

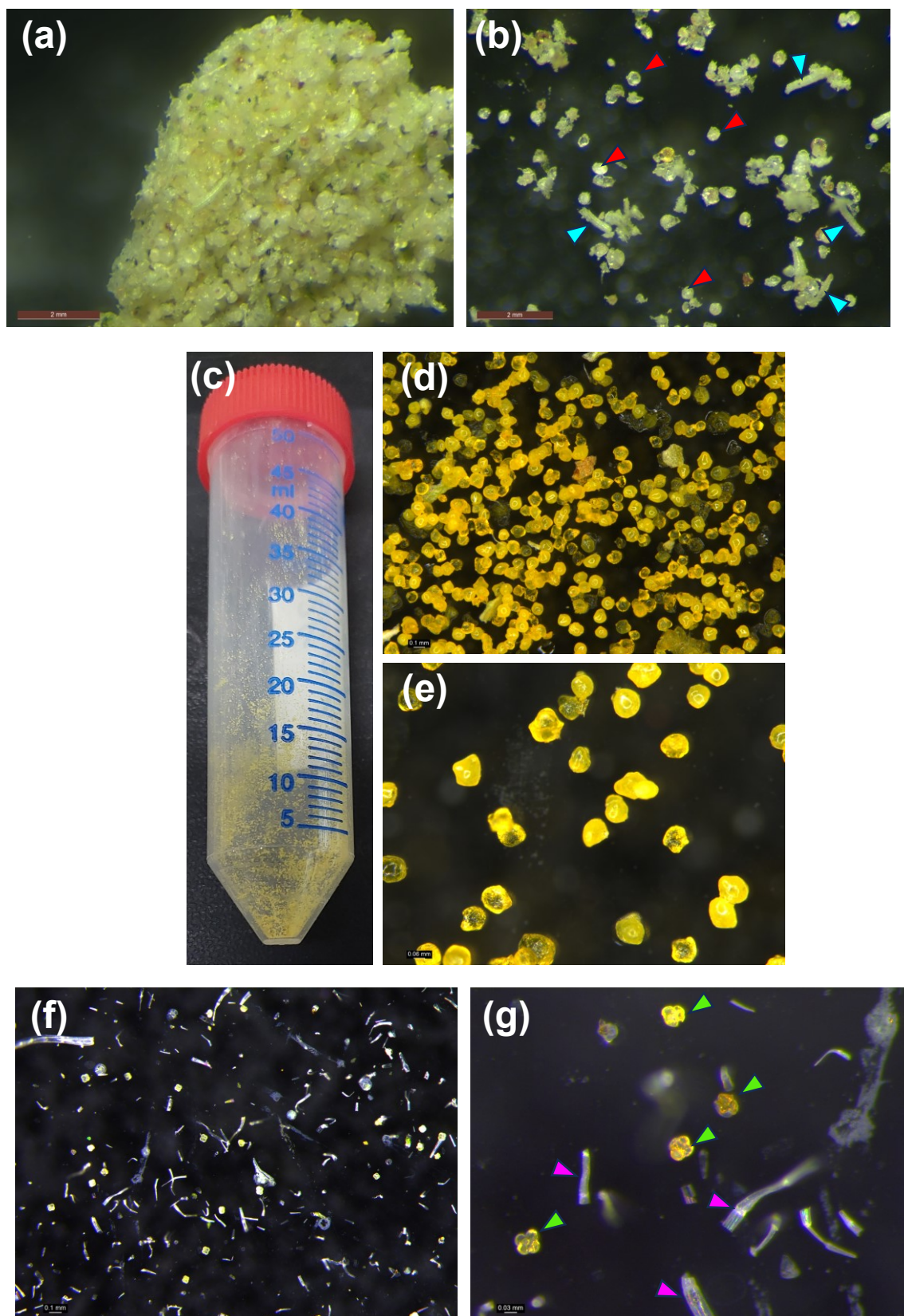
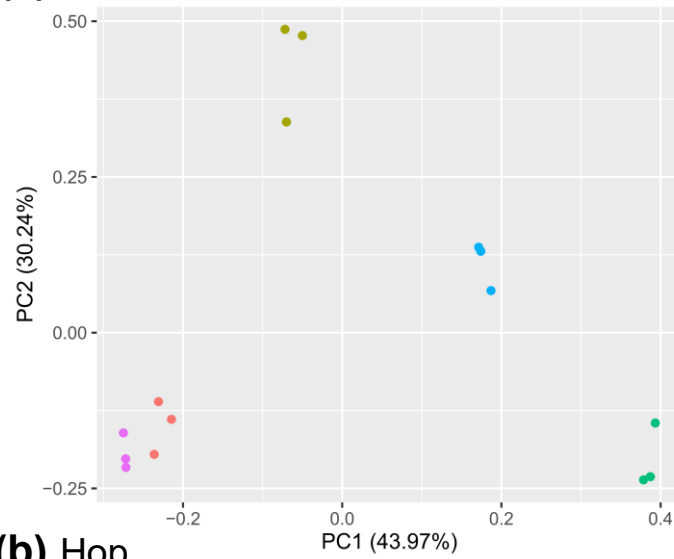
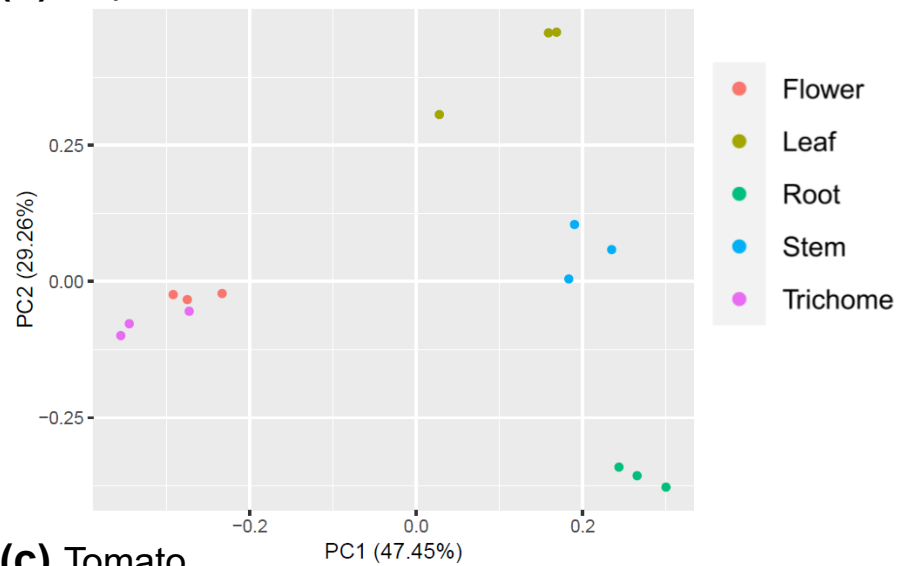


Figure S1 Trichome samples isolated by cryo-vortexing trichome-rich samples in 50 mL Eppendorf tubes containing liquid nitrogen (see **Methods**). **(a)** A clump of recovered cannabis trichomes. **(b)** Isolated cannabis GTs mainly composed of GT heads (red arrowheads) and stalks (blue arrowheads). **(c)** Hop GTs (lupulin glands) remaining adhered to tube walls after vortexing and removing all other debris. **(d, e)** Images of showing individual isolated hop GTs. **(f, g)** Images of isolated tomato trichomes showing both GTs (green arrowheads) and GT stalks/non-GTs (purple arrowheads).

(a) Cannabis



(b) Hop



(c) Tomato

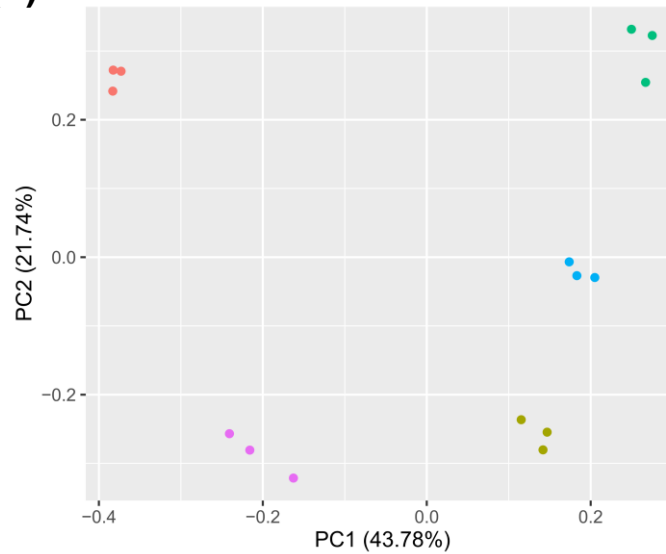
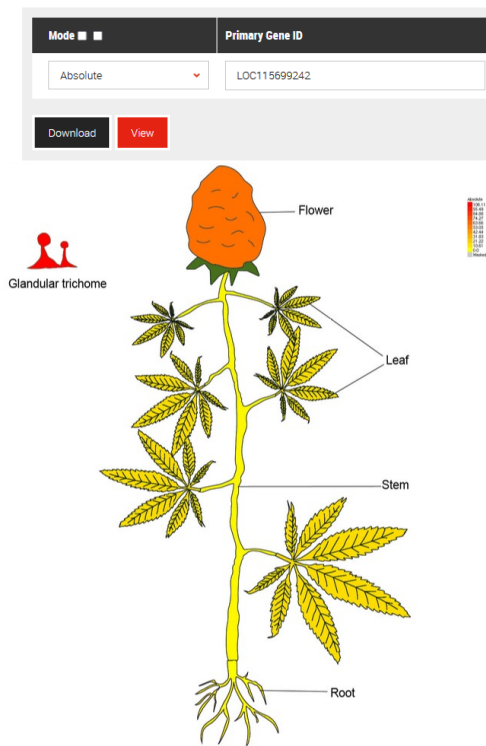


Figure S2 Principal component analysis plots of RNA-seq data showing the grouping of samples according to biological replicates and organ type (flower, leaf, root, stem and trichome).

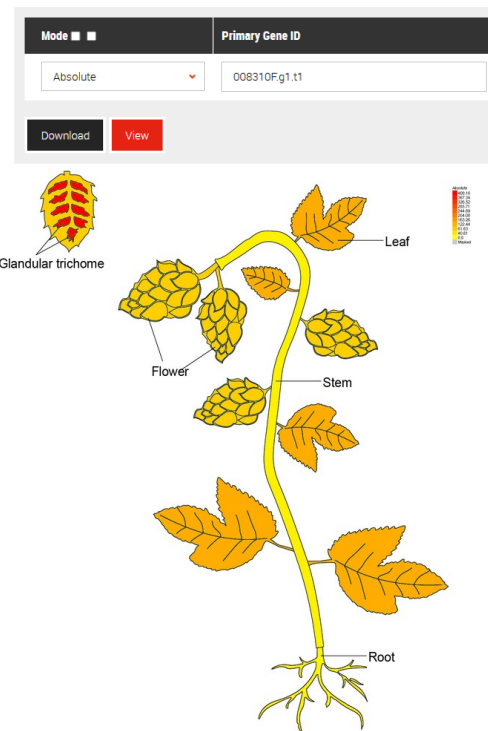
(a) Cannabis

GeneView (eFP): LOC115699242



(b) Hop

GeneView (eFP): 008310F.g1.t1



(c) Tomato

GeneView (eFP): Solyc12g044280.2.1

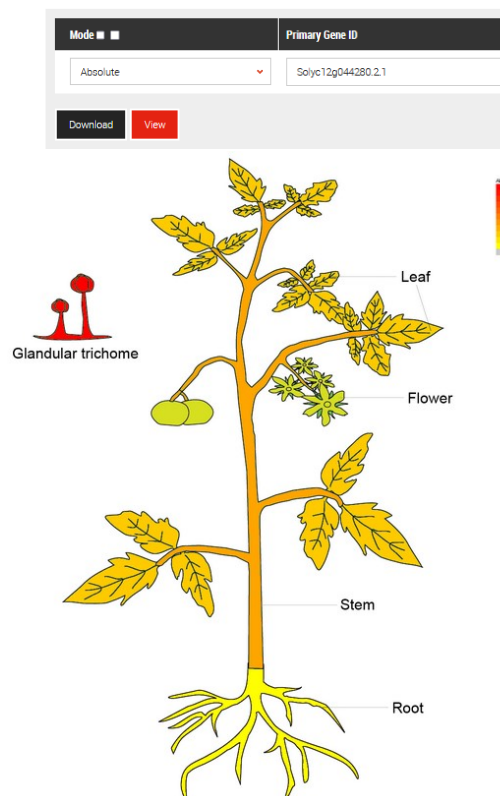
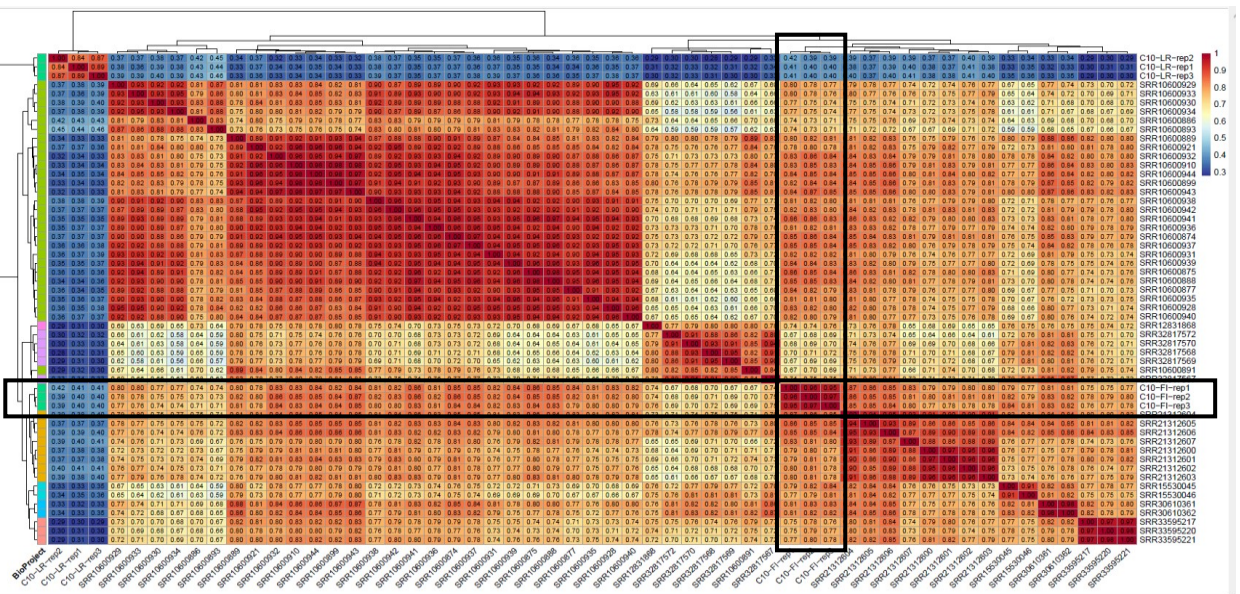


Figure S3 eFP (electronic fluorescent pictographs) images showing expression profiles of candidate (a) cannabis, (b) hop and (c) tomato genes across different organs.

(a) Flower



(b) Trichome

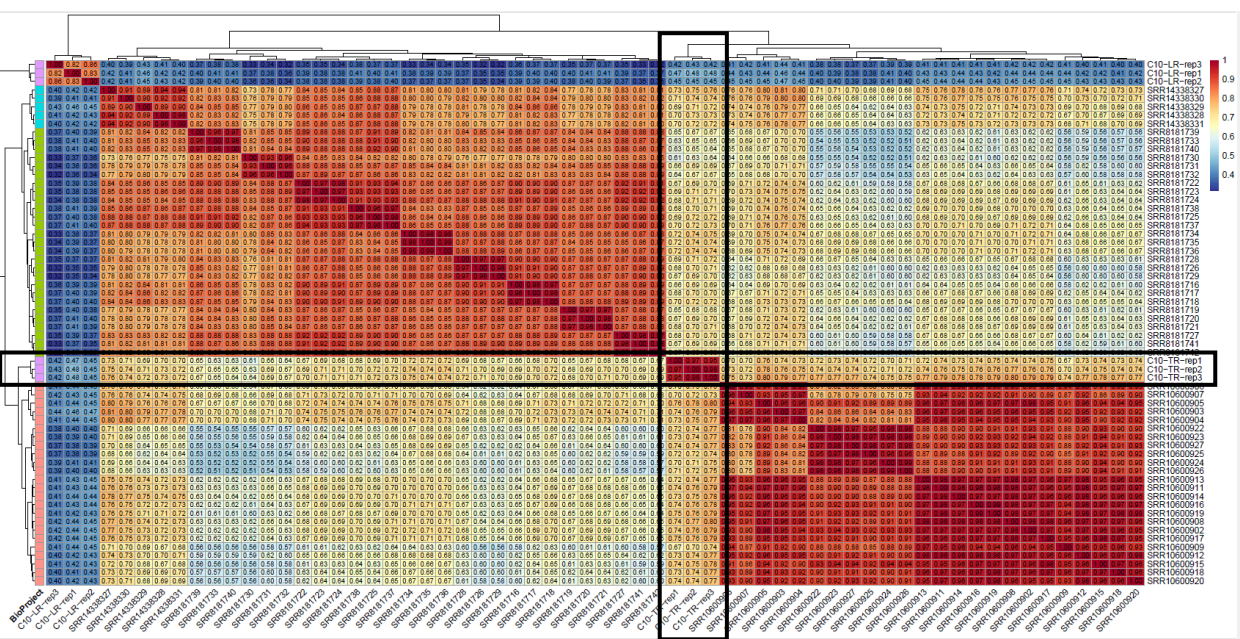
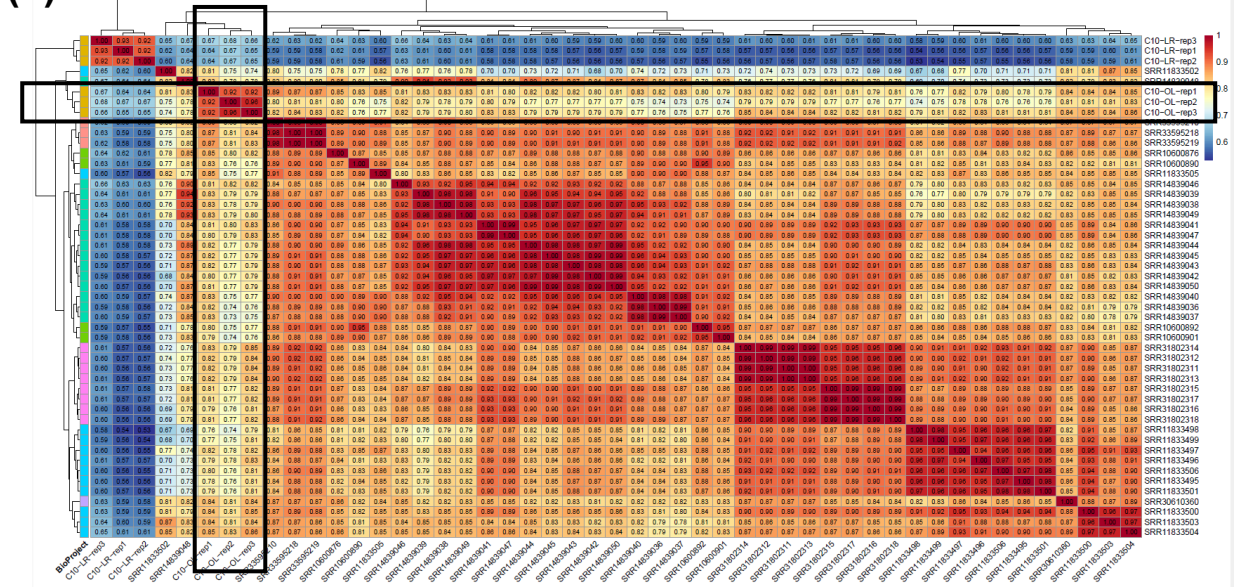
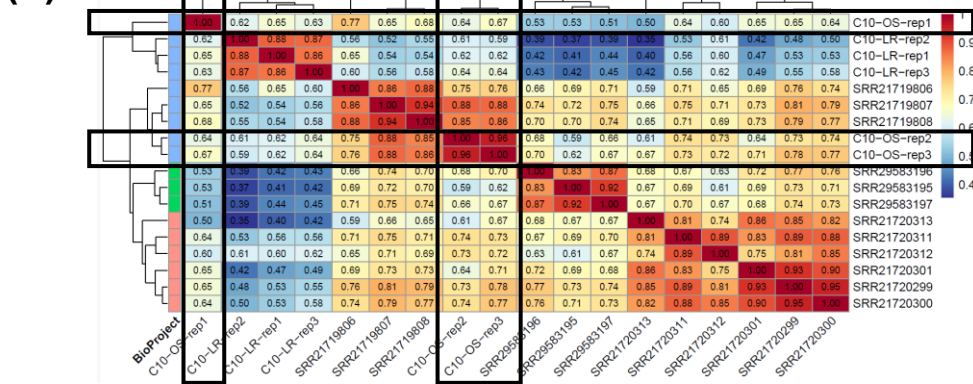


Figure S4 Spearman correlation plots illustrating organ-specific correlations of RNA-seq datasets in cannabis. The plots compare independent, publicly available cannabis RNA-seq data from **(a)** flower and **(b)** trichome samples with the corresponding flower and trichome datasets generated in this study (highlighted by black boxes). Root RNA-seq data from this study is included as a control. The colour gradient (blue to red) indicates Spearman correlation coefficient strength from low to high, with correlation coefficients shown in each cell.

(a) Leaf



(b) Stem



(c) Root

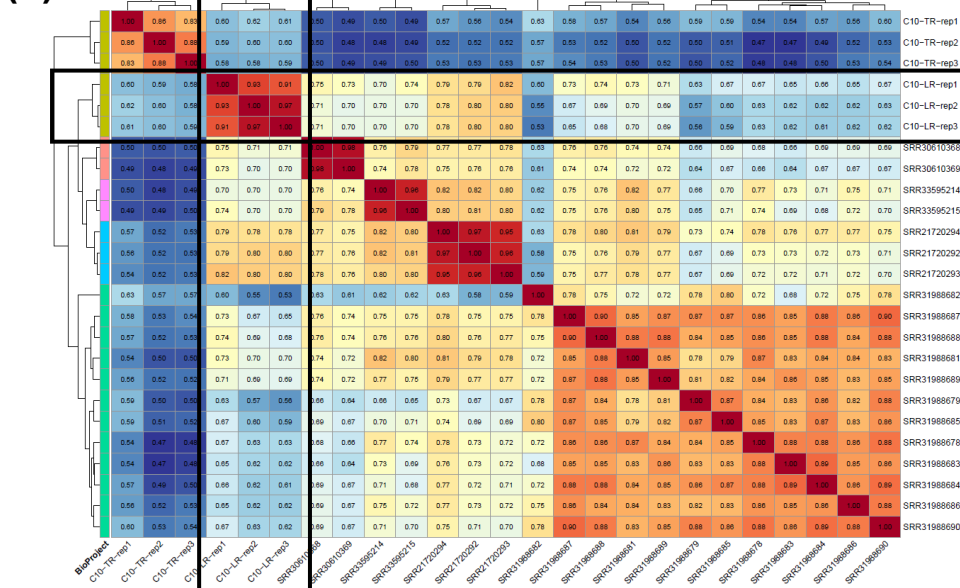
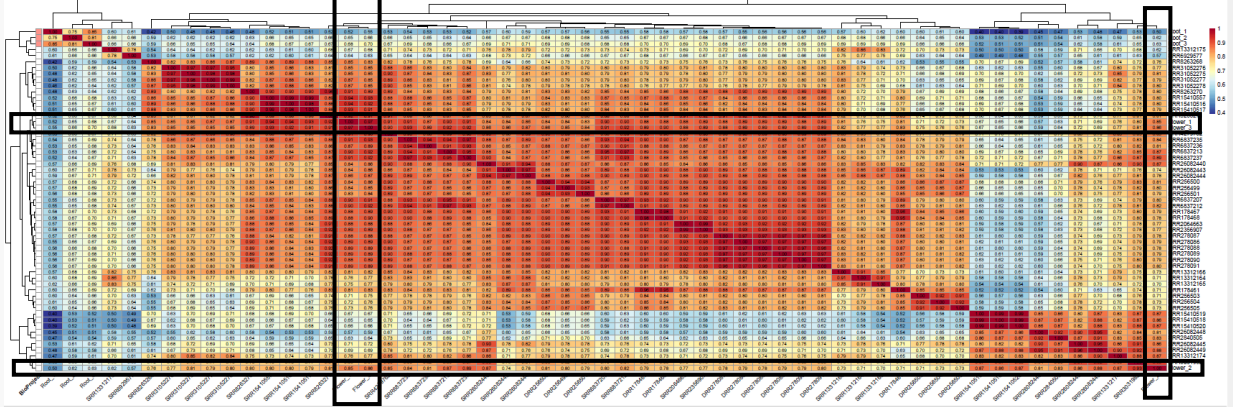
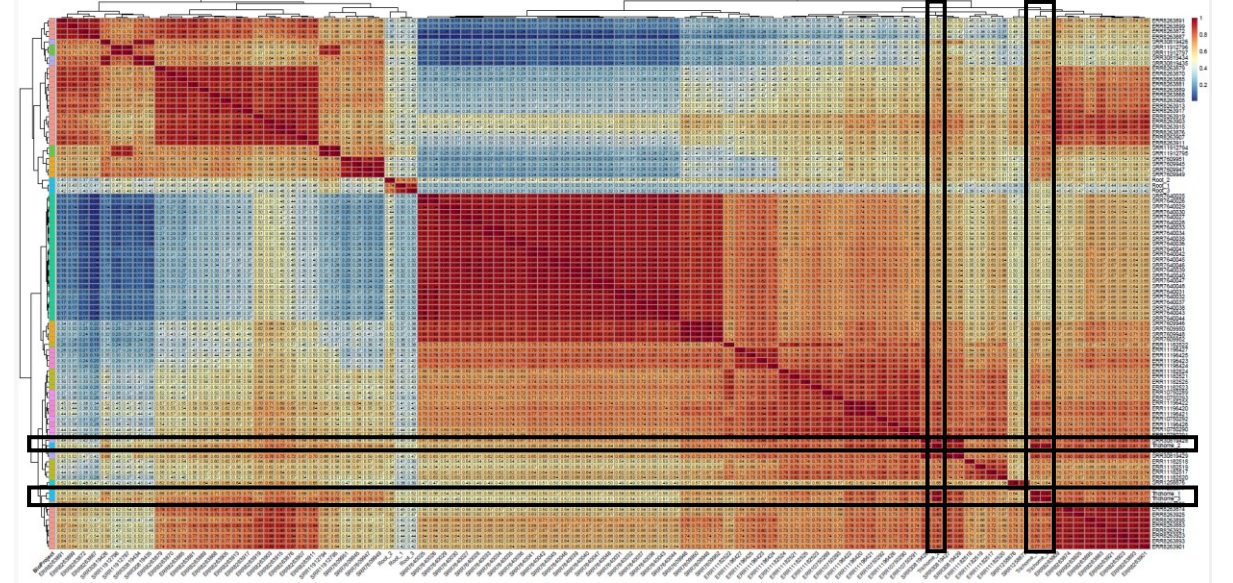


Figure S5 Spearman correlation plots illustrating organ-specific correlations of RNA-seq datasets in cannabis. The plots compare independent, publicly available cannabis RNA-seq data from (a) leaf (b) stem and (c) root samples with the corresponding leaf, root and stem dataset generated in this study (highlighted by black boxes). Root (a & b) and trichome (c) RNA-seq data from this study are included as controls. The colour gradient (blue to red) indicates Spearman correlation coefficient strength from low to high, with correlation coefficients shown in each cell.

(a) Flower



(b) Trichome



(c) Stem

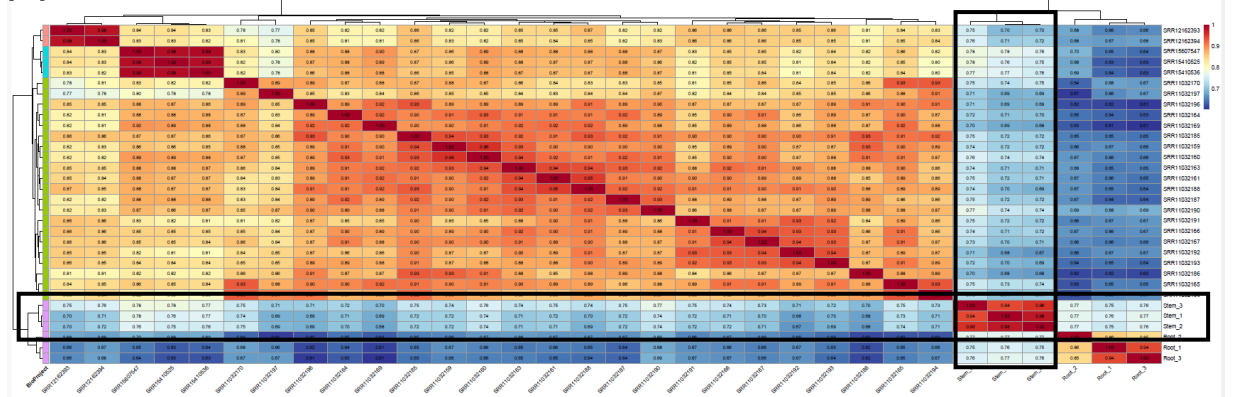
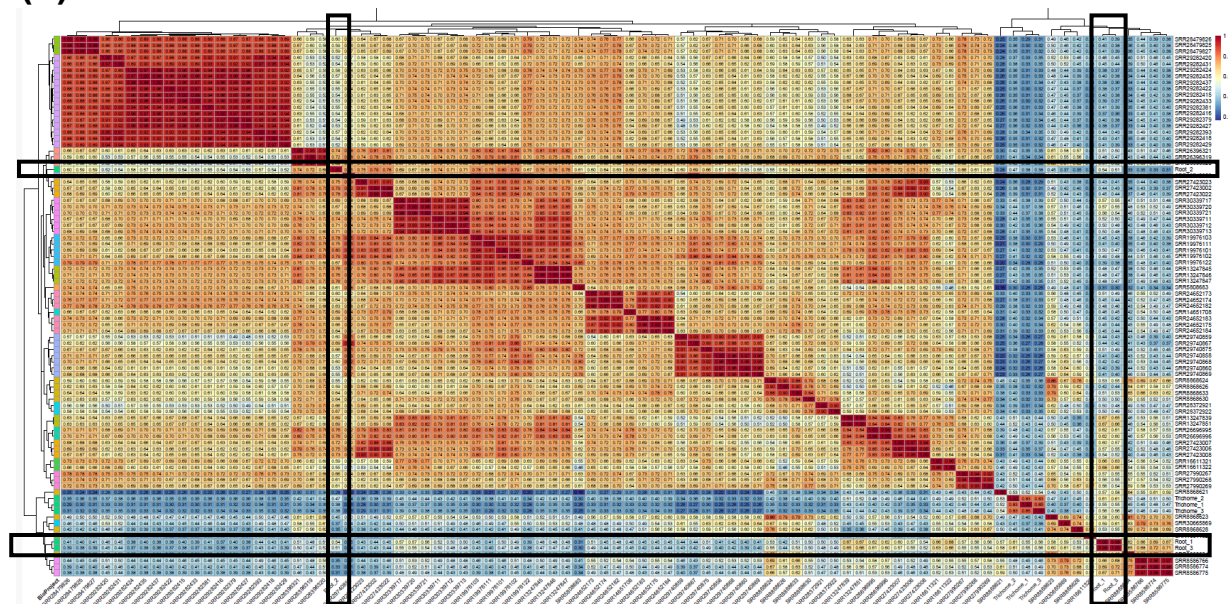


Figure S6 Spearman correlation plots illustrating organ-specific correlations of RNA-seq datasets in tomato. The plots compare independent, publicly available tomato RNA-seq data from **(a)** flower, **(b)** trichome and **(c)** stem samples with the corresponding flower, trichome and stem dataset generated in this study (highlighted by black boxes). Root RNA-seq data from this study is included as a control. The colour gradient (blue to red) indicates Spearman correlation coefficient strength from low to high, with correlation coefficients shown in each cell.

(a) Root



(b) Leaf

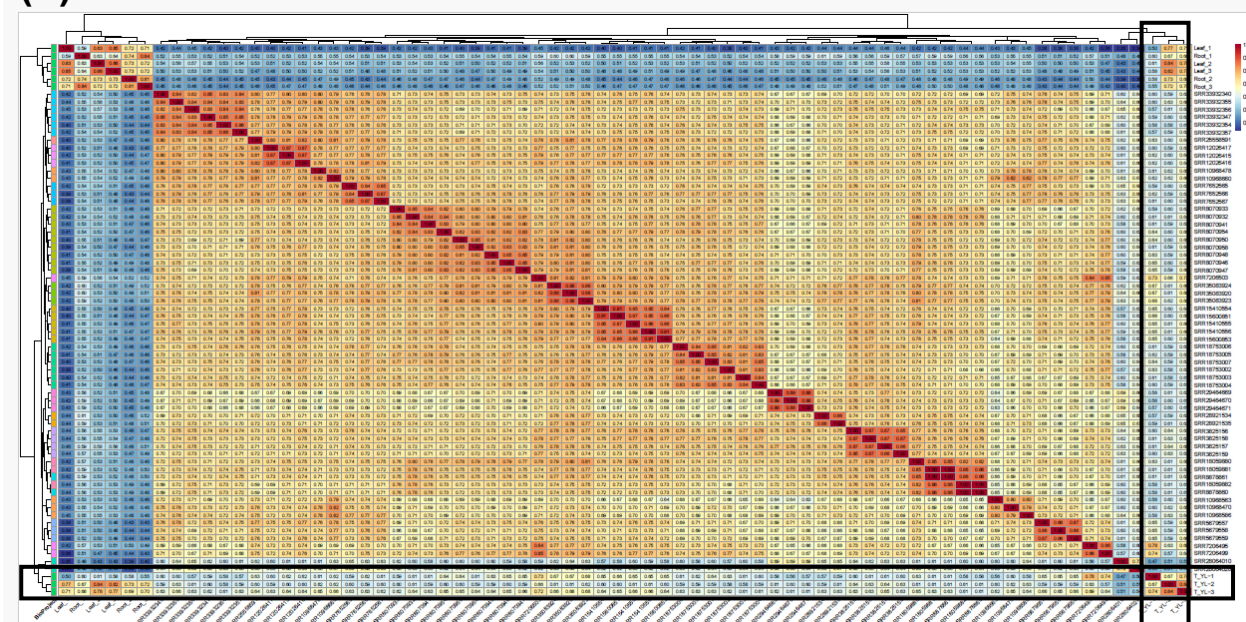
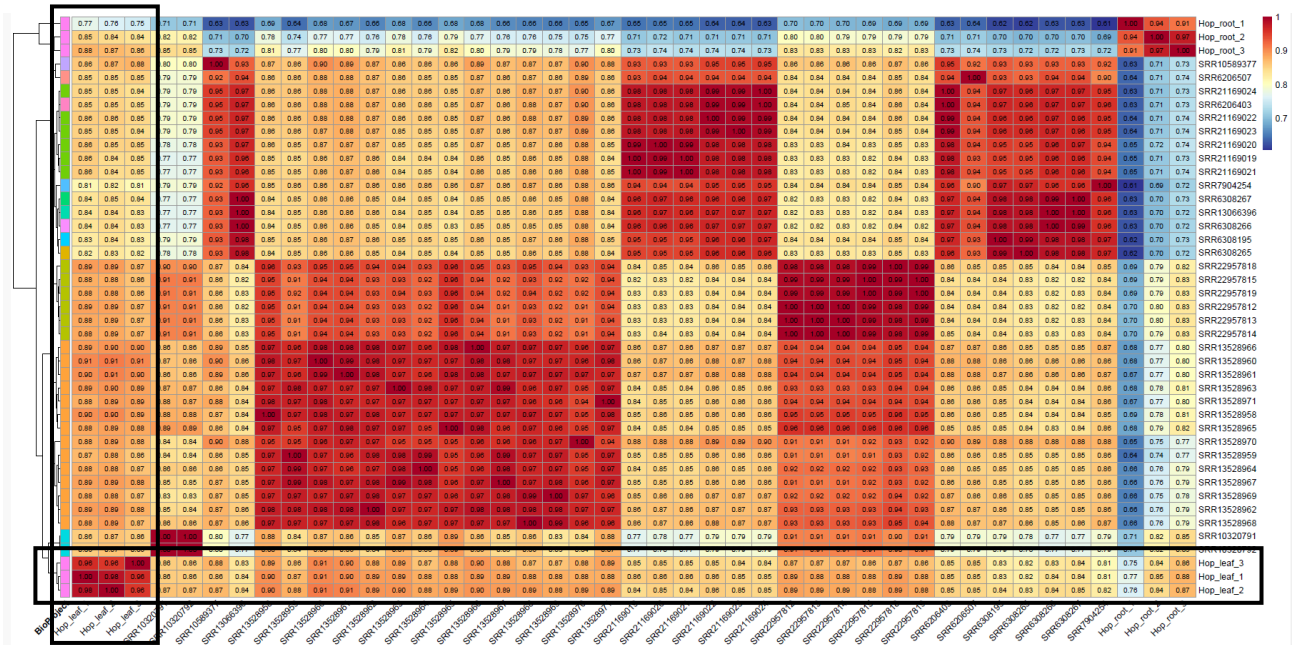


Figure S7 Spearman correlation plots illustrating organ-specific correlations of RNA-seq datasets in tomato. The plots compare independent, publicly available tomato RNA-seq data from **(a)** root and **(b)** leaf samples with the corresponding root and leaf datasets generated in this study (highlighted by black boxes). Trichome and root RNA-seq data from this study is included as a control in **(a)** and **(b)**, respectively. The colour gradient (blue to red) indicates Spearman correlation coefficient strength from low to high, with correlation coefficients shown in each cell.

(a) Leaf



(b) Trichome

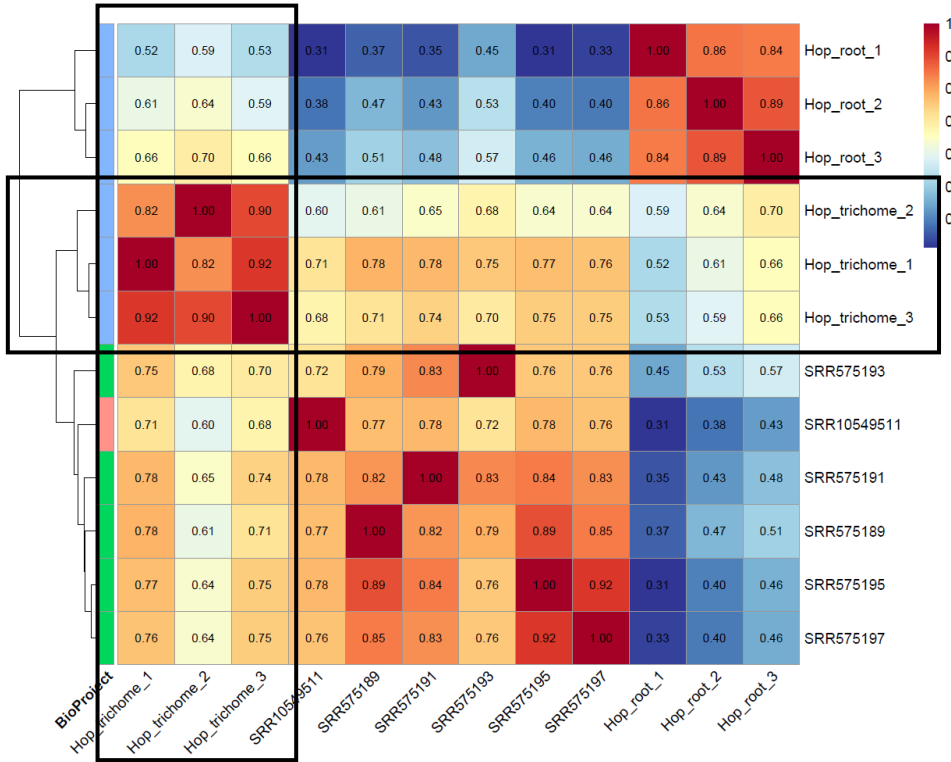


Figure S8 Spearman correlation plots illustrating organ-specific correlations of RNA-seq datasets in hop. The plot compares independent, publicly available hop RNA-seq data from (a) leaf and (b) trichome samples with the corresponding leaf and trichome dataset generated in this study (highlighted by black boxes). Root RNA-seq data from this study is included as a control. The colour gradient (blue to red) indicates Spearman correlation coefficient strength from low to high, with correlation coefficients shown in each cell.

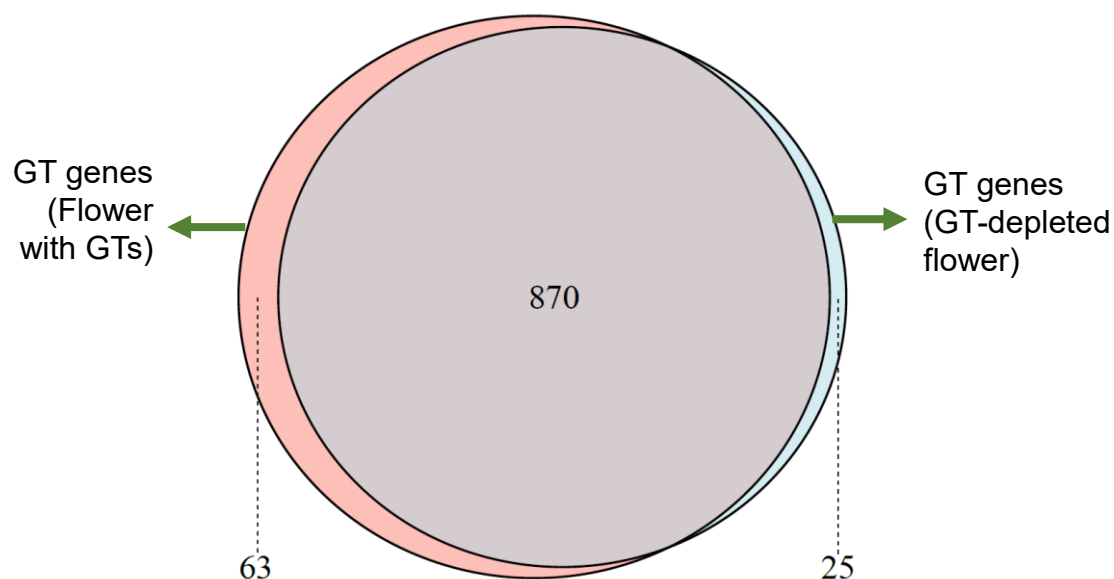


Figure S9 Venn diagram showing overlap between GT-specific gene sets identified via tau analysis. The first set (red) used transcriptomes from five organs (intact flower, trichome, root, stem and leaf), while the second (blue) used transcriptomes of trichome-depleted flower, trichome, root, stem, and leaf to assess the impact of trichomes present on flower tissue on the identification of GT-specific genes.

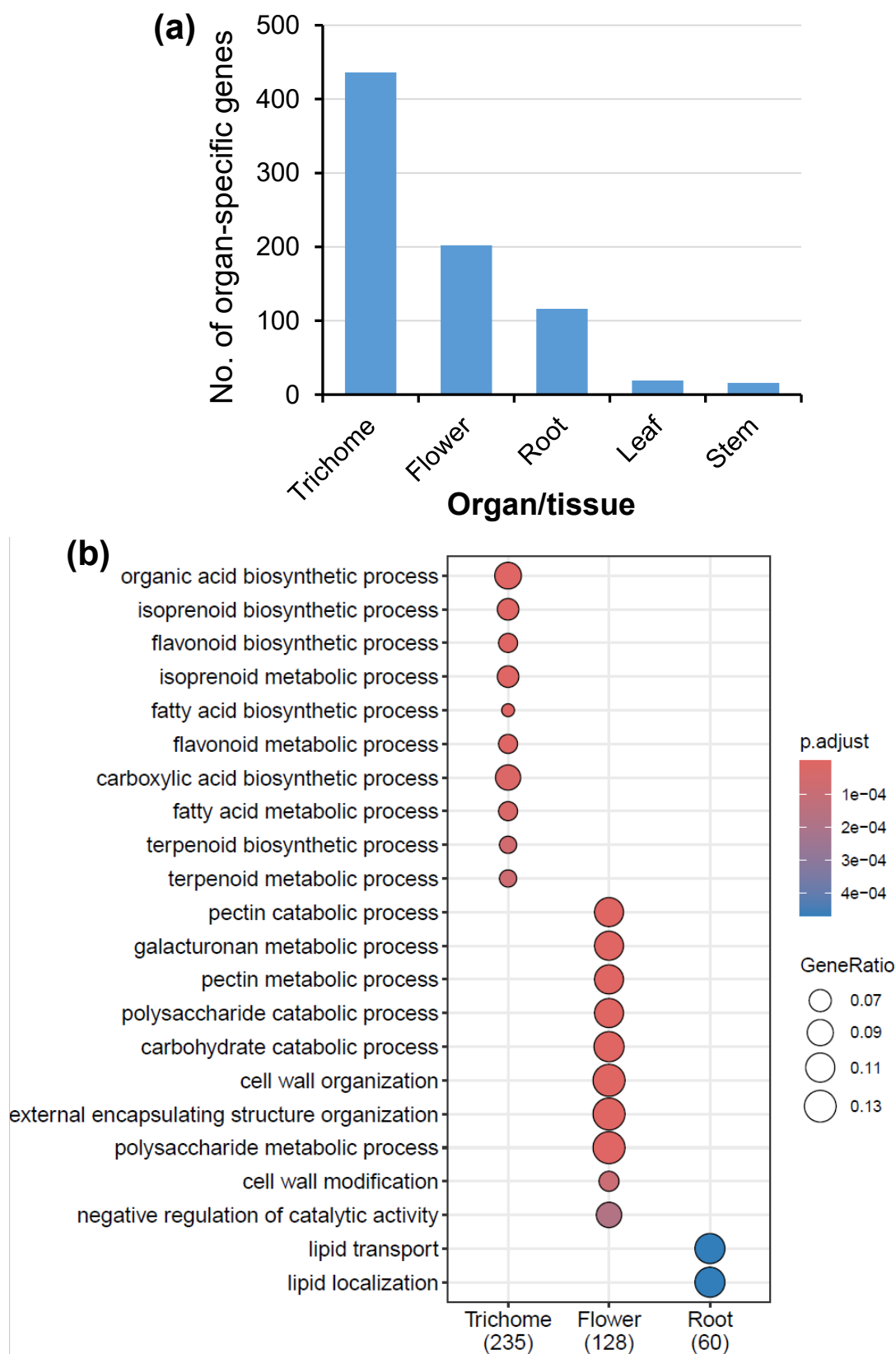


Figure S10 Independent cross-validation of organ-specific genes with published trichome data. **(a)** Distribution of tomato organ-specific genes (tau-based classification, this study) among genes upregulated in GTs compared to trichome-removed leaves from Balcke et al., 2017. **(b)** Significantly enriched GO biological process terms (adjusted p -value < 0.05) for trichome-, flower-, and root-specific gene sets. Leaf- and stem-specific genes showed no significant GO enrichment.

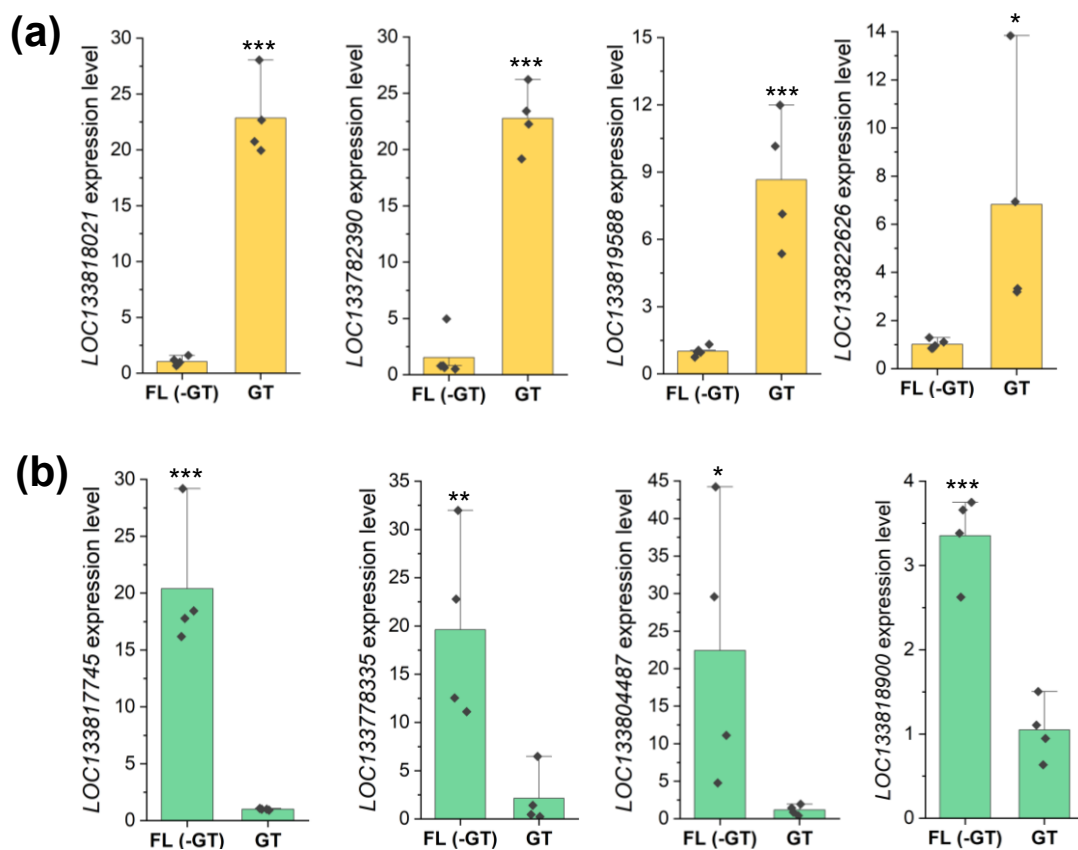


Figure S11 Quantitative real-time PCR (qRT-PCR) validation of candidate hop TFs. The relative expression of selected **(a)** trichome- and **(b)** flower-specific TFs initially identified through RNA-seq and tau analysis were validated between trichome-removed flower [FL(-GT)] and isolated GT (GT) samples. *LOC133818021* (WRKY), *LOC133782390* (C2H2), *LOC133819588* (MYB), *LOC133822626* (HB-HD-ZIP), *LOC133817745* (MYB), *LOC133778335* (GRAS), *LOC133804487* (MADS-MIKC) and *LOC133818900* (HMG). Data are presented as the mean $\pm$ standard error (SE). Statistical differences between groups were assessed using the Student's *t*-test, and significance was indicated by the following *P*-value thresholds: **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Cannabis

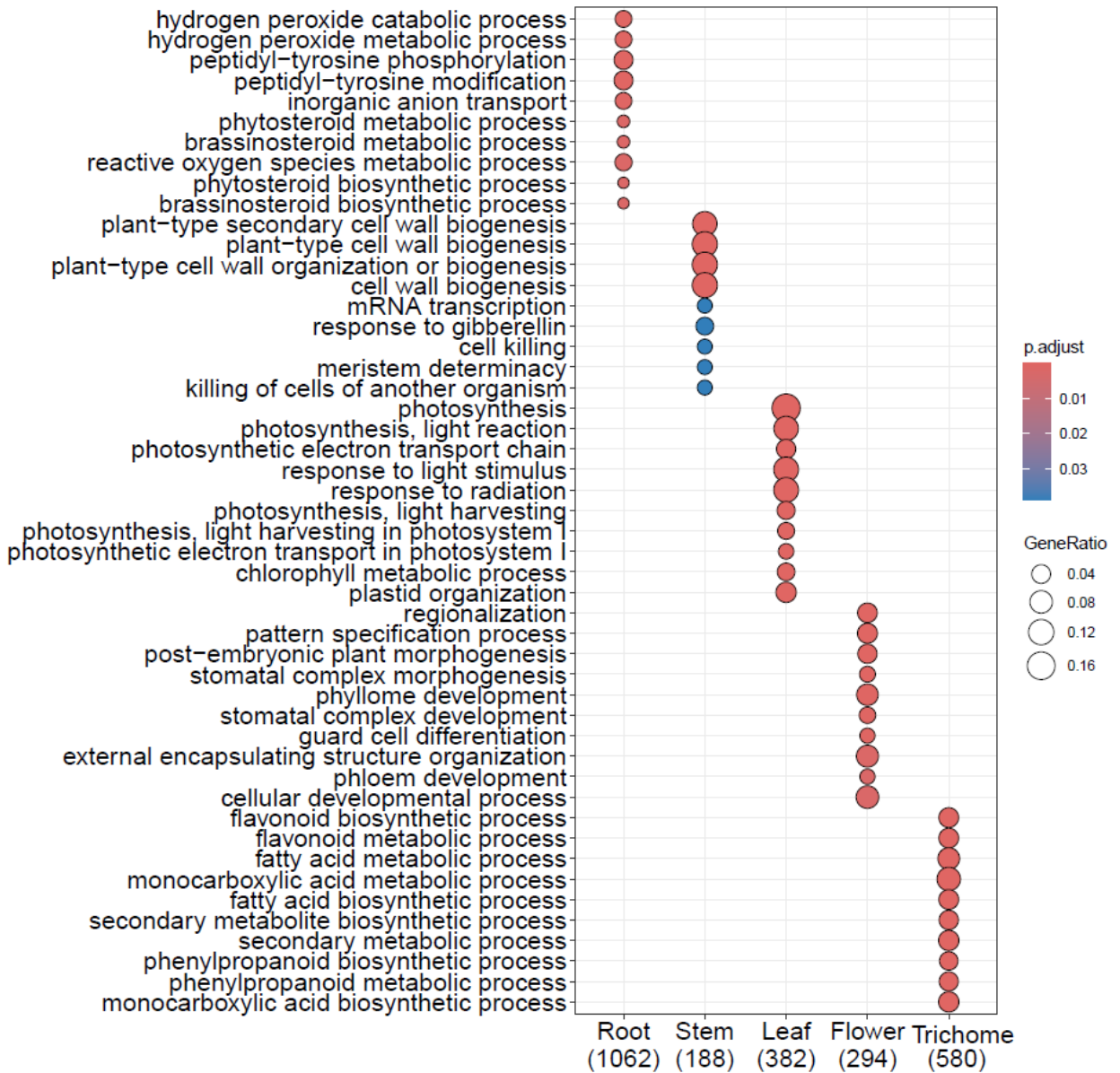


Figure S12 Dot plot showing the top 10 enriched Gene Ontology (GO) terms for organ-specifically expressed genes of cannabis. Organ names and the total number of genes within the enriched GO terms (biological process category; adjusted p -values cutoff of 0.05) selected are indicated on the x-axis. Dots are colour-coded according to the log10 (Benjamini & Hochberg) adjusted p -values and dots size represents GeneRatio (ratio of genes with enriched GO term to total genes annotated in a GO term).

Hop

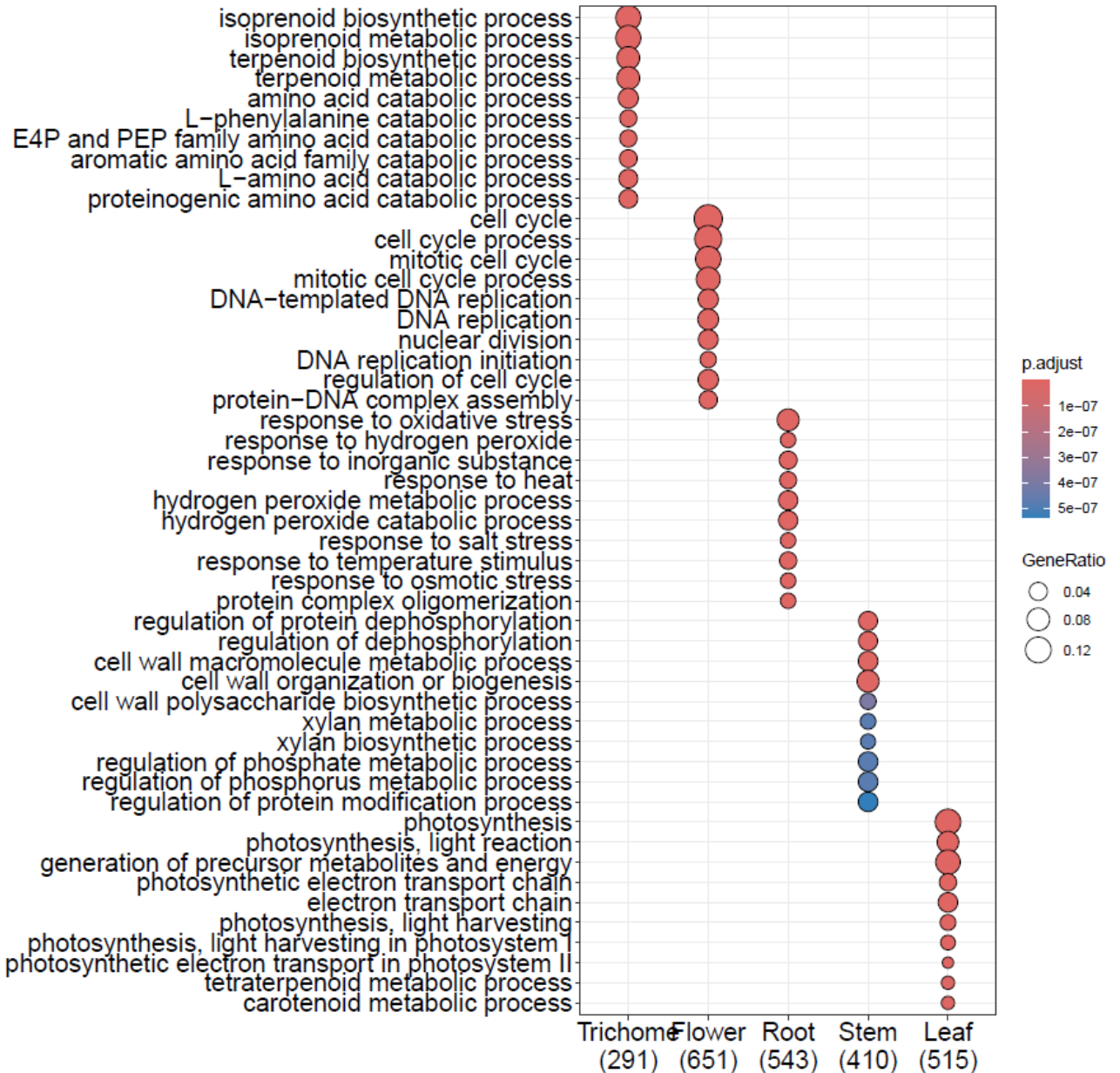


Figure S13 Dot plot showing the top 10 enriched Gene Ontology (GO) terms for organ-specifically expressed genes of hop. Organ names and the total number of genes within the enriched GO terms (biological process category; adjusted p -values cutoff of 0.05) selected are indicated on the x-axis. Dots are colour-coded according to the log₁₀ (Benjamini & Hochberg) adjusted p -values and dots size represents GeneRatio (ratio of genes with enriched GO term to total genes annotated in a GO term). E4P, erythrose 4-phosphate; PEP, phosphoenolpyruvate.

Tomato

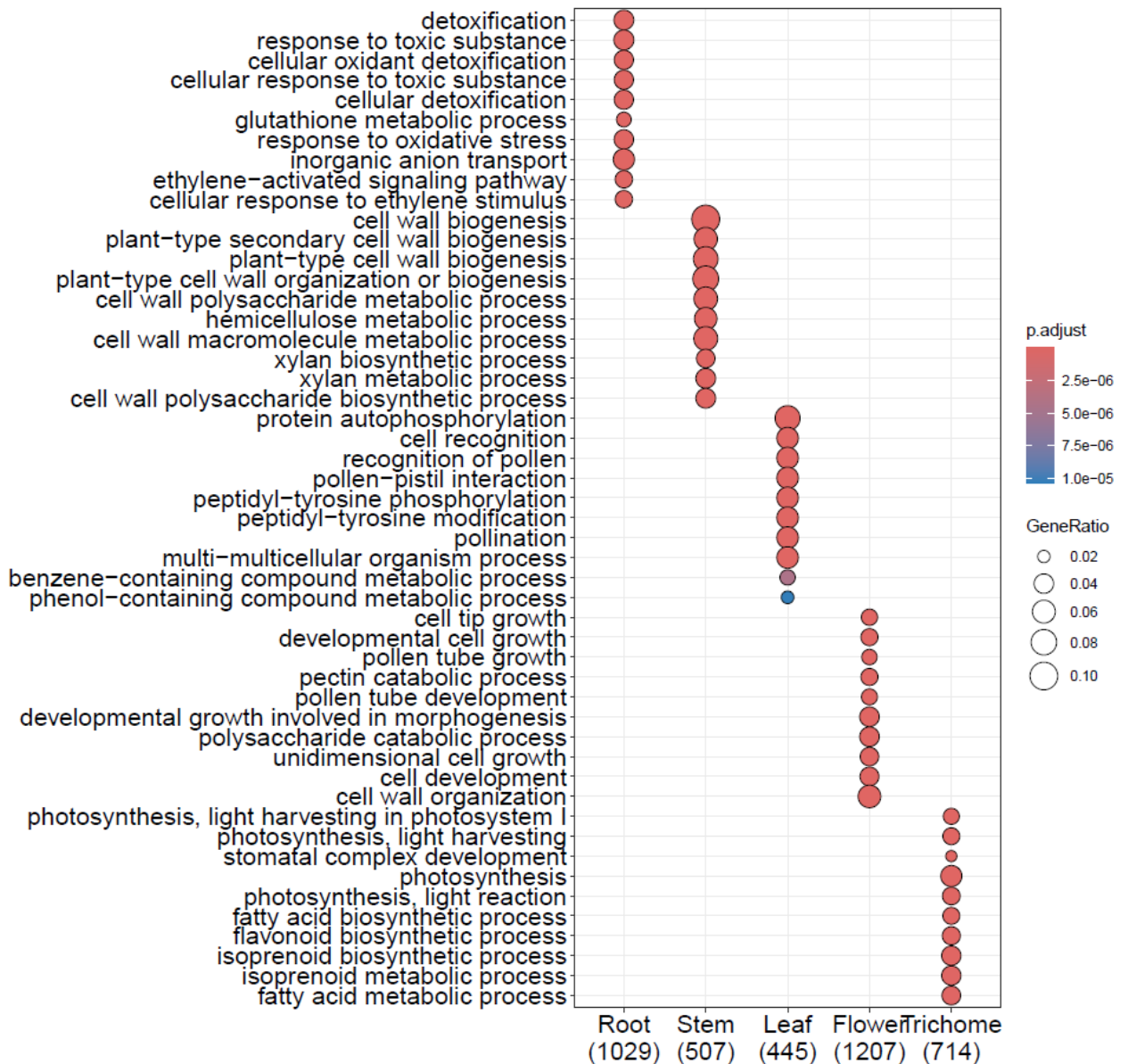


Figure S14 Dot plot showing the top 10 enriched Gene Ontology (GO) terms for organ-specifically expressed genes of tomato. Organ names and the total number of genes within the enriched GO terms (biological process category; adjusted p -values cutoff of 0.05) selected are indicated on the x-axis. Dots are colour-coded according to the log10 (Benjamini & Hochberg) adjusted p -values and dots size represents GeneRatio (ratio of genes with enriched GO term to total genes annotated in a GO term).

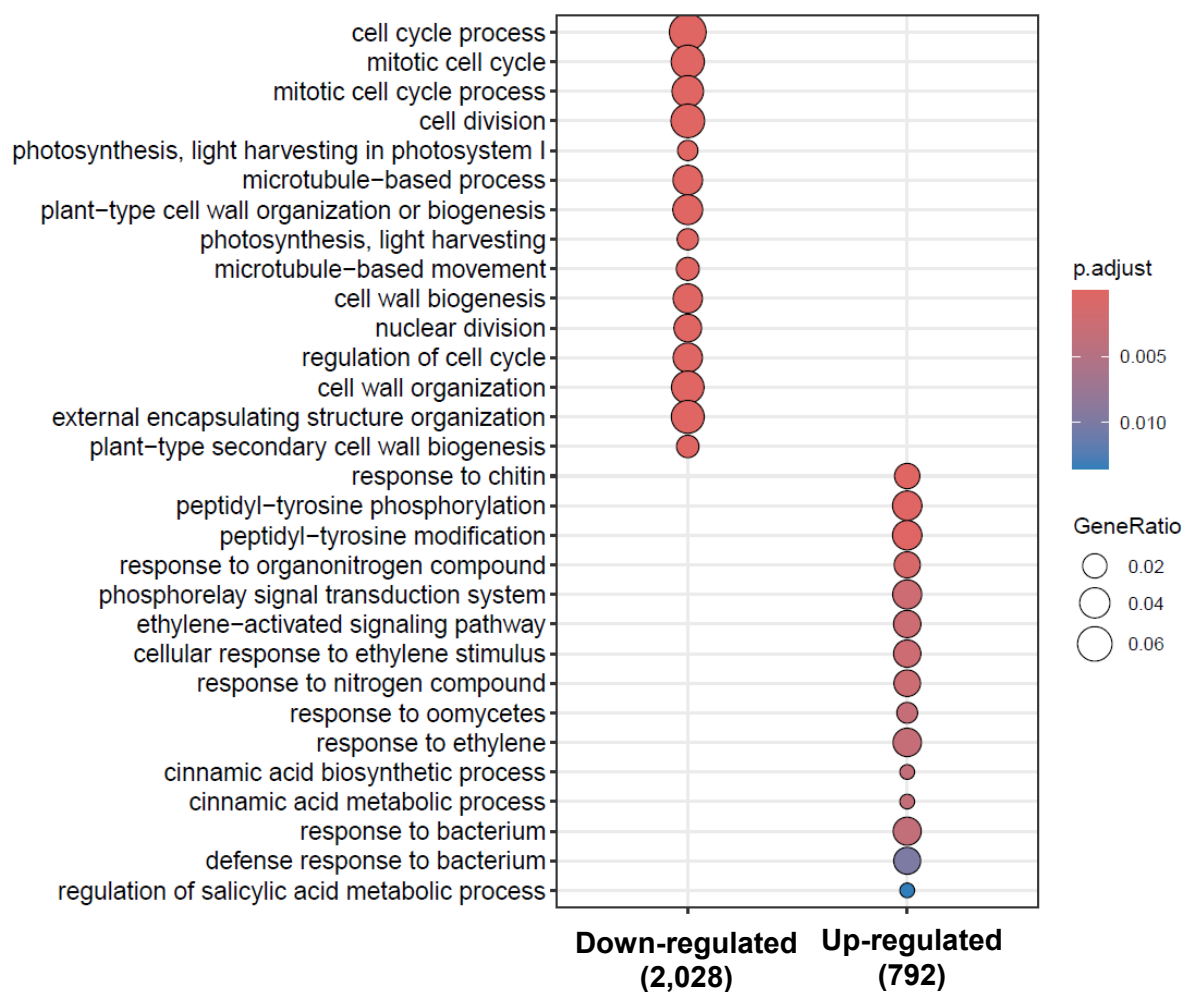


Figure S15 Top enriched GO terms for genes differentially expressed in mature tomato leaves compared to young leaves. GO terms are with respect to the biological process category (adjusted p -value < 0.05). Dots are colour-coded according to the log10 (Benjamini & Hochberg) adjusted p -values and dots size represents GeneRatio (ratio of genes with enriched GO term to total genes annotated in a GO term).

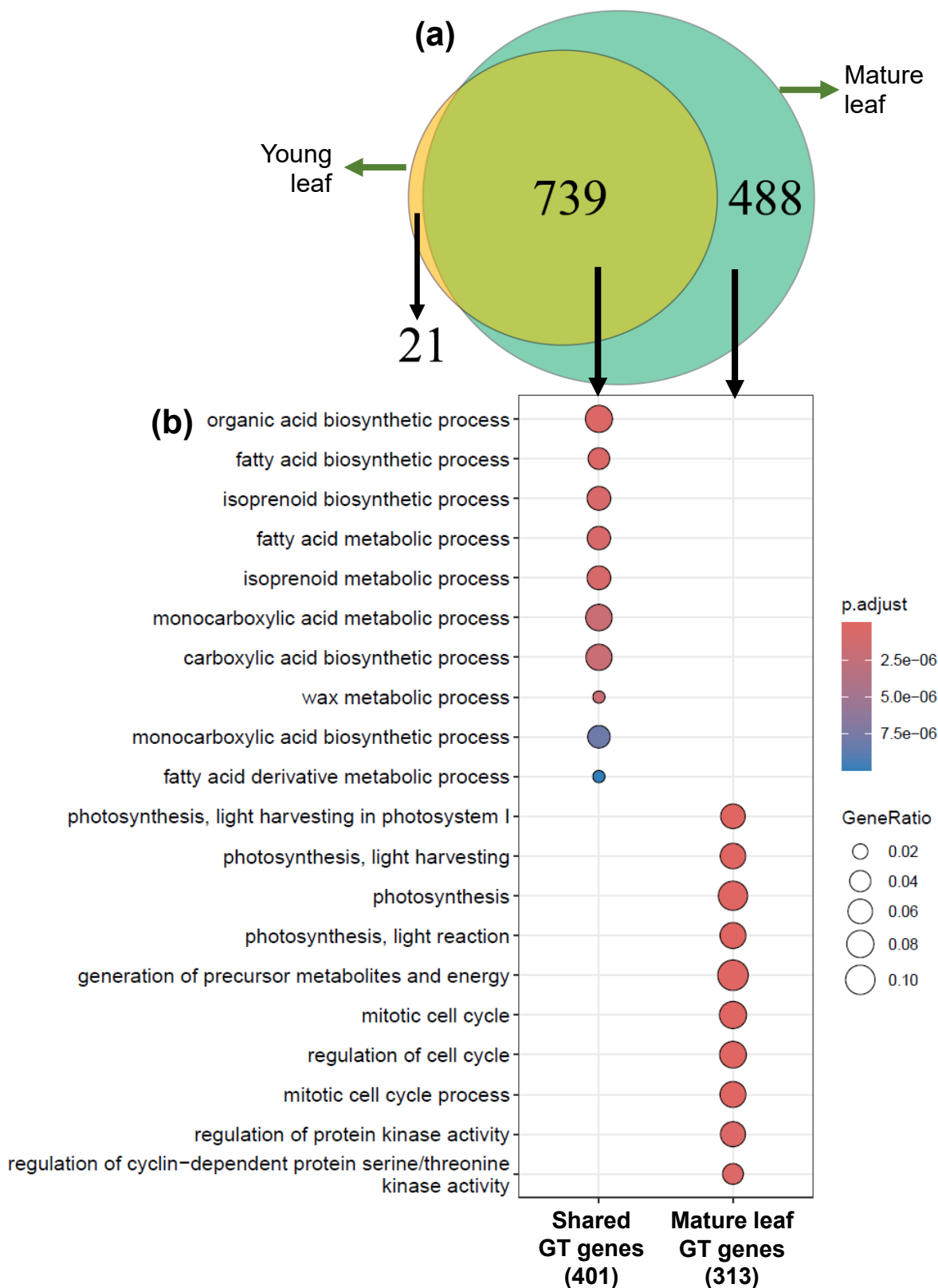


Figure S16. Comparison of GT-specific genes identified using mature versus young leaf transcriptomes. **(a)** Venn diagram illustrating overlap between GT-specific gene sets identified via tau analysis. The first set (red) used mature leaf, flower, root, stem, and GT transcriptomes; the second (blue) used young leaf, flower, root, stem, and GT transcriptomes. **(b)** Significantly enriched GO biological process terms (adjusted $p < 0.05$) for genes shared between both analyses (left panel) and genes uniquely identified when using mature leaf tissue (488 genes; right panel). Dot colour intensity indicates statistical significance ($-\log_{10}$ adjusted p -value), and dot size represents the proportion of genes annotated to each GO term (gene ratio).

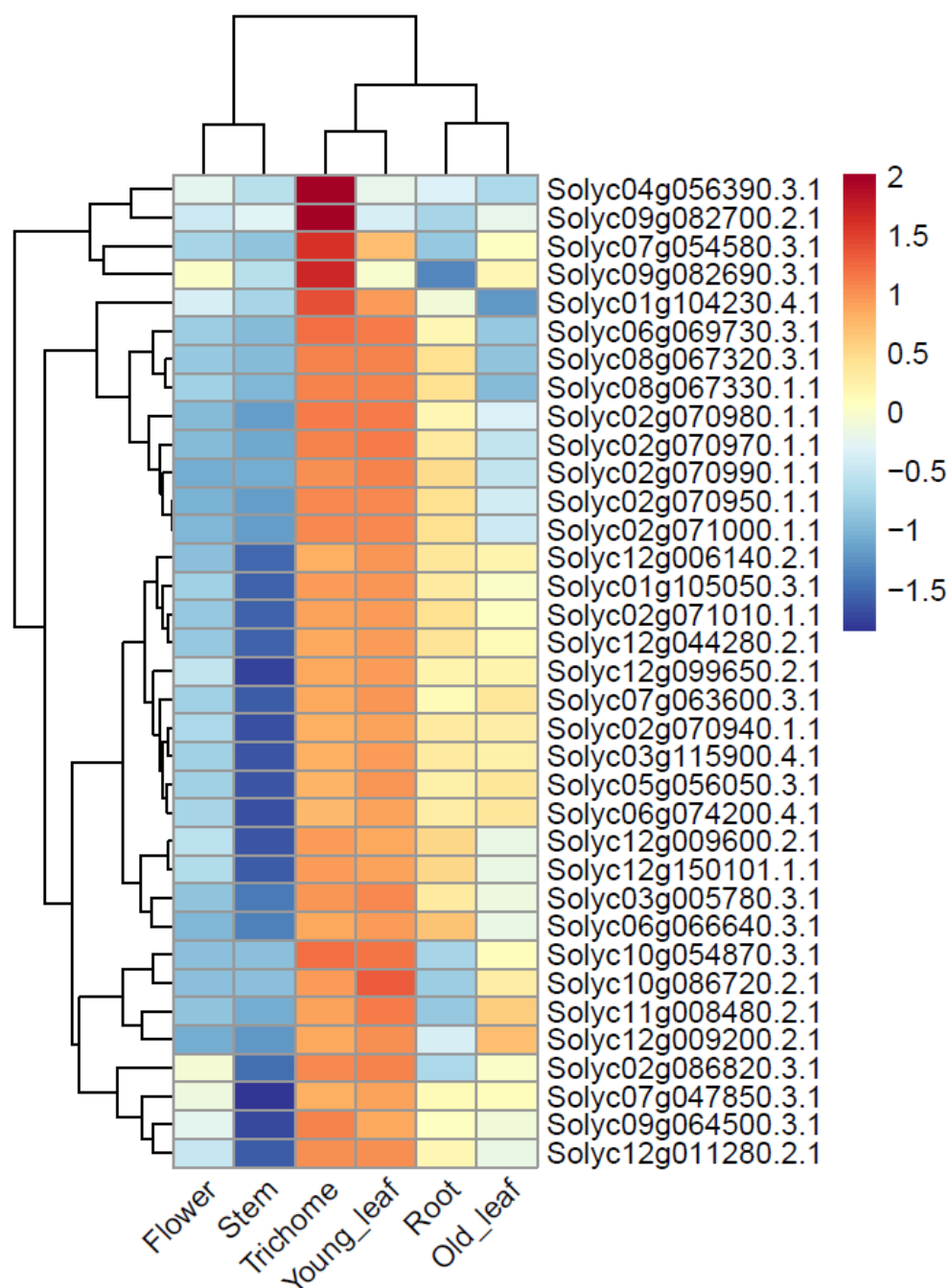


Figure S17 Heatmap showing the expression pattern of 35 genes linked to the photosynthesis and light harvesting related GO terms across different organs of tomato. These terms were associated with GT-specific genes identified using transcriptomes of mature leaf, flower, root, stem and trichomes for tau analysis. Z-score of log2-normalised transcript per million (TPM) mapped reads was used to construct the heatmaps..

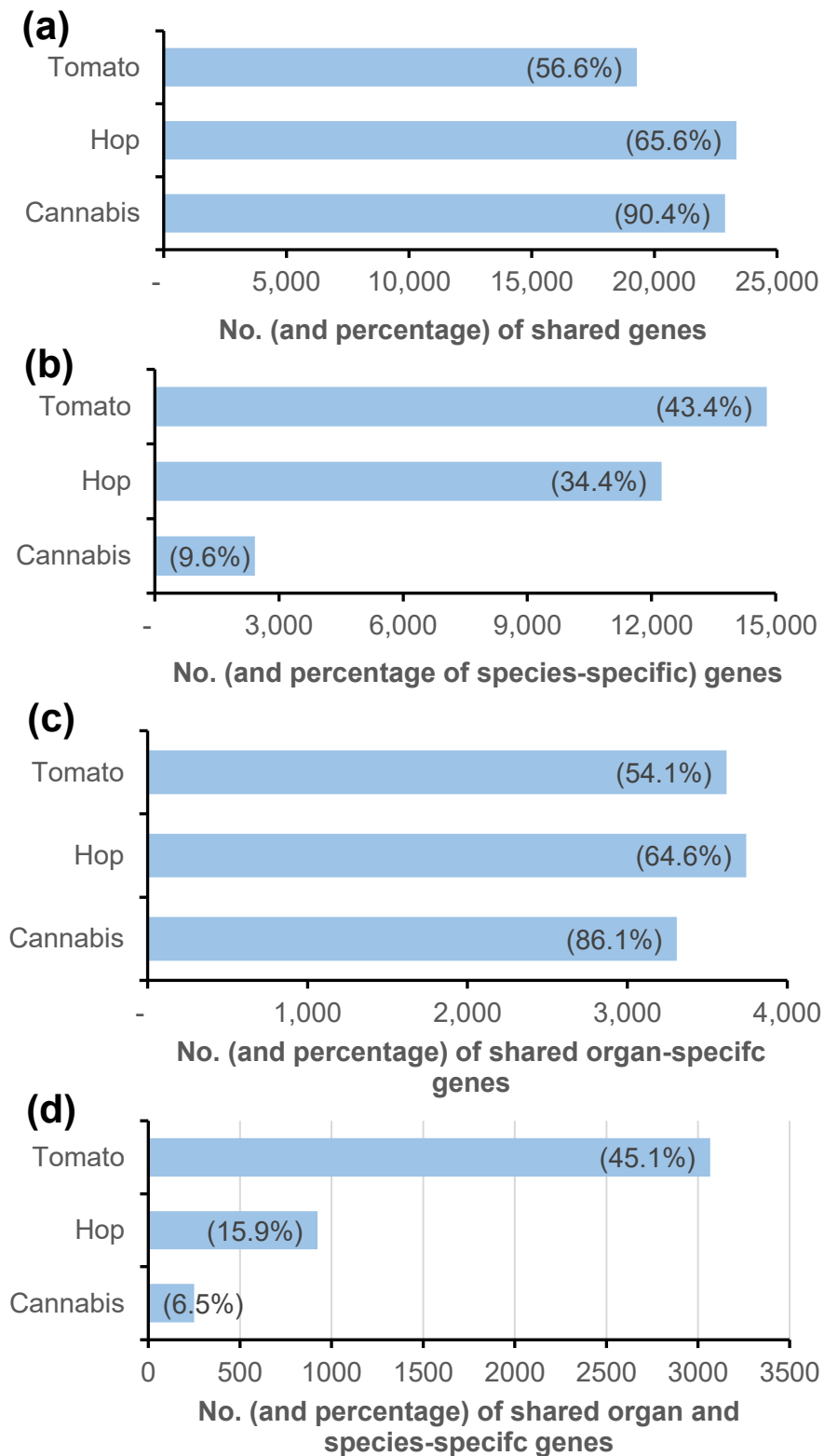


Figure S18 The conservation level of organ-specific genes determined based on HOG analysis. **(a)** The total number and percentage of cannabis, hop and tomato genes that are conserved between two or more species. **(b)** The number and percentage of cannabis, hop and tomato genes that are specific to each species. **(c)** The number and percentage of organ-specific genes that are shared across species. **(d)** The number and percentage of organ-specific genes that are species-specific. For **(a)** and **(b)**, the percentage values are based on the total number of protein-coding genes for each species (cannabis: 25,296; hop: 33,582; tomato: 34,075). For **(c)** and **(d)**, the percentage values are based on the total number of organ-specific genes for each species (cannabis: 3,844; hop: 5,796; tomato: 6,694).

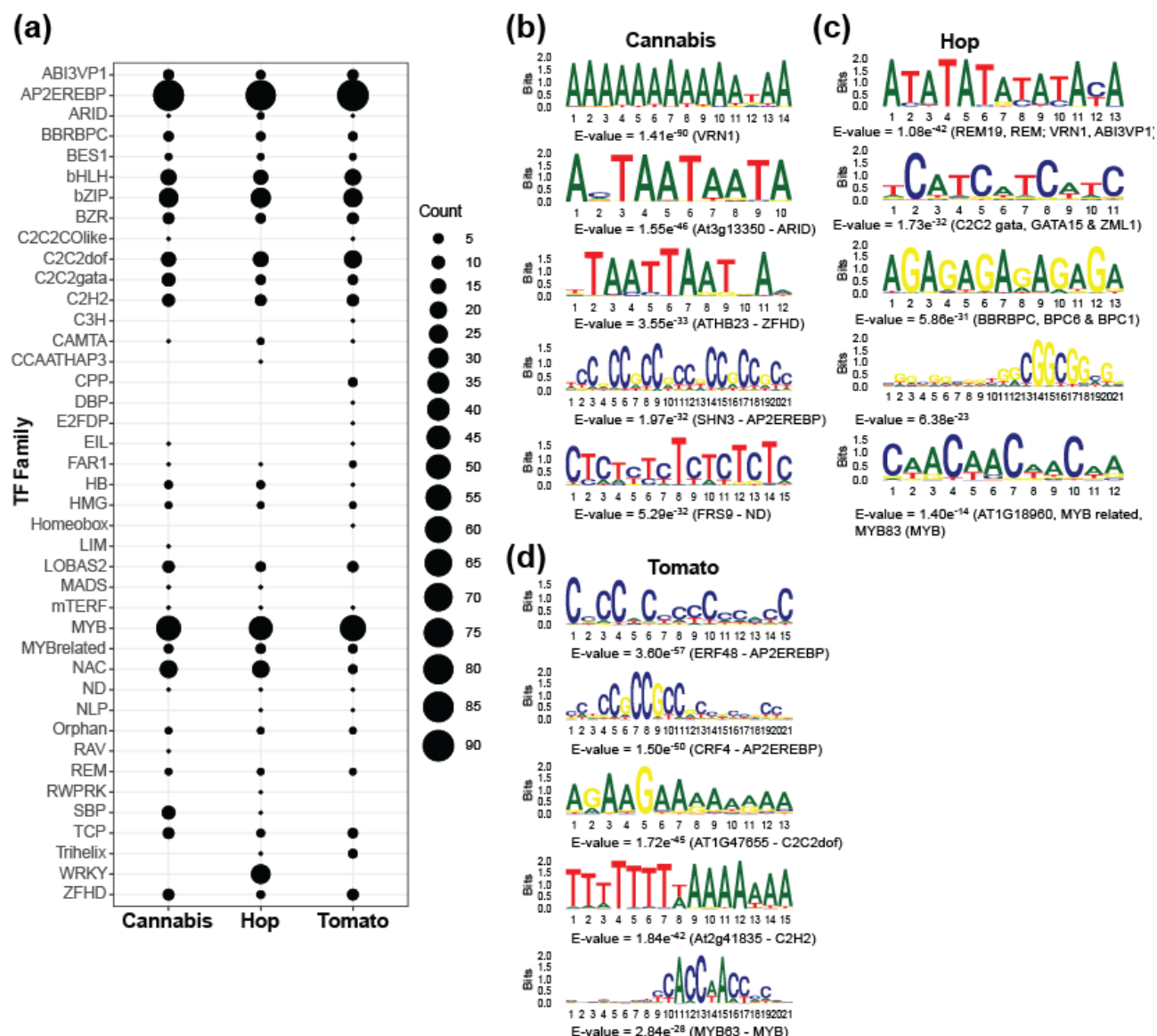


Figure S19 Motif enrichment analysis of TF targets. **(a)** Dot plot of the number of significantly enriched known motifs (E-value ≤ 0.05) detected in the promoter of the TF targets in the cannabis, hop and tomato GT GRNs and their corresponding TF families. Dot size is proportional to the number of motifs corresponding to each TF family. **(b)** Representative motifs enriched among the TF targets identified in the cannabis, **(c)** hop **(d)** tomato GT GRNs. The motifs are ordered by E-value and TF details of the most significant matches to known motifs are shown. The different font colours of the motif Logo represent the different base types, and the height of the letters corresponds to their relative frequency at a given position in the motif.

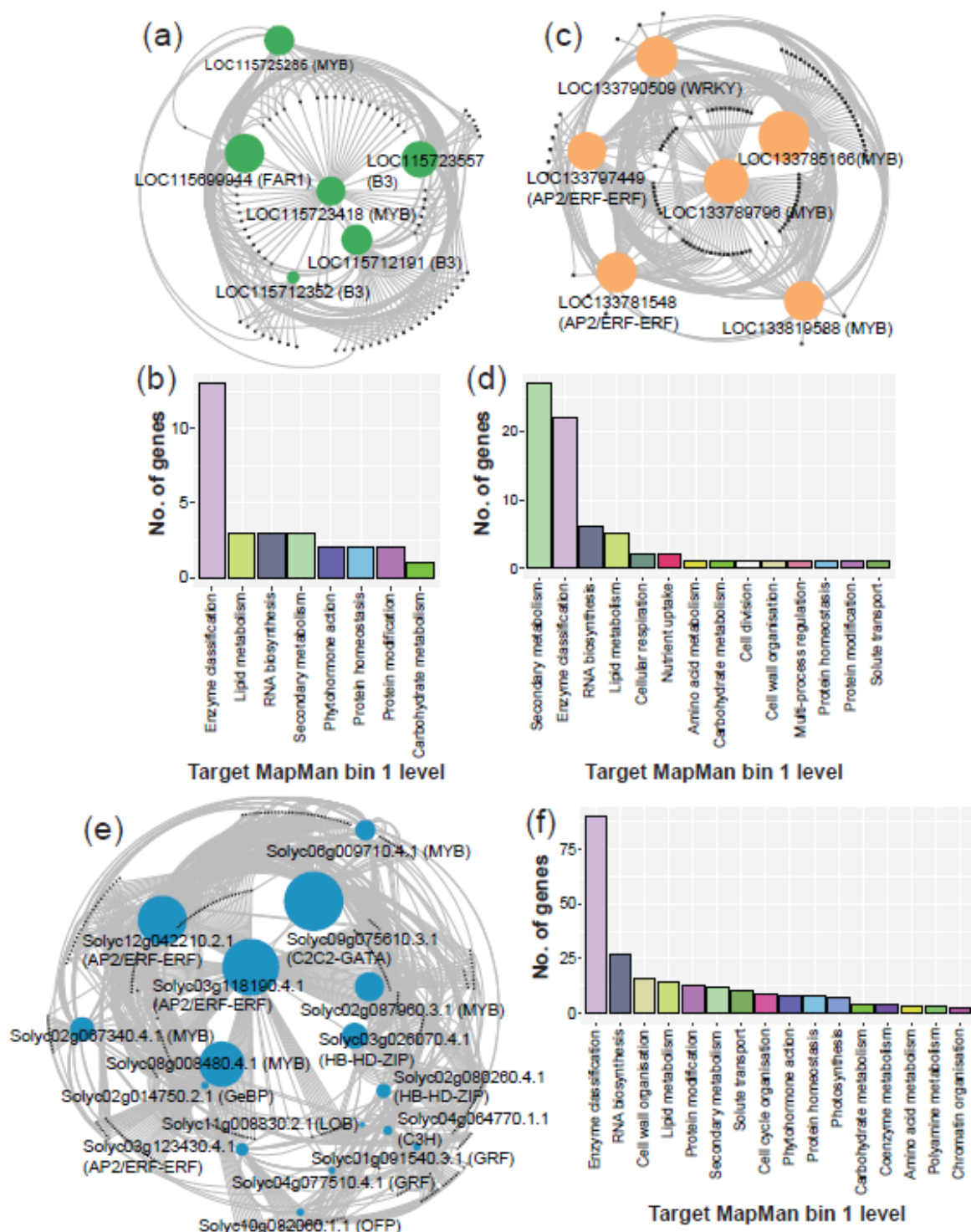


Figure S20 Species-specific GT gene regulatory sub-networks. **(a)** A gene regulatory sub-network of cannabis-specific GT TFs and target genes (no orthologs found in hop and tomato). **(b)** Barplot showing gene counts of each MapMan bin (1 level) for the gene targets included in **(a)**. **(c)** A gene regulatory sub-network of hop-specific GT TFs and target genes (no orthologs found in cannabis and tomato). **(d)** Barplot showing gene counts of each MapMan bin (1 level) for the gene targets included in **(c)**. **(e)** A gene regulatory sub-network of tomato-specific GT TFs and target genes (no orthologs found in cannabis and hop; edges representing predicted interactions were trimmed based on a weight cutoff of 0.6 for ease of visualisation). **(f)** Barplot showing gene counts of each MapMan bin (1 level) for the gene targets included in **(e)**. In **(a)**, **(c)** and **(e)**, TFs and downstream target genes are represented by coloured and black nodes, respectively. Lines indicate the edges, and TF node size is proportional to the number of interactions multiplied by a scaling factor of 5. In **(b)**, **(d)** and **(f)**, Barplots are color-coded according to the MapMan bins identified. 'Not assigned.not annotated' and 'not assigned.annotated' bins were excluded.

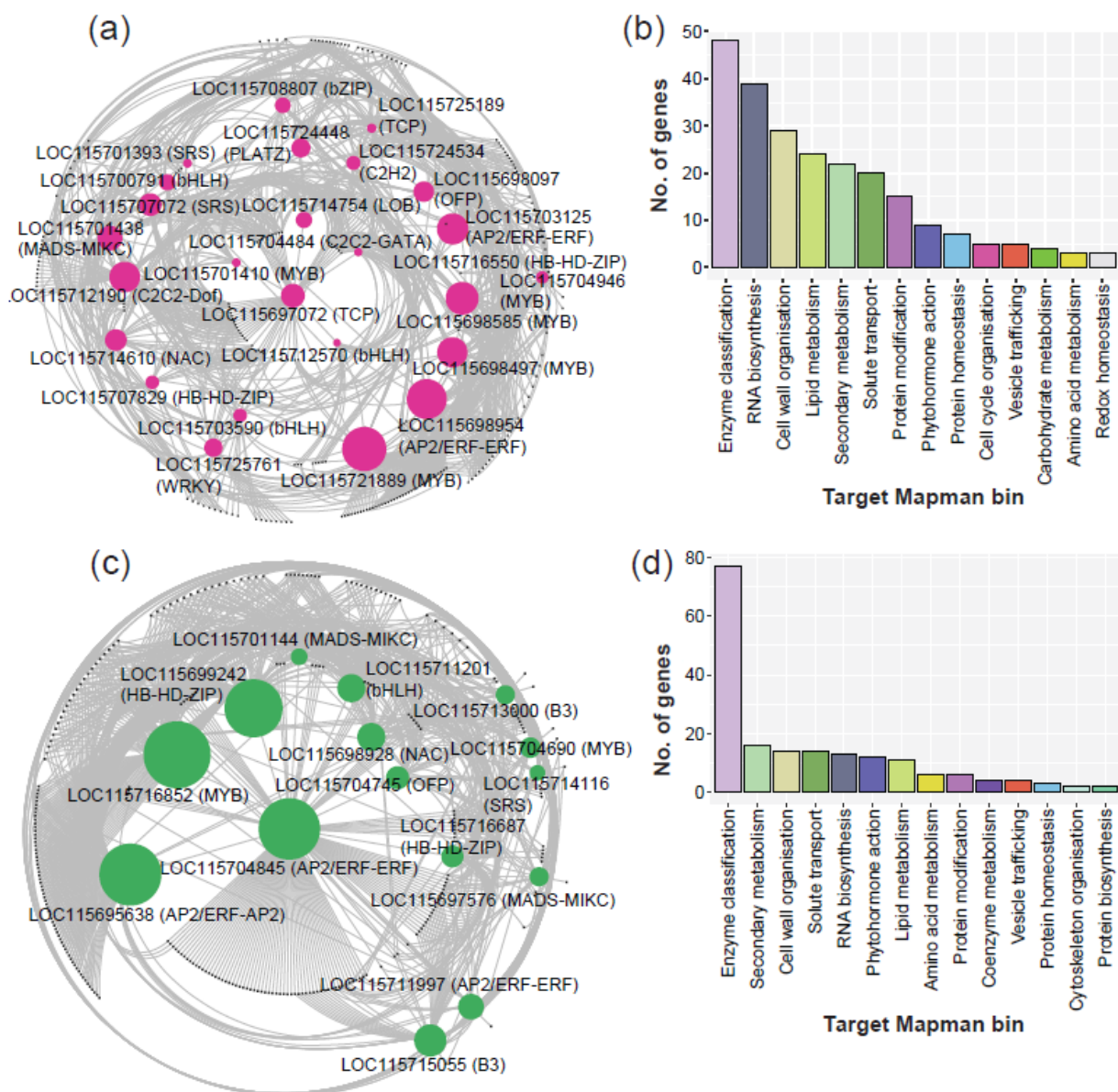


Figure S21 Shared GT-specific gene regulatory sub-networks. **(a)** A gene regulatory sub-network of cannabis GT TFs and target genes with predicted orthologs in hop and tomato. **(b)** Barplot showing gene counts of each MapMan bin (1 level) for the target genes included in **(a)**. **(c)** A gene regulatory sub-network of cannabis GT TFs and target genes with predicted orthologs in hop GT only. **(d)** Barplot showing gene counts of each MapMan bin (1 level) for the target genes included in **(c)**. For **(a)** and **(c)**, coloured and black nodes represent TFs and their targets, respectively. Grey lines indicate the predicted interactions (edges), and TF node size is proportional to the number of TF-target interactions. An edge weight cut-off of 0.5 and 0.7 was applied to **(a)** and **(c)**, respectively and nodes having fewer than 15 interactions were excluded for ease of visualisation in **(a)**. Barplots are colour-coded according to the MapMan bins identified. 'Not assigned.not annotated' and 'not assigned.annotated' bins were excluded.

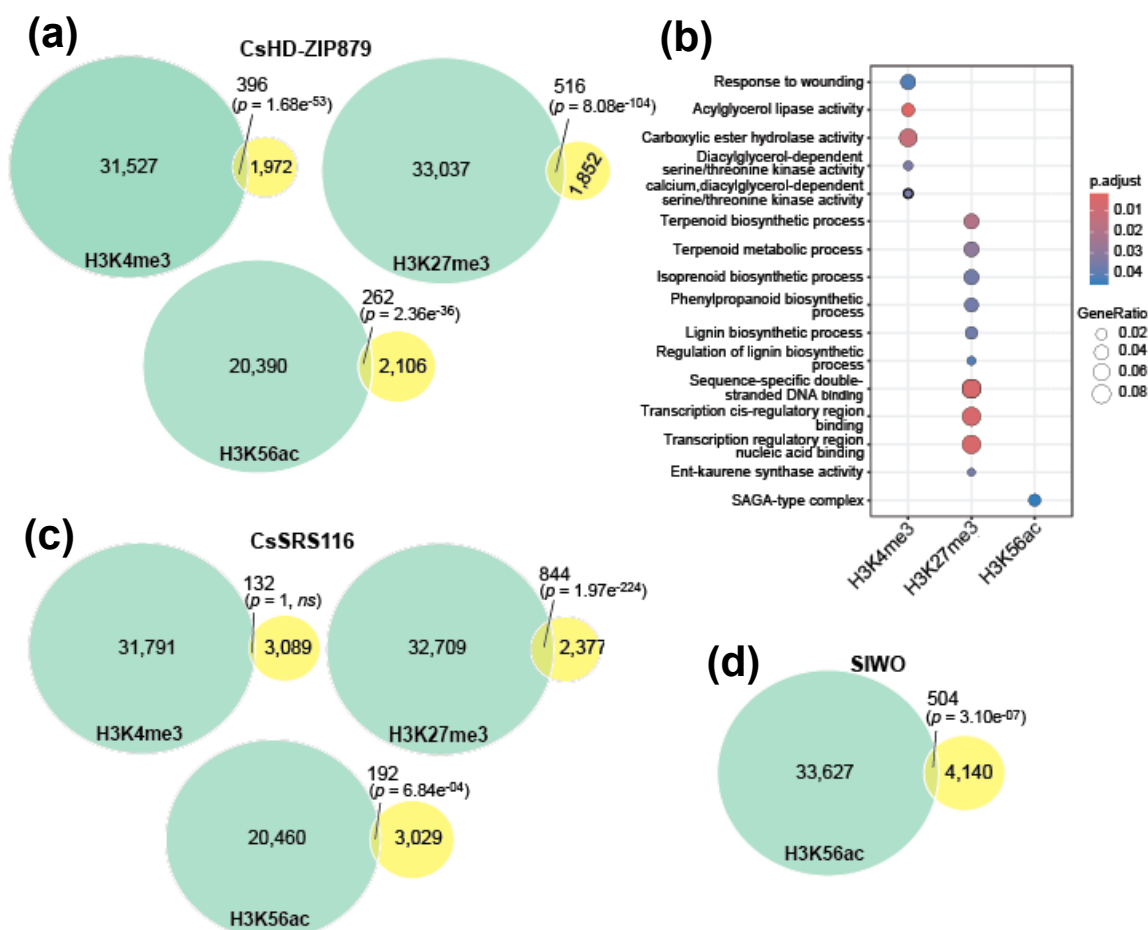


Figure S22 DAP-seq validation using ChIP-seq data for histone marks. **(a)** Venn diagrams showing overlap between 2-kb bins containing CsHD-ZIP879 DAP-seq binding sites (shown in yellow) and ChIP-seq peaks for H3K4me3 (396 bins), H3K27me3 (516 bins), and H3K56ac (262 bins) (in yellow). **(b)** Top significantly enriched GO biological process terms (adjusted $p < 0.05$) for genes at histone-marked sites overlapping with CsHD-ZIP879 binding sites. **(c)** Venn diagrams showing overlap between 2-kb bins containing CsSRS116 DAP-seq peaks and ChIP-seq peaks for H3K4me3 (132 bins), H3K27me3 (844 bins), and H3K56ac (192 bins). **(d)** Venn diagram showing overlap between 2-kb bins containing SIWO DAP-seq peaks and H3K4me3 peaks (504 bins). Overlap significance in (a), (c), and (d) was assessed using hypergeometric test ($p < 0.05$).

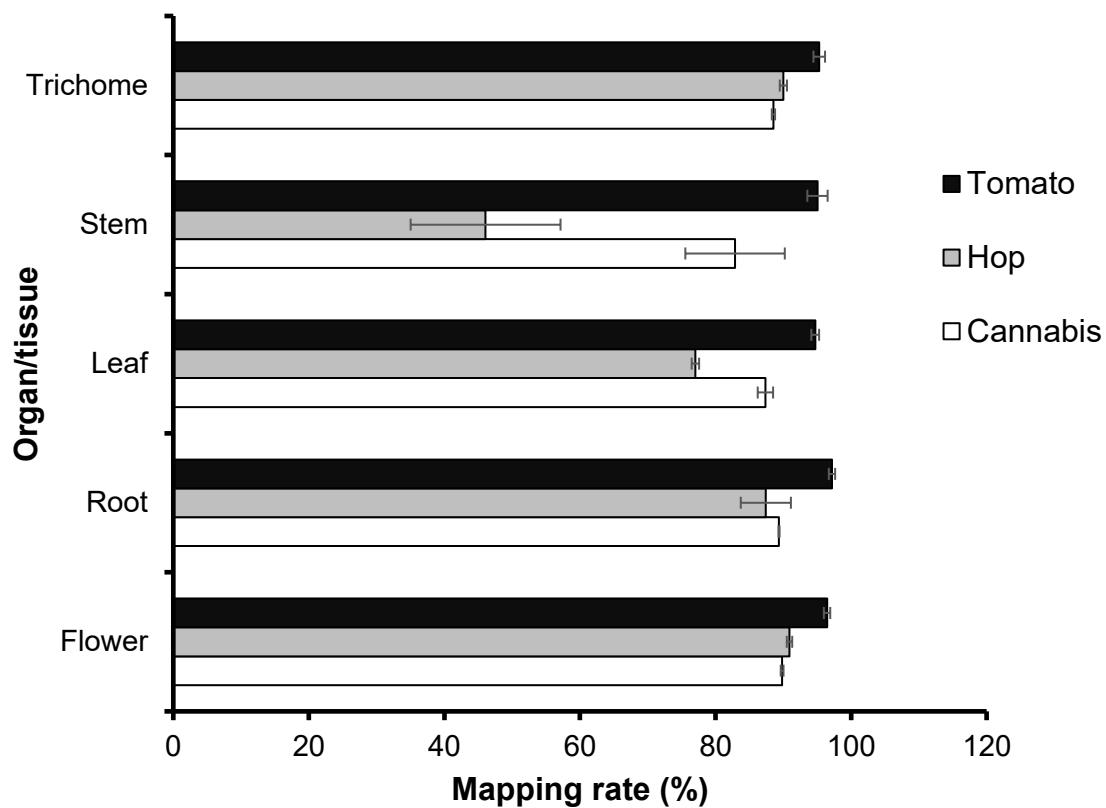


Figure S23 Hisat2 mapping statistics for RNA-seq data used to determine organ-specificity in cannabis, hop and tomato. Values are based on mean $\pm$ SE of three biological replicates.