ADVANCEMENTS IN COMPREHENDING AND ENHANCING OIL CONTENT AND QUALITY IN SEED CROPS

Dr. Suvanish Kumar V.S

Dated: 18th August, 2023
Keywords: Oil crop, oil seed, plant embryo, fatty acid elongase complex, metabolic engineering, Carbon conversion efficiency

INTRODUCTION

The world population is expected to increase by two billion people by 2050, raising worries about food and energy scarcity. Oilseed crops have emerged as a critical answer to these problems. These crops store lipids, mostly in the form of TGs, in their seeds, which may be utilised for food, animal feed, renewable fuels, and a variety of industrial purposes. As a result, there is a major emphasis on increasing both the quantity and type of oil in seeds.

Significant progress has been achieved in scientific studies to unravel the regulatory processes of fatty acid production. Identifying prospective targets for metabolic engineering is a critical component of this work. This article provides an in-depth look at our present understanding of oil metabolism. It dives into how photosynthetic activity and unusual routes can play critical roles in seed carbon conversion efficiency. Furthermore, it emphasises the importance of 13C-metabolic

flux analysis as a significant tool for learning about the complicated processes that drive oil production in seeds.

Furthermore, the review sheds light on essential genes and regulators that have recently been the focus of efforts to boost seed oil production. It also suggests potential new targets within the metabolic pathways, offering a roadmap for achieving desired levels of lipid content and quality in seeds.

Different plant species can collect varied quantities of biomass components in their seeds, such as lipids, carbohydrates, and proteins. Seeds store oils in the form of TGs, that act as the major source of energy during germination and development into a plantlet. These oil-rich seeds are extremely important in agriculture and industry, particularly in food processing, renewable fuel generation, and a variety of industrial applications. Structural similarity of TGs to long-chain hydrocarbons, enable them as a substitute for petroleum-based products, including diesel fuel, lubricants, and otherss. Additionally, biofuels derived from oilseeds, when used as an alternative to petroleum-based diesel fuels, emit significantly fewer carcinogens during combustion, offering both environmental and health advantages. With the global population expanding and improved living standards, there is a growing requirement for seed oil. To address this increasing demand, it is imperative to create new oilseed varieties that offer higher oil content and improved composition. Numerous research projects have been dedicated to improving seed oil using traditional breeding methods, tissue culture-based breeding approaches, and targeted genetic change.

The process of choosing candidate genes for genetic engineering depends on a comprehensive understanding of the biochemical mechanisms that govern the distribution of carbon during the synthesis of new fatty acids (FAS) within seeds.Triacylglycerol (TG) production begins with FAS inside plastids and involves a series of events including condensation, reduction, and dehydration. These processes lengthen acyl chains that are linked to an acyl carrier protein

(ACP). These fatty acids (FAs) are subsequently absorbed into TGs within the endoplasmic reticulum (ER) or utilised in chain elongation and acyl editing pathways or others. Acetyl-CoA, which is produced by the pyruvate dehydrogenase complex by oxidative decarboxylation of pyruvate, is the primary carbon source for the synthesis of fatty acids (FAS).

Carbon conversion efficiency (CCE), or the efficiency with which this carbon source is transformed into oil and other biomass components, is critical for improving seed oil quality. CCE is the result of numerous metabolic processes, both catabolic and anabolic, and its magnitude varies among developing embryos in different oilseeds. These oilseeds are classified as "green" or "non-green" depending on whether they contain chlorophyll during the seed-filling stage. To gain a complete understanding of the biochemical mechanisms driving CCE in each species, a more quantitative investigation of carbon flux via central metabolism is necessary. Steady-state metabolic flux analysis (MFA), which uses 13C-labeling, has shown to be a useful approach for quantifying the flow of carbon through central metabolism. This method gives critical information that may be used to drive genetic engineering efforts.

Concurrently, numerous technologies for genetically modifying fatty acid composition and plant lipid metabolism have been used for enhancing the concentration of fatty acids (FA) in oilseeds. Enhancing FA synthesis, boosting processes involved in triacylglycerol (TG) assembly, improving FA storage in lipid droplets (LDs), reducing lipid breakdown, or a combination of these techniques are among the options under consideration.

Synthesis and Extension of Fatty Acids in Seeds

Carbon produced from photosynthesis is largely carried in oilseeds in the form of the disaccharide sucrose. Hexose phosphates, which are formed during the breakdown of sucrose, can enter metabolic pathways in both the cytosol and the plastids.

The cytosolic glycolytic pathway is a key method by which carbon enters the process of Fatty Acid Synthesis (FAS). Through the decarboxylation of pyruvate, this route eventually leads to the formation of acetyl-coenzyme A (acetyl-CoA) within plastids. FAS is initiated by an enzyme called Acetyl-CoA carboxylase (ACCase), which uses energy to convert acetyl-CoA to malonyl-CoA. An enzyme known as malonyl-CoA-ACP transacylase subsequently transfers malonyl-CoA to the Acyl Carrier Protein (ACP). Following this, a series of molecules, enzymes and co-enzymes interplay to produce 3-ketoacyl-ACP. This sequence of reactions is repeated to extend saturated fatty acid (FA) chains, resulting in the formation of 16:0-ACP. Eventually, the last elongation step to synthesize 18:0-ACP is performed by 3-ketoacyl-ACP synthase II (KAS II). The conversion of 18:0-ACP into 18:1-ACP is achieved through the action of stearoyl-ACP Δ9-desaturase. The plastidic de novo FAS process concludes when the fatty acid thioesterase removes the ACP group from the acyl backbones.

The complex interplay between the OPPP and glycolytic pathways forms a detailed network that presents additional metabolic routes for supplying the building blocks needed for de novo Fatty Acid Synthesis (FAS) within plastids. During the conversion of pyruvate into acetyl-CoA, the simultaneous release of pyruvate's carboxylic group as carbon dioxide (CO_2) has a substantial impact on the Carbon Conversion Efficiency (CCE) during embryo development. This metabolic pathway compensates for the loss of CO_2 in reactions like the OPPP and pyruvate dehydrogenase, thereby enhancing CCE during embryo development.

The carbon required for fatty acid elongation is obtained from citrate lyase's breakdown of cytosolic citrate into oxaloacetate (OAA) and acetyl-CoA. This process uses ATP. Acetyl-CoA carboxylase uses the cytosolic acetyl-CoA generated to synthesise malonyl-CoA, which serves as the carbon source for fatty acid elongation.

The fatty acid elongase complex facilitates four sequential enzymatic steps in the ER elongation of acyl-CoAs:

1. **Ketoacyl-CoA synthase (KCS)** condenses malonyl-CoA with the elongating acyl-CoA using ATP.
2. NAD(P)H is used by **3-ketoacyl-CoA reductase (KCR)** to reduce 3-ketoacyl-CoA.
3. **3-hydroxyacyl-CoA dehydrate (HCD)** catalyses 3-hydroxyacyl-CoA dehydration.
4. **Enoyl-CoA reductase (ECR)** reduces enoyl-CoA by using NAD(P)H.

Elongation of fatty acids, like FAS, is an energy intensive process that requires reductants. ATP are harnessed from mitochondrial oxidative phosphorylation process or via the glycolytic process along with NADH. Furthermore, the pentose-phosphate pathway can also promote energy supply with the use of glucose-6-phosphate and 6-phosphogluconate dehydrogenases, which may contribute to NADPH generation during embryo development.

Membrane-bound acyltransferases help in the sequential esterification of acyl-CoAs onto the glycerol 3-phosphate (G3P) backbone. The acylation process begins with the formation of lysophosphatidic acid (LPA), which is subsequently acylated to yield the important metabolite phosphatidic acid (PA). The enzyme PA phosphatase (PAP) then converts PA to diacylglycerol (DAG).

Through the activity of diacylglycerol acyltransferase (DGAT), an enzyme uniquely dedicated to TG production, a third fatty acid (FA) is added to the accessible position on DAG in the Kennedy route (Cao and Huang, 1986; Cao and Huang, 1987; Li-Beisson et al., 2013). Alternatively, by transferring an acyl group from PC to DAG, acyl chains from phosphatidylcholine (PC) can be used for TG production. The enzyme phospholipid:diacylglycerol acyltransferase (PDAT) catalyses this reaction.

TGs collect within lipid droplets (LDs) or oil bodies after TG assembly in the Endoplasmic Reticulum (ER). These LDs are made up of a TG core surrounded by a phospholipid monolayer. The aliphatic chains of TGs inside these LDs are orientated towards the interior, whereas the phosphate groups face the cytosol (Yatsu and Jacks, 1972; Chapman and Ohlrogge, 2012).

Isolated Plastids to Determine the Origins of Carbon Precursors and Reductants for Fatty Acid Synthesis (FAS)

Apart from ATP, which can be produced through oxidative phosphorylation and transported across biological membranes, the generation of carbon precursors (acetyl-CoA) and reductants is compartmentalized within cells. These essential molecules need to be synthesized separately in plastids for fatty acid synthesis (FAS) and in the cytosol for fatty acid elongation. The process of producing acetyl-CoA and NAD(P)H for FAS involves several biochemical steps, and their significance varies depending on the plant species. Understanding the sources of these carbon precursors and reducing agents is critical for enhancing oil production in developing seeds and identifying potential bottlenecks in the process.

Early investigations involved the incubation of radiolabeled substrates with plastids from plant cells to identify carbon precursors the pathways that enhance de novo FAS rates. Pyruvate, glucose 6-phosphate (G6P), dihydroxyacetone phosphate (DHAP), malate, acetate, and phosphoenolpyruvate (PEP) were identified to be FAS precursors when examining *Brassica napus* plastids. Malate, on the other hand, was found to be an effective FAS precursor in castor and sunflower embryos. This is due to malate producing extra NADPH via the plastic specific NADP-dependent malic enzyme (pNADP-ME). However, adding G6P with malate had no effect on FAS rates in isolated plastids.

Metabolic flux analysis: fatty acid synthesis in developing embryos

In vivo C partitioning with ^{13}C-MFA

The primary aim of metabolic flux analysis (MFA) is to quantitatively evaluate all the metabolic fluxes occurring within a specific organ or cell, such as developing embryos, in order to construct a comprehensive metabolic flux map. To determine intermediary carbon fluxes, the use of 13C-labeling is essential. Therefore, it's crucial to establish culture conditions that closely mimic the natural development of embryos within the plant to ensure the accuracy of carbon flux maps.

For instance, certain species within the Brassicaceae family have been observed to thrive in liquid cultures during the development of embryos. In these cultures, substrates from the parent plant are supplied to the embryo via the endosperm liquid. Knowing the endosperm nutrient composition is crucial in producing a liquid growth media to suit planta environment. Other crucial factors are nutrient composition of substrate, total osmotic pressure, and light intensity and photoperiod. It is critical to maintain metabolic steady state and homeostasis in cultured embryos in order to conduct 13C-MFA research properly.

Successful plant embryo culture conditions are indicated by an increase in dry weight and biomass composition similar to those in plants. In practise, parallel labelling studies utilising different 13C-substrates are carried out to provide full coverage of the metabolic network. Labelled embryos are collected after the labelling pattern in intermediate metabolites and products has stabilised. Nuclear magnetic resonance and/or mass spectrometry (MS) methods are then used to identify the labelling pattern in distinct metabolites .

Several extremely sensitive approaches for directly tracking labelling in critical metabolic intermediates such as carbon sources, phosphorylated chemicals, free amino acids, and organic acids have been developed utilising gas chromatography-mass spectrometry (GC-MS) and liquid chromatography tandem mass spectrometry (LC-MS/MS). Different labelling patterns of chosen metabolites and hydrolyzed macromolecules whose production occurs in discrete compartments are used to investigate compartmentalization inside plant cells. The entire labelling data set is then incorporated into a mathematical model assisted by software, to determine each network flux specific to the observed label distribution. 13C-based MFA has proven to be a successful approach in plant systems, offering valuable insights into in vivo carbon fluxes within critical metabolic pathways in fatty acid synthesis in developing embryos.

Carbon Utilization Efficiency in Developing Embryos

Carbon conversion efficiency (CCE), or the efficiency with which growing embryos convert carbon inputs into diverse biomass components, is critical for understanding and improving seed oil output. CCE is the total of catabolic and anabolic metabolic processes in developing embryos of different soybean species. Embryos are cultivated in circumstances that mirror their normal development to assess CCE, as previously described. There are two basic ways that are often used:

1. Radiolabeling Method: Embryos are exposed to 14C-labeled substrates in enclosed containers in this procedure, allowing researchers to assess the radiolabeling emitted as 14CO2 and integrated into various biomass components such as lipids, proteins, and carbohydrates. This method sheds light on the carbon movement within embryos as they develop.
2. Substrate Depletion Method: This approach involves quantifying the depletion of carbon substrates from the culture medium and the biomass accumulation during the incubation

period. By tracking the changes in substrate concentration and biomass composition, researchers can calculate CCE.

The presence of light, especially for green embryos capable of photosynthesis, influences CCE. Higher light levels during embryo development enhance CCE by stimulating photosynthesis, leading to increased production of NADPH and ATP, crucial for energy-intensive processes like fatty acid synthesis (FAS).

Differences in CCE and biomass composition among oilseed species are attributed to variations in carbon flow through central metabolic pathways. Understanding CCE differences requires analysing carbon flows in different pathways. As a result, 13C-based metabolic flux analysis (13C-MFA) is a useful approach for quantifying carbon flow rates and identifying active metabolic pathways in growing embryos during FAS.

MFA is a strong tool for determining the origins of carbon sources and reductants in FAS throughout embryo development. MFA combines a variety of methodologies and technologies, including 13C-labeling, to give a complete knowledge of the embryonic metabolic network and its function in FAS.

To create a metabolic flux map quantifying all in vivo metabolic fluxes in developing embryos, 13C-labeling is essential. Accurate carbon flux mapping requires culture conditions that mimic natural embryo development, such as liquid cultures observed in Brassicaceae family species. Analyzing endosperm or vascular tissue constituents helps design a growth medium that replicates in planta conditions.

Optimizing factors like substrate composition, osmotic pressure, and light intensity maintains metabolic stability in cultured embryos. Typically, photoperiod conditions are not applied during

13C-MFA studies. Successful culture conditions are confirmed when cultured embryos closely resemble those grown in planta in terms of dry weight gain and biomass composition.

Multiple labeling experiments using various 13C-labeled substrates cover the entire metabolic network. Harvesting labeled embryos at isotopic steady state, where labeling patterns stabilize in metabolites and products, is crucial. Nuclear magnetic resonance and mass spectrometry are advanced methods for detecting labelling patterns in metabolites such as sugars, phosphorylated substances, free amino acids, and organic acids.

Different labelling patterns of individual metabolites and macromolecules synthesised in separate cellular compartments are monitored in the context of plant cell compartmentalization. The entire labelling data set is included into a mathematical model that describes the metabolic network. Equations representing metabolic and isotopic stable states are included in this model. Finally, mathematical techniques, which are frequently aided by software, compute fluxes through the network that match to observable labelling patterns.

MFA: Developing embryo tracking of carbon sources and FAS reductants

13C-Metabolic Flux Analysis (13C-MFA) was utilised to determine the sources of carbon and reductants used in Fatty Acid Synthesis (FAS) in experiments using growing embryos from several oilseed species. It also aims to discover novel routes that improve CCE.

Notably, in developing B. napus embryos, 13C-labeling experiments revealed an unconventional pathway called the "Rubisco shunt." This pathway involves Rubisco, the enzyme involved in photosynthesis, fixing plastidic 13CO2 to produce phosphoglycerate (pPGA). Rubisco's pCO2 fixation acts as an extra carbon source for FAS, compensating for decarboxylation stages and

channeling more substrates into FAS, thereby boosting CCE. Under physiological circumstances, CCE in B. napus embryos was 86% due to CO2 refixation by Rubisco into pPGA. Rubisco generated up to 64% of the pPGA, contributing to the synthesis of acetyl-CoA for de novo FAS and minimising carbon loss as CO2.

Similar processes were observed in *G. max* and *T. arvense* embryo development, where Rubisco played a role in contributing to pPGA, albeit to varying degrees (14% and 25%, respectively). These findings highlight the importance of unconventional pathways, such as the Rubisco shunt, in enhancing CCE during FAS in developing oilseed embryos.

The use of 13C-MFA revealed an unanticipated role for isocitrate dehydrogenase (IDH) in the incubation and growth of photosynthetic embryos. Contrary to earlier assumptions about the thermodynamic irreversibility of this reaction, IDH was found to catalyse the carboxylation of -ketoglutarate into isocitrate in growing Brassica napus embryos. This discovery has major ramifications since IDH's CO2 fixation has the potential to improve CCE.

The surprising phenomenon was attributed to the heightened demand for citrate in Fatty Acid (FA) elongation in B. napus embryos and the abundant availability of CO2 (40 mM) within developing oilseeds. Interestingly, the reversibility of IDH was not limited to B. napus but was also observed in developing green embryos from various species, including Glycine max, Thlaspi arvense, Camelina sativa, and Linum usitatissimum.

Carbon is a critical need in the context of FAS, and reductants must be synthesised inside the plastid. The plastidic pyruvate dehydrogenase complex yields one mole of NADH for every mole of acetyl-CoA generated, which is directly utilised in FAS. The OPPP, pNADP-ME, and, in the case of photosynthetic embryos, light-driven processes can all help to produce NADPH, another important reductant for de novo FAS. However, it is vital to note that the operation of the OPPP and pNADP-ME may result in CO2 production, which may influence CCE. To determine the

relative contributions of the OPPP and pNADP-ME to NADPH production in developing embryos across different species, researchers have employed 13C-labeling and MFA. In general, the OPPP was found to contribute more significantly to NADPH production compared to pNADP-ME. However, in species like Glycine max and Brassica napus, the combined activities of these two pathways were insufficient to meet the NADPH demands for FAS in developing embryos. These species likely rely on additional NADPH from photosynthetic light reactions and/or catabolic pathways to make up for the shortfall. In contrast, in *Zea mays*, the OPPP and pNADP-ME played the role of limiting factor producing NADPH to support FAS in developing embryos, with pNADP-ME functioning at its maximum capacity. On the other hand, embryos from *Helianthus annuus, Camelina sativa*, and *Linum usitatissimum* produced an excess of NADPH through the OPPP and pNADP-ME, but this resulted in the generation of CO2 and led to the lowest CCE in these species.

Metabolic engineering: seed oil content and composition

To boost oil yield and fatty acid (FA) composition in oilseed crops, one must first understand how plant systems work to produce store lipids. Furthermore, species-specific insights into the TG synthesis pathway are critical in optimising FA composition. Several techniques for genetically enhancing FA composition and TG metabolic pathways in oilseeds have been explored. Several techniques are outlined below:

The Push Strategy

The "push" strategy is designed to enhance the flow of carbon through the fatty acid synthesis (FAS) pathway, thereby increasing the production of acyl chains within plastids for subsequent incorporation into triglycerides (TGs) within the ER. This strategy is widely employed to manipulate oil content and involves the overexpression or sometimes downregulation of

transcription factors (TFs) that can simultaneously regulate multiple reactions in the biosynthesis of oil.

Some of the most notable TFs utilized in this strategy include LEAFY COTYLEDON1 (LEC1), LEC1-LIKE (L1L), LEC2, etc. TFs including GRF2, LEC1, and WRI1 have demonstrated remarkable effectiveness in increasing seed oil content, resulting in levels 40% to 48% higher than those observed in wild-type plants.

Furthermore, specific enzymes participating in the initial stages of de novo FAS, such as acetyl-CoA carboxylase (ACCase) and malonyl CoA-ACP malonyltransferase (MCAMT), have been targeted for genetic manipulation.

The Pull Strategy

The "pull" strategy is designed to facilitate the efficient incorporation of fatty acids (FAs) generated within plastids into triglycerides (TGs) in ER. Numerous metabolic engineering studies aimed at optimizing this step in seed oil production have primarily focused on the overexpression of DGAT. DGAT is a key enzyme responsible for committing acyl chains to storage TGs. Additionally, phospholipid: diacylglycerol acyltransferase 1 (PDAT1) has been another target for manipulation in this "pull" strategy).

Package strategy

The accumulation of TGs within the ER phospholipid bilayer stretches the outer layer into the cytoplasm, resulting in the formation of nascent LDs that bud out to produce LDs. The "package" technique maximises droplet stability by targeting LD biogenesis. Proteomic studies of seed proteins indicated the presence of 20-30 coat proteins involved in LD formation and/or stability. These coat proteins may help restricting lipases access TG within the LD core, resulting in increased LD formation. Seed-specific overexpression or down-regulation of genes such as lipid

droplet-associated protein (LDAP), oleosin, lipid droplet-associated protein (LDAP), seipins, and LDAP-interacting protein (LDIP) enhanced LD size and/or number, according to research.

Protect strategy

Endogenous proteases and lipases can hydrolyze coat proteins and target TGs to create free FAs, leading in the generation of acetylCoA through oxidation during seed germination and seedling development. Inhibiting the enzymatic breakdown processes of coat proteins and TGs (also known as the "protect" method) is thus one of the best ways to increase seed oil content. The first stage of TG mobilisation by lipases includes the ubiquitination of certain coat proteins, most notably oleosin and caleosin, followed by their destruction by the proteasome.

Several genes have been identified as prospective targets for the "protect" approach, which tries to boost seed oil content by preventing TG degradation. Seed fatty acid reducer (SFAR), Gly-Asp-Ser-Leu (GDSL)-motif lipases, plastid lipase1 (PLIP1), and Sugar-dependent1 (SDP1) are among the genes involved. Notably, among the genes examined as part of the "protect" strategy, downregulation of PLIP1 in Arabidopsis resulted in the most significant increase in seed oil content (+45%).

Surprisingly, a new study has found that overexpression of specific genes involved in TG breakdown might improve seed oil content, which may look paradoxical at first. Patatin-related phospholipase III (pPLAIII) and nonspecific phospholipase C6 (NPC6) are two of these genes. The discovery raises an important issue about how overexpressed proteins cause greater lipid buildup in seeds.

Modifying FA composition

Interaction of A. fumigatus with human proteins is a critical aspect of its immune evasion strategies. One such interaction involves the human protein p11, which plays a role in the regulation of intracellular calcium levels. Researchers have discovered that A. fumigatus employs a sophisticated mechanism to hijack p11, manipulating its function to evade host immune responses and establish infection.

Intracellular calcium signaling is crucial for various cellular processes, including immune responses and cell survival. A. fumigatus can exploit this pathway by targeting p11, which is an annexin A2 (ANXA2) binding protein involved in calcium homeostasis. The interaction between A. fumigatus and p11 occurs during the early stages of infection when the fungus comes into contact with host cells, particularly in lung epithelial cells.

Studies have shown that A. fumigatus secretes a specific protein, termed gliotoxin, which acts as a virulence factor and facilitates the hijacking of p11. Gliotoxin is a secondary metabolite produced by the fungus and serves as a potent immunosuppressive agent. It binds to p11, forming a complex that alters intracellular calcium dynamics. As a result, the manipulation of calcium signaling by the A. fumigatus-p11-gliotoxin complex helps the fungus to evade host immune defenses and dampens the inflammatory response within the infected cells.

By disrupting the normal calcium signaling pathways in the host cells, A. fumigatus can inhibit various immune mechanisms that rely on intracellular calcium, such as phagocytosis, cytokine production, and activation of immune cells. This manipulation of host cell calcium dynamics aids the fungus in surviving and proliferating within the host, contributing to its ability to cause invasive infections.

Understanding the specific mechanisms by which A. fumigatus interacts with human protein p11 and other host factors is crucial for developing targeted therapeutic approaches to combat invasive fungal infections. By disrupting these interactions and immune evasion strategies,

researchers hope to improve the treatment and management of fungal diseases, particularly in immunocompromised individuals who are at higher risk of severe fungal infections.

Combination strategies

Several studies have reported a combined approach to increase TG levels by incorporating genes from different strategies. This approach is suitable when applied to non-seed tissues like leaves and roots . Instances exist where combinations of specific genetic modifications resulted in higher seed oil content in comparison to individual gene alterations.

It is crucial to note, however, that the success of this combinatorial method is not assured. Attempts to boost TG content in seeds by combining several procedures did not always provide the intended results. Overexpression of WRI1 (the "push" method) and DGAT1 (the "pull" strategy) from Arabidopsis in soybean, for example, did not result in a rise in TG content, suggesting that certain species may use counteracting mechanisms to maintain constant oil content.

Combining various techniques, on the other hand, has been effective in changing FA composition in seeds. For example, co-expression of Cuphea viscosissima FATB (the "push") and LPAT (the "pull") with Cuphea avigera DGAT (the "pull") resulted TGs accumulation, of mostly medium-chain FAs (C6-C14) in pennycress, which is valuable for industrial applications, jet fuel, and improved biodiesel (Esfahanian et al., 2021).

CONCLUSION

The increasing demand for seed based oils in food and animal feed is the result of population growth, urbanization, and industrialization. To meet this growing demand, metabolic engineering

offers a hopeful method for enhancing oil production derived from seeds and fine-tuning the fatty acid (FA) composition in oilseed crops. However, the key challenge lies in identifying the most suitable targets for these modifications.

The application of 13C-Metabolic Flux Analysis (13C-MFA) has been instrumental in enhancing our understanding of plant Fatty Acid Synthesis (FAS) on a systemic level. The flux maps it creates for primary metabolism offer valuable information about regulatory processes and pathways throughout the entire metabolic network. This, in turn, aids in the identification of potential genes that may be altered to improve oil output. Furthermore, recent advances in mass spectrometry imaging methods, when paired with 13C-isotopic pulse labelling, allow for enhanced spatial and temporal resolution in the measurement of metabolic fluxes.

There has been a significant increase in the discovery of multiple genes linked to the stages of "push, pull, package, and protect" in oil production. This has greatly aided the successful modification of oil levels and composition in different crops. Successful approaches often entail increasing the expression of transcription factors (TFs) that promote Fatty Acid Synthesis (FAS) and genes responsible for assembling triacylglycerols (TGs). Simultaneously, there is a reduction in the activity of enzymes involved in breaking down TGs.It's crucial to acknowledge that a strategy that works well in one plant species may not produce similar outcomes in others. This is exemplified by 13C-Metabolic Flux Analysis (13C-MFA) investigations, which reveal that developing embryos from various species utilize unique pathways, and in some cases, even unconventional reactions, during FAS.

A thorough grasp of various omics technologies and advanced genome-editing tools provides the opportunity to quickly integrate multiple candidate genes to further enhance the quality of seed oil. Prominent genome-editing methods like CRISPR/Cas9 can be utilized to eliminate or diminish metabolic competition, channeling metabolic flow toward the synthesis of TGs.

Furthermore, they can be applied to modify particular amino acids in enzymes engaged in oil biosynthesis, thus enhancing or altering their enzymatic functions (Park and Kim, 2022).

Reference

Sagun JV, Yadav UP, Alonso AP. Progress in understanding and improving oil content and quality in seeds. Front Plant Sci. 2023 Jan 26;14:1116894. doi: 10.3389/fpls.2023.1116894.

Acket, S., Degournay, A., Rossez, Y., Mottelet, S., Villon, P., Troncoso-Ponce, A., et al. (2019). 13c-metabolic flux analysis in developing flax (Linum usitatissinum l.) embryos to understand storage lipid biosynthesis. Metabolites 10, 14. doi: 10.3390/metabo10010014

Allen, D. K., Laclair, R. W., Ohlrogge, J. B., Shachar-Hill, Y. (2012). Isotope labelling of rubisco subunits provides in vivo information on subcellular biosynthesis and exchange of amino acids between compartments. Plant Cell Environ. 35, 1232–1244. doi: 10.1111/j.1365-3040.2012.02485.x

Alonso, A. P., Goffman, F. D., Ohlrogge, J. B., Shachar-Hill, Y. (2007). Carbon conversion efficiency and central metabolic fluxes in developing sunflower (Helianthus annuus l.) embryos. Plant J. 52, 296–308. doi: 10.1111/j.1365-313X.2007.03235.x

Cahoon, E. B., Shockey, J. M., Dietrich, C. R., Gidda, S. K., Mullen, R. T., Dyer, J. M. (2007). Engineering oilseeds for sustainable production of industrial and nutritional feedstocks: solving bottlenecks in fatty acid flux. Curr. Opin. Plant Biol. 10, 236–244. doi: 10.1016/j.pbi.2007.04.005

Cai, G., Fan, C., Liu, S., Yang, Q., Liu, D., Wu, J., et al. (2020). Nonspecific phospholipase C6 increases seed oil production in oilseed brassicaceae plants. New Phytol. 226, 1055–1073. doi: 10.1111/nph.16473

Dong, Z., Zhao, H., He, J., Huai, J., Lin, H., Zheng, J., et al. (2002). Overexpression of a foxtail millet acetyl-CoA carboxylase gene in maize increases sethoxydim resistance and oil conten. Afr J. Biotechnol. 10, 3986–3995. doi: 10.5897/AJB11.053

Du, H., Huang, M., Hu, J., Li, J. (2016). Modification of the fatty acid composition in arabidopsis and maize seeds using a stearoyl-acyl carrier protein desaturase-1 (ZmSAD1) gene. BMC Plant Biol. 16, 1–10. doi: 10.1186/s12870-016-0827-z
18

Ettaki, H., Troncoso-Ponce, M. A., To, A., Barthole, G., Lepiniec, L., Baud, S. (2018). Overexpression of MYB115, AAD2, or AAD3 in arabidopsis thaliana seeds yields contrasting omega-7 contents. PloS One 13, 1–19. doi: 10.1371/journal.pone.0192156

Fan, J., Yan, C., Zhang, X., Xu, C. (2013). Dual role for Phospholipid:Diacylglycerol acyltransferase: Enhancing fatty acid synthesis and diverting fatty acids from membrane lipids to triacylglycerol in arabidopsis leaves. Plant Cell 25, 3506–3518. doi: 10.1105/TPC.113.117358
Focks, N., Benning, C. (1998). wrinkled1: A novel, low-Seed-Oil mutant of arabidopsis with a deficiency in the seed-specific regulation of carbohydrate metabolism. Plant Physiol. 118, 91. doi: 10.1104/PP.118.1.91

Zhao, N., Zhang, Y., Li, Q., Li, R., Xia, X., Qin, X., et al. (2015). Identification and expression of a stearoyl-ACP desaturase gene responsible for oleic acid accumulation in xanthoceras sorbifolia seeds. Plant Physiol. Biochem. 87, 9–16. doi: 10.1016/j.plaphy.2014.12.009

Zhou, B., Fei, W., Yang, S., Yang, F., Qu, G., Tang, W., et al. (2020). Alteration of the fatty acid composition of brassica napus l. via overexpression of phospholipid: Diacylglycerol acyltransferase 1 from sapium sebiferum (L.) roxb. Plant Sci. 298, 110562. doi: 10.1016/j.plantsci.2020.110562

Zhu, Y., Xie, L., Chen, G. Q., Lee, M. Y., Loque, D., Scheller, H. V. (2018). A transgene design for enhancing oil content in arabidopsis and camelina seeds. Biotechnol. Biofuels 11, 46. doi: 10.1186/s13068-018-1049-4