Current Biology, Volume *35*

Supplemental Information

Auxin and tryptophan trigger common responses in the streptophyte alga *Penium margaritaceum*

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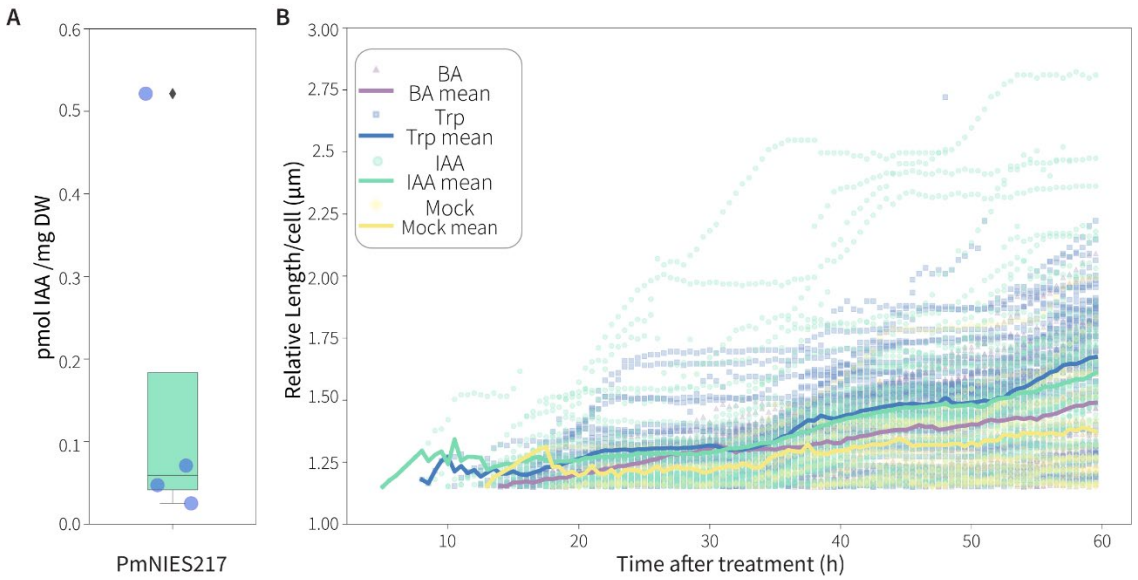

Figure S1. Auxin measurements and single-cell relative length traces obtained from growth experiments of *Penium* treated with IAA, Trp, BA or DMSO (related to Figure 1 and 2). (A) Quantification of Auxin (Indole-3-acidic acid (IAA) in dry weight (DW) *Penium margaritaceum NIES217* cultures through Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS). Each dot represents a biological replicate. (B) Single-cell relative length traces showing cell growth over time under continuous exposure to different treatments: purple triangles (benzoic acid, BA), blue squares (tryptophan, TRP), green circles (IAA), and yellow diamonds (DMSO, mock control). Each curve represents an individual cell, with dots indicating measurements taken every 30 minutes over 60 hours.

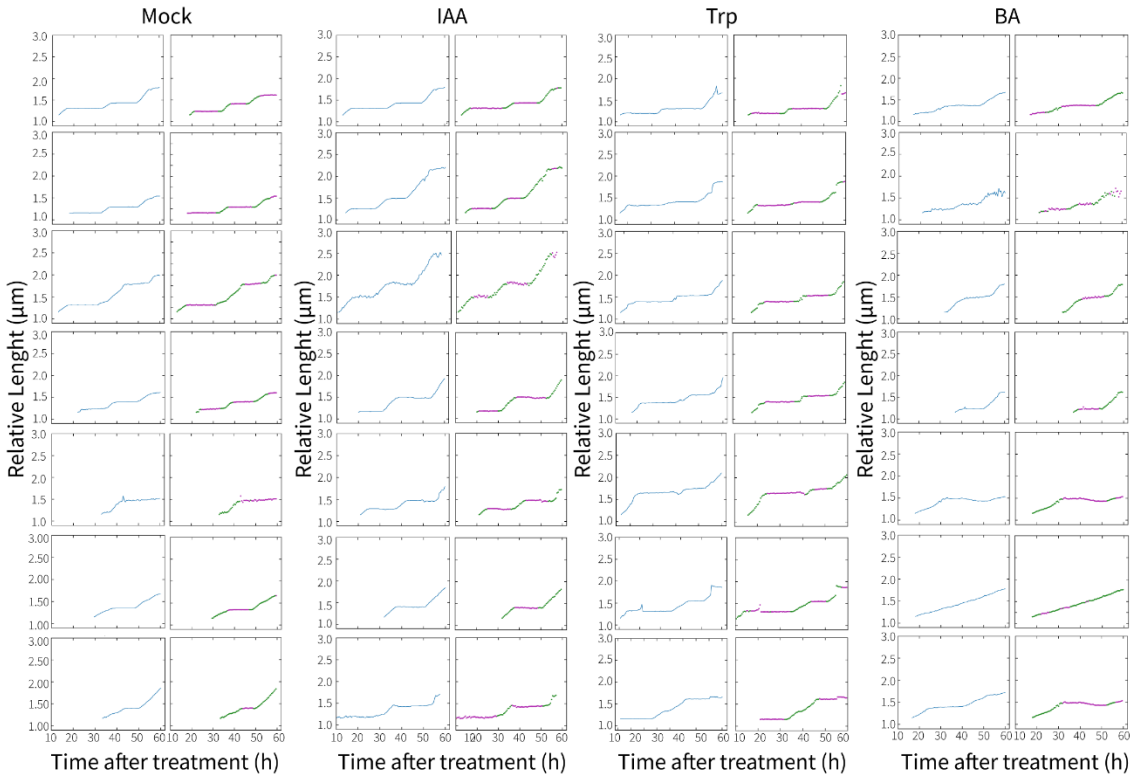

Figure S2. Single-cell relative length traces adjusted using a displacement pipeline to distinguish growing and resting phases (related to Figure 2). The figure shows single-cell traces of relative length over time of multiple cells treated with IAA, BA, Trp or DMSO (mock control). In the right panel of each treatment, continuous cell traces are displayed. In the left panel, traces are shown after applying a displacement pipeline that separates growing (green) and resting (magenta) phases point by point. The threshold to distinguish between growing and resting regimes was set at a relative growth rate of 0.012 µm/h. Green-marked points (growing phases) were used to calculate the growth rate for all treatments.

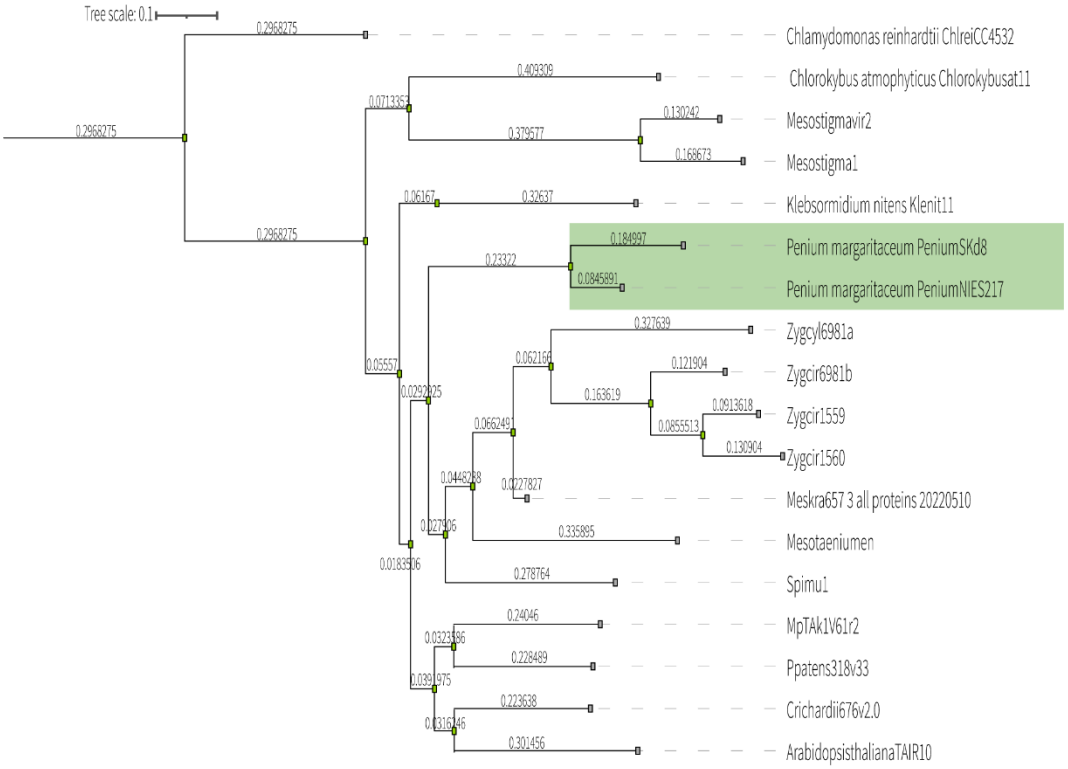

Figure S3. Species tree of streptophyte algal species with available proteomes, depicting the placement of *Penium margaritaceum* isolates PmSk#8[S1] **and PmNIES217(related to Figure 3)**. The tree was generated in Orthofinder with the complete proteome of all included species, following the pipeline indicated on Ref.[S2]. Both *P. margaritaceum* strains are highlighted in green.

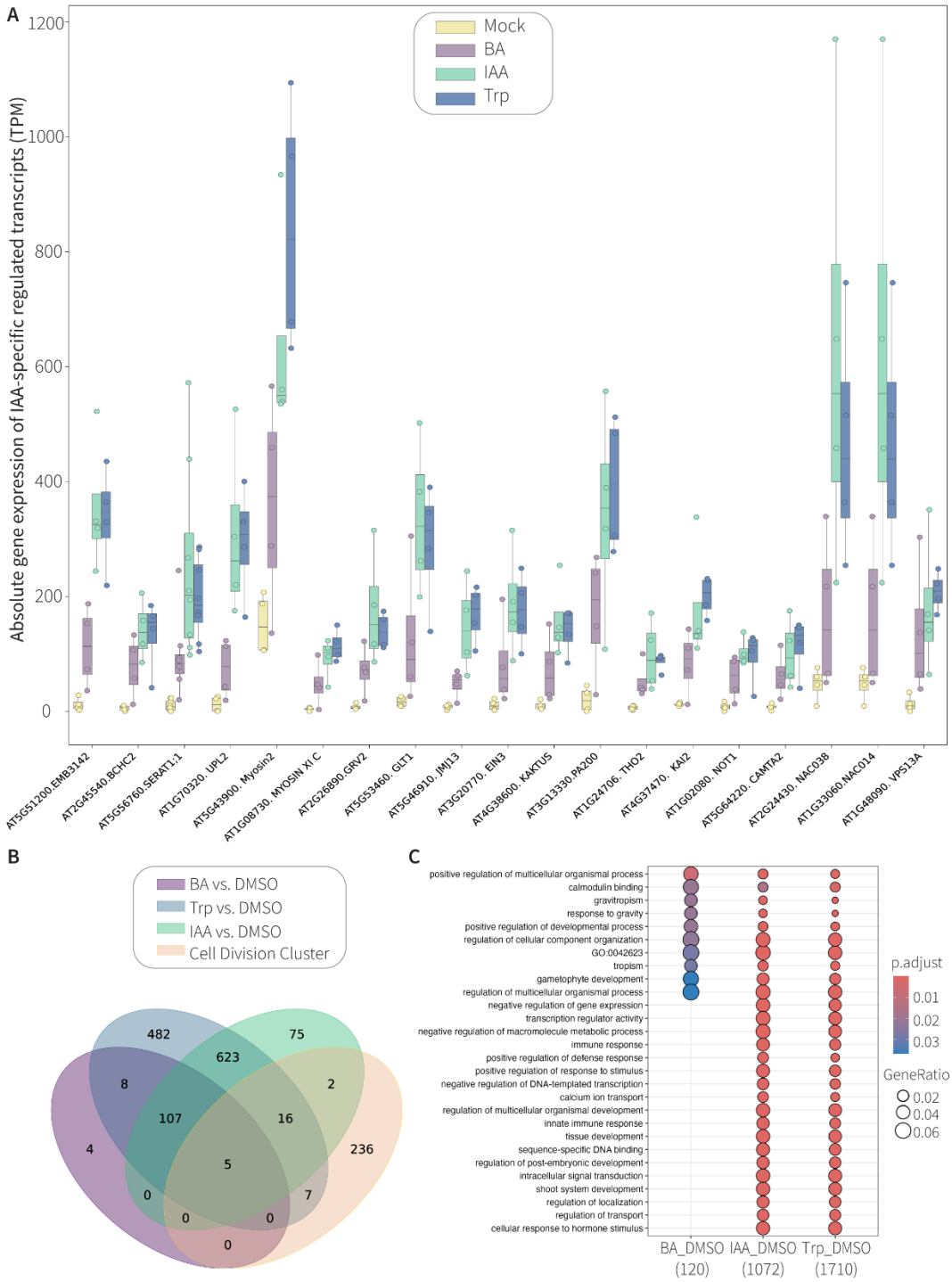

Figure S4. Complementary transcriptomic analysis of Penium cells treated with IAA, Trp, BA or mock control (related to Figure 3). (A) Expression of the top 20 IAA regulated genes containing an *Arabidopsis* ortholog with a baseMean threshold (>50) ploted along with the gene expression in DMSO, Trp or BA treatments. (B) Venn diagram indicating the overlap between differentially expressed genes in IAA, BA and Trp treatments and a cell division gene cluster derived from research in *Mesotaenium* [S3]. The numbers correspond to regulated transcripts that contain an *Arabidopsis* ortholog. (C) GO term analysis comparing functional enrichment between DEGs of TRP, IAA or BA-treated Penium cells with respect to DMSO. Significant genes (padj<0.05 & log2(foldchange)>1) were used for the analysis. Gene ratio and p-value of the GO enrichment are indicated on right.

Treatment	Average percentage of growing cells	Average percentage of dividing cells	Growth rate (measured from intermittent growth)	Track mean speed µm/s
Mock	18%	6%	0.025	18.6
IAA	41%	31%	0.033	22.5
Trp	66%	45%	0.035	25.8
BA	51%	21%	0.021	19.31

Table S1. Summary of parameters used to quantify growth, division and changes in cytoplasmic streaming in *Penium* cells treated with different chemicals (related to Figure 2).

Supplemental references

S1. Jiao, C., Sørensen, I., Sun, X., Sun, H., Behar, H., Alseekh, S., Philippe, G., Palacio Lopez, K., Sun, L., Reed, R., et al. (2020). The Penium margaritaceum Genome: Hallmarks of the Origins of Land Plants. Cell *181*, 1097-1111.e12. https://doi.org/10.1016/j.cell.2020.04.019.

S2. Emms, D.M., and Kelly, S. (2019). OrthoFinder: phylogenetic orthology inference for comparative genomics. Genome Biol *20*, 238. https://doi.org/10.1186/s13059-019-1832-y.

S3. Dadras, A., Fürst-Jansen, J.M.R., Darienko, T., Krone, D., Scholz, P., Sun, S., Herrfurth, C., Rieseberg, T.P., Irisarri, I., Steinkamp, R., et al. (2023). Environmental gradients reveal stress hubs pre-dating plant terrestrialization. Nat. Plants *9*, 1419–1438. https://doi.org/10.1038/s41477-023-01491-0.