Identification of rooting genes using bioinformatics tools

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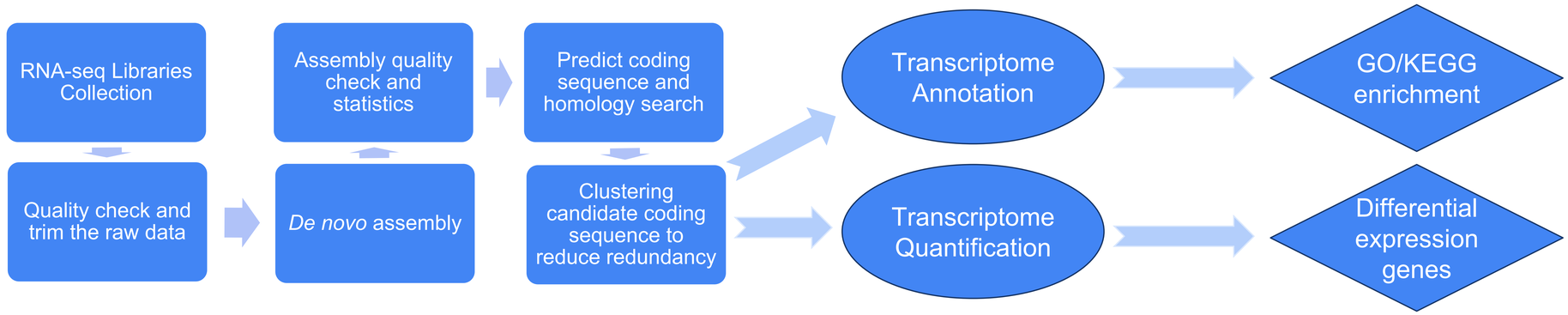
Background

The golden camellia (*Camellia nitidissima* Chi.) is a well-known ornamental plant, often referred to as "the queen of camellias" due to its golden yellow flowers. While most camellia species and cultivars have red, pink, white, or purple flowers, yellow flowers are rare. This rarity is primarily because *C. nitidissima* is difficult to propagate under natural conditions and is distributed in very limited areas. To address this issue, we used the *de novo* assembly method with publicly available RNA-seq raw data to obtain a reference transcriptome. We then aim to investigate the genes involved in adventitious root formation in *C. nitidissima* to facilitate developing an effective method for its rapid clonal propagation.

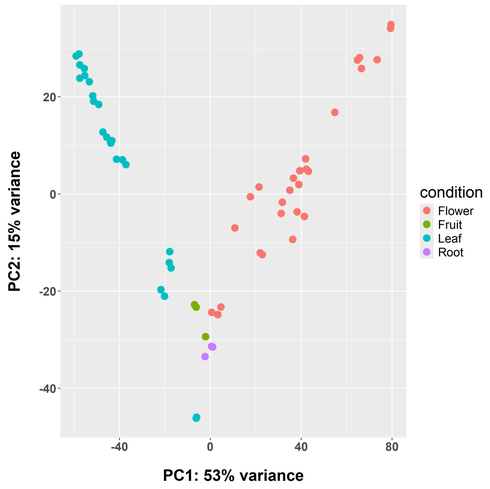
Method



Lignified YC stem with AR primordia

Figures of yellow camellia (*Camellia nitidissima* Chi.) buds/flowers and rooted cuttings 1.5 months after auxin induction

PCA for RNAseq Libraries



Principal component analysis of trimmed RNA-seq data shows that 57 of 58 libraries cluster by tissue type, with one Leaf sample as an exception. Most Flower and Leaf libraries group in the top left or right corners.

Differential expressed genes results

Group	Down_regulated	Up_regulated	Total_DEGs
Flower_vs_Leaf	5605	4505	10110
Root_vs_Leaf	2196	1632	3828
Fruit_vs_Leaf	1895	651	2546
Fruit_vs_Root	765	365	1130
Flower_vs_Root	1853	1897	3750
Fruit_vs_Flower	569	1769	2338

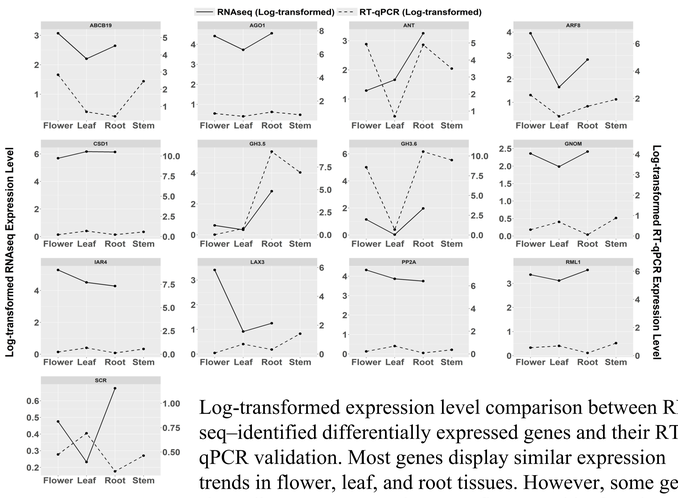
The table above presents the DEG results, filtered with criteria "padj < 0.001 and log2FC >= 2". The "Flower_vs_Leaf" group shows the highest number of differential genes, with 4,505 up-regulated and 5,605 down-regulated. Compared to leaf, the other three tissues have more down-regulated than up-regulated genes, with "Fruit_vs_Leaf" having the least DEGs. And for the other three comparisions, "Flower_vs_Root" has the largest number of DEGs.

Conclusion and Future works

The yellow-flowering camellia (*Camellia nitidissima* Chi.) was used as a case study to demonstrate how publicly available genomic data can be leveraged to identify candidate genes involved in rooting. Using bulk RNA-seq data, we assembled a de novo transcriptome that provides a reference for differential gene expression (DEGs) analysis, functional annotation, and enrichment studies in C. nitidissima. Selected auxin response factors (ARF) were using RT-qPCR to check the relative expression levels and compared with These results establish a foundation for future investigations into gene function and the molecular mechanisms underlying adventitious root development.

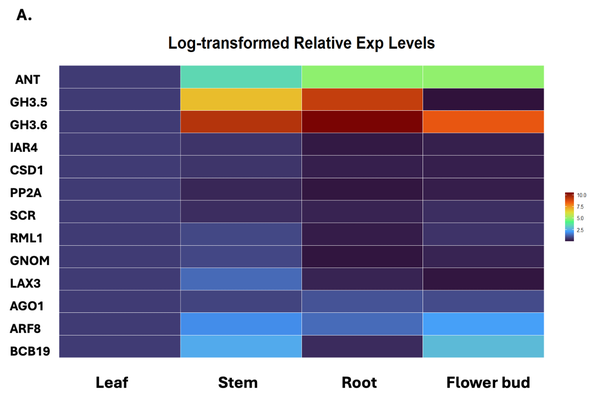
Building on this work, our next phase in American chestnut research will integrate multiple approaches, including advanced RNA sequencing platforms, confocal imaging, and molecular biotechnology. By mining and characterizing key transcription factors and validating their functions in established model systems such as Arabidopsis and poplar, we aim to uncover the regulatory networks that govern root formation and contribute to tree improvement.

Expression Level Comparison

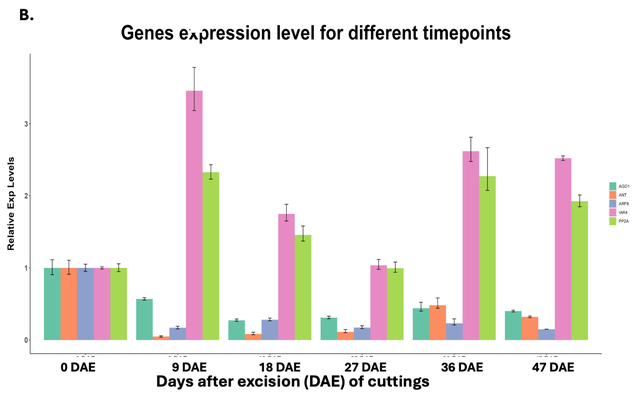


Log-transformed expression level comparison between RNA-seq–identified differentially expressed genes and their RT-qPCR validation. Most genes display similar expression trends in flower, leaf, and root tissues. However, some genes show divergent patterns between flower and leaf, and RML1 exhibits a distinct difference between leaf and root.

RT-qPCR Verification



RT-qPCR using leaf as the calibrator reveals differential expression of 13 auxin response factors. Most genes in the other three tissues show higher expression than in leaf, with GH3.5 and GH3.6 exhibiting the highest expression in root.



RT-qPCR results from stem samples, using 0-day as the calibrator, show that IAR4 and PP2A are most expressed—peaking on day 9. In contrast, AGO1, ANT, and ARF8 exhibit lower expression, with ANT being the lowest overall.

Acknowledgment