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Exploring *Brassica* for low viscosity biodiesel production through genetic engineering

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Energy crises and environmental concerns are driving researchers to develop viable alternative fuels from renewable sources. The use of *Brassica juncea* oil as an alternative fuels suffers from problems such as high viscosity, low volatility and poor cold temperature properties. The seed of Euonymus alatus produces unusual triacylglycerol (TAGs) called acetyl triacylglycerol (acTAGs) where the sn^{-3} position is esterified with acetate instead of a long chain fatty acid. The enzyme Euonymus alatus diacylglycerol acetyltransferase (EaDacT) present in these plants is an acetyltransferase that catalyzes the transfer of an acetyl group from acetyl-CoA to diacylglycerol (DAG) to produce acetyl TAG (AcTAG). In order to reduce the viscosity of *Brassica juncea* oil by synthesizing acTAG, we have optimized an efficient and simple agrobacterium mediated floral dip transformation method to generate transgenic *Brassica juncea* plants with EaDacT gene. A binary vector containing the EaDacT gene under the transcriptional control of a glycinin promoter and with a basta selection marker was transformed into *Agrobacterium tumefaciens* strain GV-3101 through electroporation and subsequently to *B. juncea* through floral dip method. The basta resistant putative transgenic plants were further confirmed by PCR. The developed transgenic *B. juncea* seeds showed altered TAG fatty acid composition with enhanced level of oleic acid (from 41% to 63%) and reduced Erucic acid level (11.18%), which is an ideal composition of fatty acids to be used as biodiesel. The results showed that the Agrobacterium-mediated floral-dip transformation can be a successful strategy to develop transgenic *Brassica Juncea* having oil with modified fatty acids profile that could directly be used as biodiesel. Further, the developed protocols could be used to accumulate unusual acTAG in *B. juncea* seed, providing a direct way of biodiesel production from plant oil.

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How does genetic modification impact the fractionation, depolymerization and catalytic upgrading of lignin from engineered plants?

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Developing biomass feedstocks with desirable traits for cost effective conversion is one of the main focus areas in biofuels research. Pretreatment is a crucial step for making biomass feedstocks more amenable to biological conversion by unlocking sugars for fermentation. Nevertheless, as suggested by techno-economic analyses, the success of a lignocellulose-based biorefinery largely relies on the utilization of lignin to generate value-added products, i.e., fuels and chemicals. The fate of lignin and its structural/compositional changes during pretreatment have received increasing attention of late; however, the effect of genetic modification on the fractionation, depolymerization and catalytic upgrading of lignin from engineered plants is not well understood. This study aims to fractionate and characterize the lignin streams from wild-type and engineered switchgrass species (with low/high lignin content and high S or G lignin content) using three different pretreatment methods, i.e. dilute acid, ammonia hydroxide, and ionic liquid (cholinium lysinate). The molecular weight of the lignin fractions recovered from the liquid and solids streams after pretreatment and enzymatic hydrolysis was determined by gel permeation chromatography (GPC), while the cleavage of inter-unit lignin linkages was tracked by H^1C^{13} HSQC NMR, results being compared with lignin in untreated switchgrass. Analytical-scale pyrolysis of lignin streams was carried out in a pyrolysis-GC/MS instrument to characterize the lignin pyrolysates and provide information about lignin structure and composition. Results from this study provide a better understanding of how lignin engineering of switchgrass influences lignin fractionation and upgrading during conversion processes based on different pretreatment technologies.

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