

# Engineering Triterpene Methyltransferase in Tobacco

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*Botryococcus braunii* race B is a green algae that generates large amounts of triterpene oils, such as squalene, which are known progenitors of modern combustible fuels. Triterpene methyltransferases (TMTs) are enzymes characteristic of the algae that convert triterpenes (C<sub>30</sub>) to methylated triterpenes (C<sub>31</sub>-C<sub>32</sub>), which produce higher quality fuels through hydrocracking. However, this algae cannot be a production host for renewable biofuels because of its slow growth rate. Engineering triterpene biosynthesis genes from *B. braunii* to a larger, faster growing foundation plant could increase biofuel production to usable levels. In this study, *Agrobacterium*-mediated plant transformation was conducted to transfer TMT genes into the tobacco genome, and independent T<sub>0</sub> transgenic tobacco plants were generated in antibiotic selection medium. Leaf material from each transgenic line was collected and chemically analyzed for terpene contents by gas chromatography-mass spectrometry (GC-MS). In the event that TMT genes are successfully expressed and function properly, methylated squalene (C<sub>31</sub>-C<sub>32</sub>) will be detected in the transgenic plants, but not in wild type plants. The results will provide strong evidence whether the algal triterpene methyltransferases can be successfully engineered in higher plants, such as tobacco, for biofuel production.