Commentary

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Gene Editing for Enhanced Triacylglycerol Production in Plants and Microbes

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Description

Triacylglycerol (TAG) is a vital lipid molecule serving as an energy reservoir in organisms and a valuable resource for food, biofuels, and industrial applications. Given the growing global demand for sustainable energy and bioproducts, enhancing TAG production in plants and microbes has emerged as a promising strategy. Advances in gene editing technologies, particularly CRISPR-Cas systems, have revolutionized the ability to manipulate metabolic pathways with precision, offering unprecedented opportunities to increase TAG yield. This article explores the mechanisms and applications of gene editing in optimizing TAG production in plants and microbial systems. Plants naturally accumulate TAGs in seeds as an energy source for germination. However, these reserves are often insufficient to meet industrial demands. Gene editing technologies enable targeted modifications in lipid biosynthesis pathways, enhancing TAG production in both seed and non-seed tissues.

Gene editing in plants for tag enhancement

TAG biosynthesis in plants involves a well-characterized pathway centered around key enzymes like Diacylglycerol Acyltransferase (DGAT) and Phospholipid: Diacylglycerol Acyltransferase (PDAT). By using CRISPR-Cas9, researchers have successfully upregulated or altered these enzymes to boost TAG accumulation. For instance, editing the DGAT gene in Arabidopsis thaliana has shown a significant increase in TAG content by improving the conversion efficiency of Diacylglycerol (DAG) to TAG. Similarly, suppressing enzymes involved in competing pathways, such as phospholipid biosynthesis, reallocates metabolic flux toward TAG production. While seeds are the primary sites of TAG accumulation, gene editing has enabled its production in vegetative tissues like leaves and stems. For example, introducing transcription factors such as WRINKLED1 (WRI1) into non-seed tissues enhances TAG biosynthesis by activating downstream lipid synthesis genes. CRISPR-Cas9 has been employed to overexpress WRI1 and related genes, leading to the successful engineering of oil-rich leaves in crops like tobacco and camelina. These advancements pave the way for large-scale biomass-based TAG production. Gene editing also allows for the customization of fatty acid profiles within TAGs, tailoring them for specific industrial applications. For instance, modifications in Fatty Acid Desaturase (FAD) genes have resulted in higher proportions of oleic acid, a desirable component for biodiesel production due to its stability and low oxidation rate. In edible oil crops, such as soybean and canola, CRISPR-mediated editing of *FAD2* and *FAD3* genes has enhanced the content of omega-3 fatty acids, improving nutritional value. Environmental stresses like drought and salinity often limit plant productivity. Gene editing has been used to increase the stress tolerance of TAG-producing plants. For example, manipulating genes involved in Abscisic Acid (ABA) signaling pathways has enhanced drought resilience, ensuring stable TAG yields under adverse conditions. Additionally, CRISPR-Cas systems have been applied to edit genes linked to oxidative stress responses, improving lipid stability and quality.

Gene editing in microbes for industrial tag production

Microorganisms, particularly oleaginous microbes such as *Yarrowia lipolytica*, *Rhodosporidium toruloides*, and *Chlorella vulgaris*, are efficient TAG producers. Their rapid growth rates and ability to utilize diverse carbon sources make them ideal candidates for industrial lipid production. Gene editing tools like CRISPR-Cas9 and CRISPR interference (CRISPRi) have significantly advanced microbial metabolic engineering for enhanced TAG biosynthesis. The microbial TAG biosynthesis pathway relies on Acetyl-CoA as a precursor, which is converted into malonyl-CoA and subsequently into fatty acids. By overexpressing or editing key enzymes, such as Acetyl-CoA Carboxylase (ACC) and Fatty Acid Synthase (FAS), researchers have achieved increased TAG accumulation. For instance, CRISPR-mediated deletion of competing metabolic pathways, such as glycolysis or ethanol production, redirects carbon flux toward TAG biosynthesis in *Yarrowia lipolytica*. This targeted approach has resulted in lipid yields surpassing natural limits. Oleaginous microbes naturally accumulate TAGs under nitrogen-limited conditions. By using CRISPR-Cas systems, researchers have identified and manipulated genes involved in nitrogen sensing and signaling pathways to enhance TAG production without the need for nutrient stress. For example, editing regulatory genes like Nitrogen Catabolite Repression Kinase (NCRK) has enabled continuous TAG synthesis in *Rhodosporidium toruloides* under nutrient-replete conditions, improving overall productivity. Microbes capable of utilizing diverse carbon sources, including agricultural waste and industrial byproducts, offer cost-effective

solutions for TAG production. Gene editing has been employed to expand the metabolic capabilities of microbes, allowing them to process complex sugars, glycerol, and lignocellulosic biomass. For example, introducing cellulolytic enzymes into *Yarrowia lipolytica via* CRISPR has enabled direct conversion of plant biomass into lipids, reducing production costs and environmental impact. Similar to plants, microbes can be engineered to produce TAGs with specific fatty acid compositions. By editing desaturase and elongase genes, researchers have customized microbial TAGs for applications in biofuels, cosmetics, and nutraceuticals. For instance, CRISPR-Cas9 has been used to increase the production of medium-chain fatty acids in *Escherichia coli*, creating a more efficient biofuel precursor. The accumulation of TAGs in microbial cells is limited by lipid droplet formation and storage capacity. Gene editing has been employed to enhance the expression of genes involved in lipid droplet assembly, such as seipin and perilipin, leading to higher intracellular TAG content. These modifications ensure efficient lipid storage and extraction for industrial applications. Gene editing has unlocked new frontiers in enhancing TAG production in plants and microbes, offering sustainable solutions to meet global demands for biofuels, nutraceuticals, and industrial bioproducts. By precisely targeting key metabolic pathways, scientists can optimize TAG yields, tailor fatty acid compositions, and improve resilience under varying environmental conditions. Continued advancements in gene editing technologies promise to further expand the potential of TAG-producing organisms, driving innovation in agriculture, biotechnology, and sustainable energy production. The integration of these approaches into industrial-scale applications holds the key to addressing pressing challenges in food security, energy sustainability, and environmental conservation.