Investigating the Genes and Biochemical Pathway Responsible for Caffeine Production in Ilex vomitoria (Yaupon Holly)

Tong, Claire (School: Kalamazoo Area Mathematics and Science Center)
Yao, Janelle (School: Kalamazoo Area Mathematics and Science Center)
Goel, Aanya (School: Kalamazoo Area Mathematics and Science Center)

Coffee and tea are among the world's most popular drinks, containing large quantities of caffeine, a central nervous system stimulant. Many plants have been shown to produce caffeine by using three genes either labeled caffeine synthase (CS) or xanthine methyltransferase (XMT). One plant that produces caffeine is Ilex vomitoria (Yaupon Holly), one of the only two caffeine-producing plants in North America. It is closely related to Ilex paraguariensis (Yerba Mate), the only other known Ilex species capable of producing caffeine. We investigated three hypotheses: (1) we tested the presence of caffeine synthase type genes in Ilex vomitoria, (2) we tested whether the genes responsible for caffeine synthesis in Ilex vomitoria and Ilex paraguariensis were closely related, and (3) we tested for similarities in the biochemical pathways for caffeine production in the two species. To investigate these hypotheses, we first conducted transcriptome sequencing. Then, we identified caffeine synthase genes that were present in the Ilex vomitoria RNA sequence, and there was a clear lack of XMT genes. To amplify our three candidate genes, we conducted Reverse Transcriptase-Polymerase Chain Reaction. Afterwards, these genes were cloned and transformed into Escherichia coli. We used an enzyme assay to test these enzymes from our cultures, creating a phylogenetic tree to analyze this data. The tree indicated that IvRT1 is closely related to IpCS3 and that IvRT8 is closely related to IpCS2. However, there was no sequence in the Ilex vomitoria transcriptome that closely matches IpCS1. Lastly, our enzyme assay showed that IvRT8 methylates 3-methylxanthine to produce theobromine, as expected. This result is similar to the known biochemical pathway of Ilex paraguariensis.

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