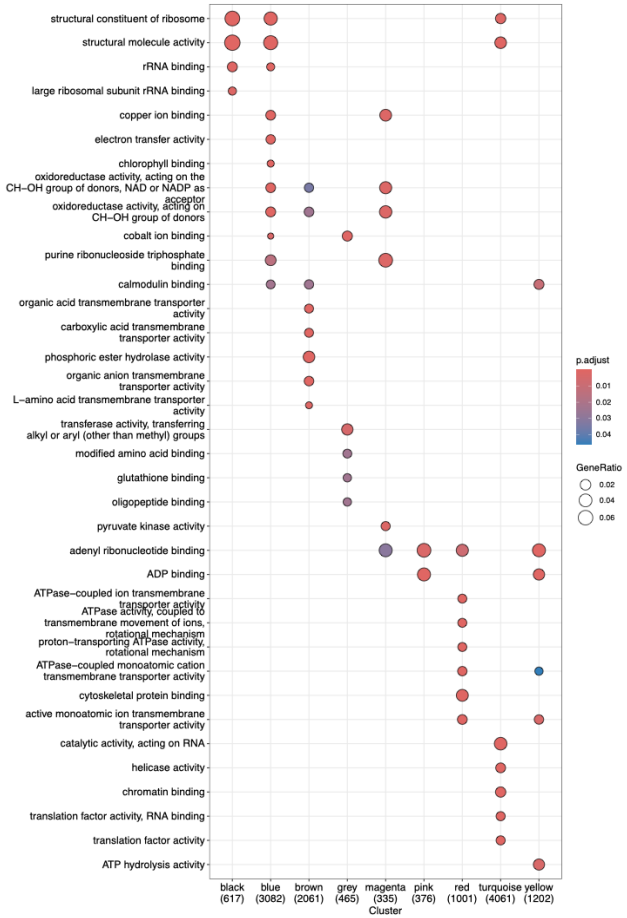
# Functional annotation in WT and *sal1* mutants of four plant species under salinity and heat stress

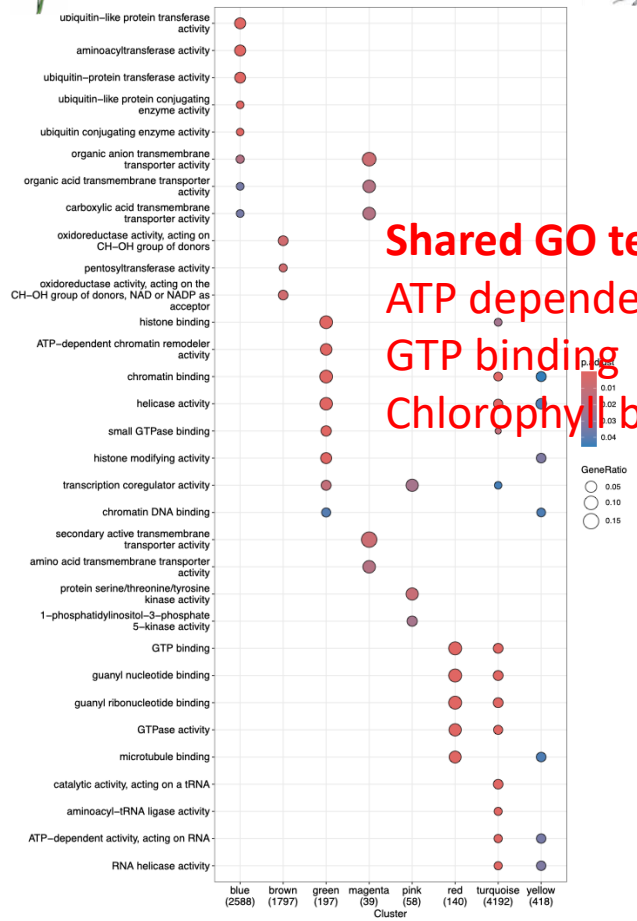
***Arabidopsis thaliana***

***Hordeum vulgare***

***Ceratopteris richardii***

***Physcomitrella patens***



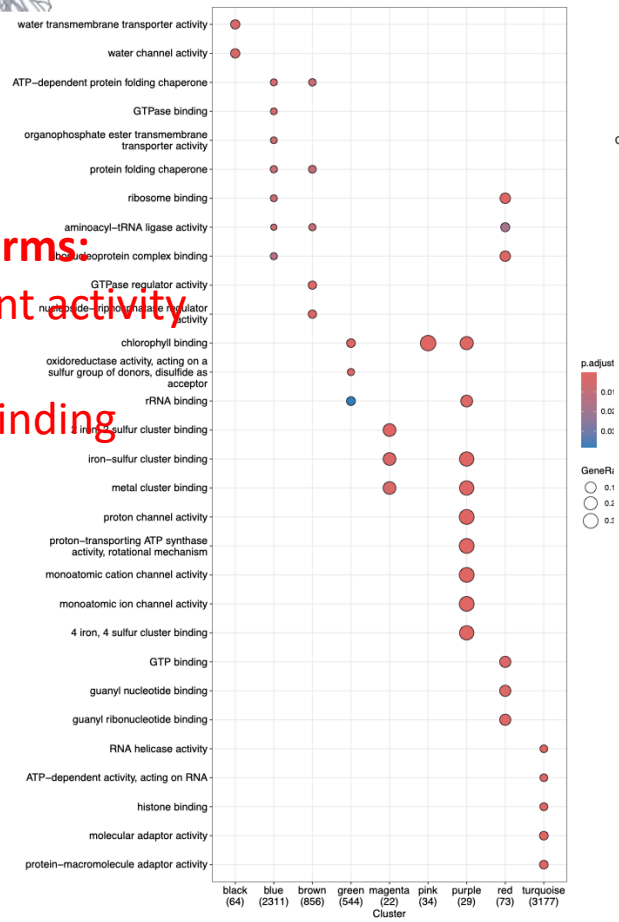



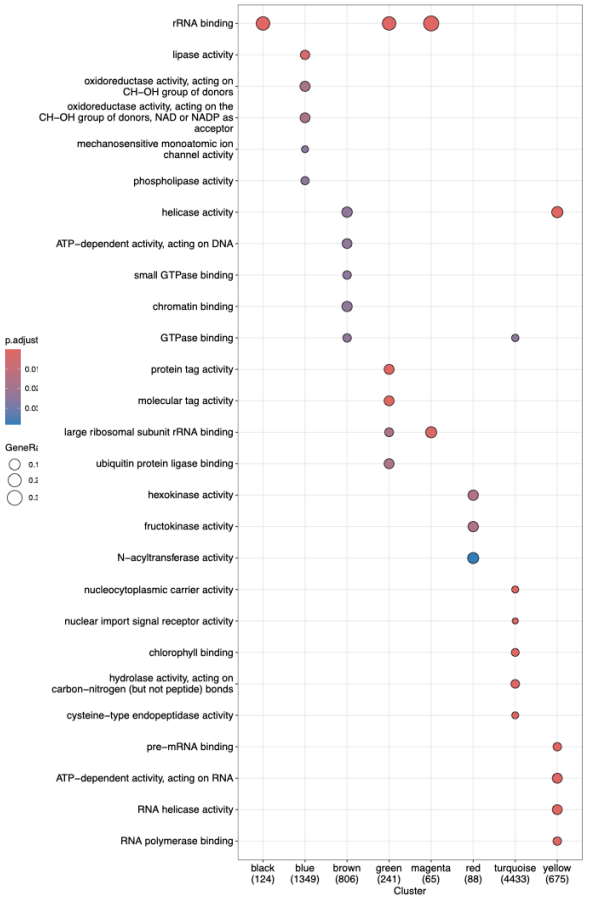

**Shared GO terms:**

ATP dependent activity

GTP binding

Chlorophyll binding




GO enrichment analysis of module genes, with bubble size indicating gene ratio and color representing adjusted *p*-values.

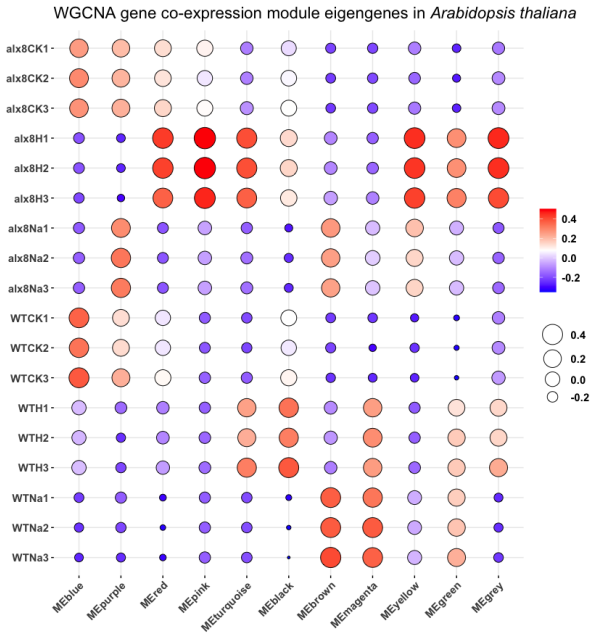
# WGCNA module eigengenes in WT and *sal1* mutants of four plant species under salinity and heat stress

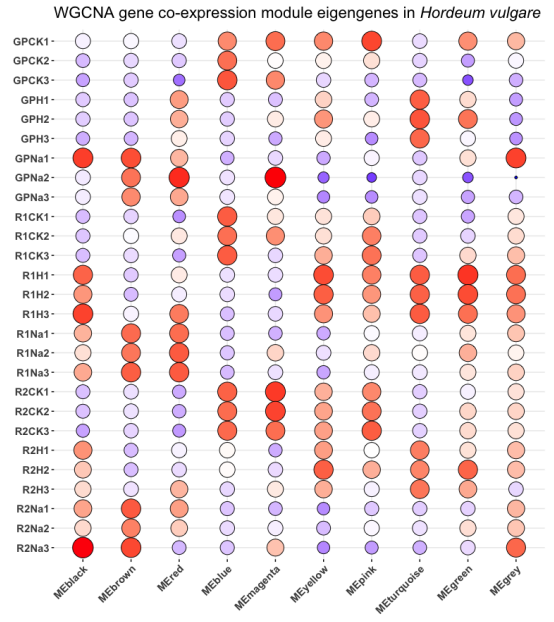
***Arabidopsis thaliana***

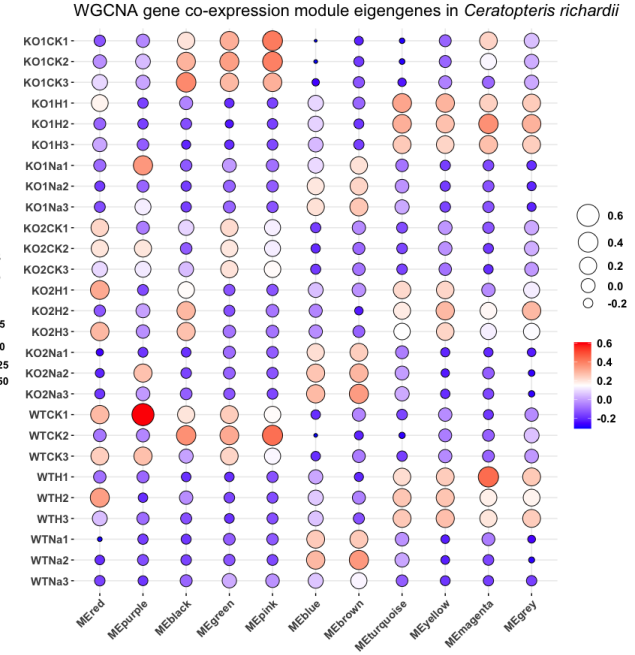
***Hordeum vulgare***

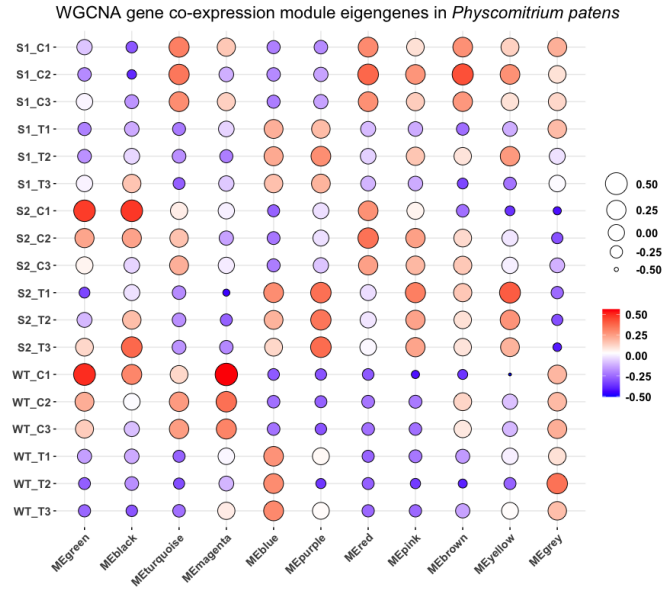
***Ceratopteris richardii***

***Physcomitrella patens***



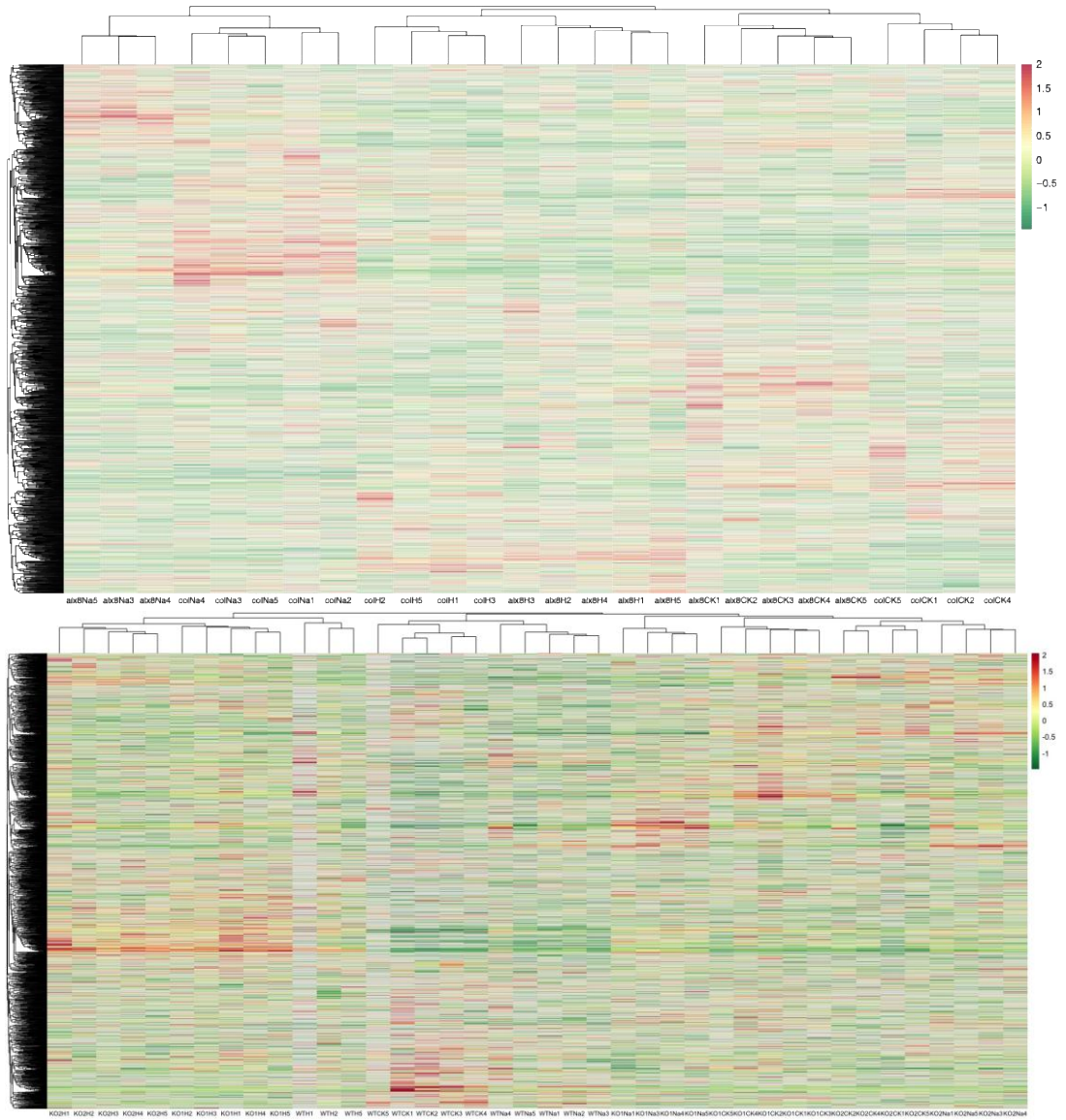



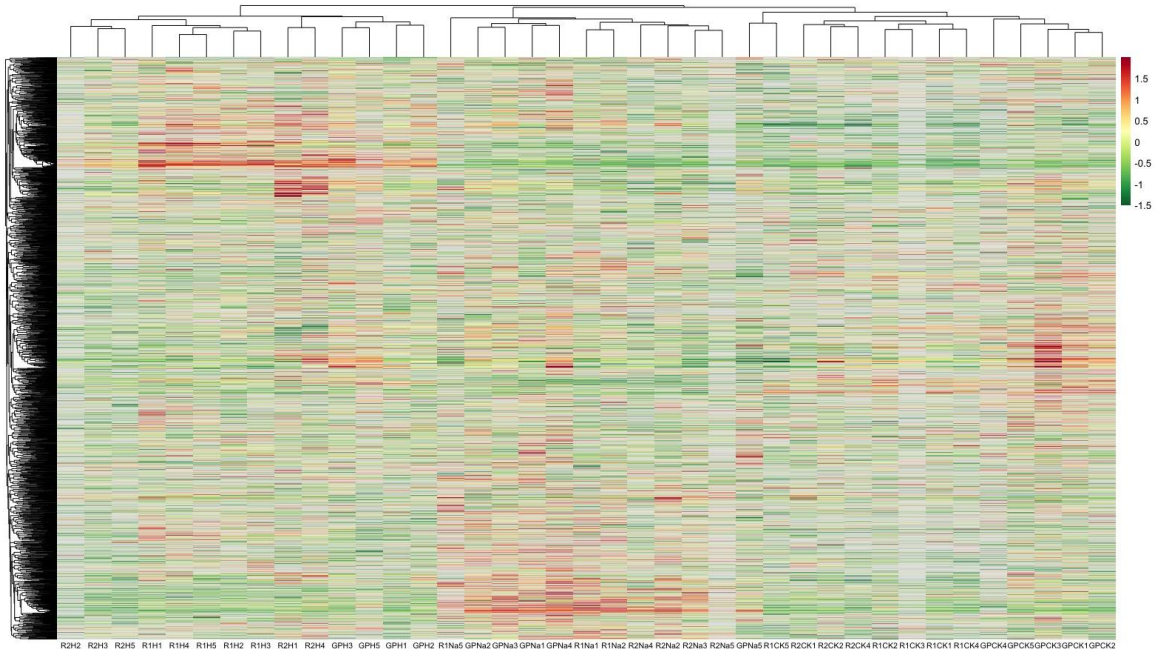




Bubble plot showing eigengene values in each of the four species of WT and mutants of co-expression modules across RNA-seq samples. Bubble size reflects the value of the eigengene, representing the overall expression of genes within each module.

# Metabolomic response of WT and *sal1* mutants to salinity and heat stress



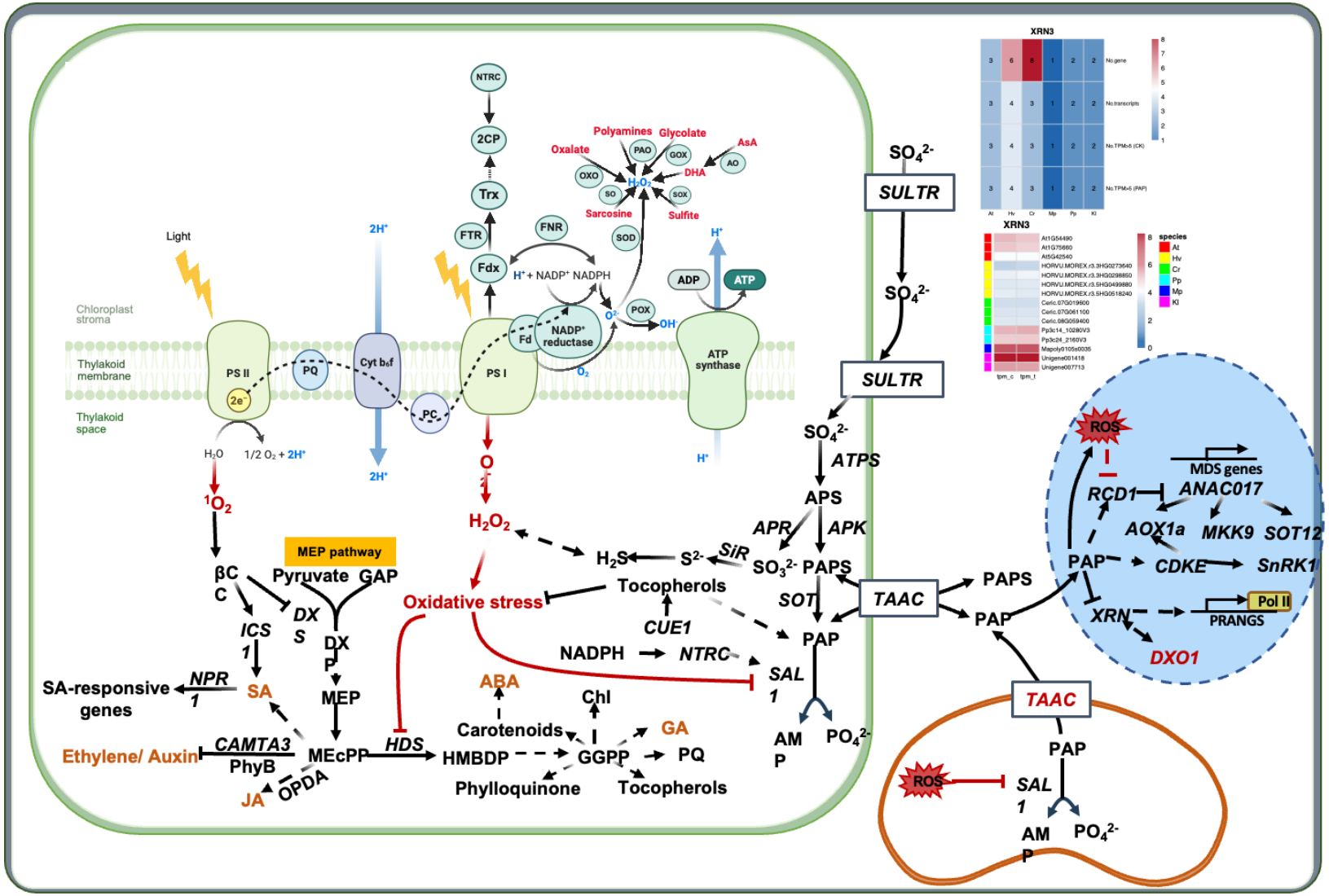


(A) Heatmap showing relative metabolite abundance across biological replicates under control and NaCl treatments. Color scale represents normalized values. (B) UpSet plot displaying the intersection of significantly differential metabolites between different genotype or treatment comparisons.

# SAL—PAP function in chloroplast retrograde signaling is conserved for salinity tolerance in green plants

- ✓ Phenotypes
- ✓ Cellular responses
- ✓ Gene expression

Metabolites?



# *Evolution of chloroplast retrograde signalling facilitates green plant adaptation to land*

- SAL1 is a highly conserved protein in plants and algae and SAL1 biochemical function is conserved in fern and moss.
- *SAL1* is an important gene for salinity tolerance of four evolutionarily distinct land plant species of eudicot, monocot, fern and moss.
- The conquest of land by plants may be enabled by rapid response to salinity and heat stress through this ancestral SAL1-PAP signaling pathway.

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