

MODERN GENETIC TOOLS AND THEIR ROLE IN ENHANCING OIL YIELD AND QUALITY IN OILSEED AND LEGUME CROPS

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Abstract

The world population is increasing and this has resulted in the rising need for vegetable oils and protein-rich legumes, which is due to industrial and nutritional necessity. alone have not been sufficient to counter this upsurge particularly for features like higher oil yield, better fatty acid content and resistance to environmental stress. Modern genetic methods such as genome editing, marker assisted selection, transgenic technologies and strong omics platforms are changing the field of crop development. These techniques enable an unprecedented level of precision in the regulation of complicated metabolic pathways controlling the synthesis and storage of oil as well as factors impacting seed quality. This study describes recent advances in modern genetics of oilseed and legume crops covering approaches, achievements, challenges and future perspectives. The necessity of joining progress in biotechnology with breeding methods for sustainable increases in oil yield and quality is stressed.

Keywords: *Modern genetic tools, oilseed crops, legume crops, CRISPR/Cas9, marker-assisted selection, genetic engineering, oil yield, fatty acid profile, biotechnology, crop improvement.*

1. Introduction

Oilseed and pulse crops are one of the most important crops of the world agriculture from the nutritional and economical view. Crops such as soybean (*Glycine max*), canola (*Brassica napus*), sunflower (*Helianthus annuus*), peanut (*Arachis hypogaea*) and chickpea (*Cicer arietinum*) supply fats, proteins and minerals to millions of people. These crops are utilised for cooking oils, food processing, biofuels and industrial uses. Hence, researchers and breeders have been focusing on boosting the oil yield and changing the fatty acid composition to make it healthy.

Traditional breeding has focused on high yield and stress tolerant plants in the past. But the routes to oil synthesis and storage are a little messy, regulated by networks of interacting genes and subject to the vagaries of environmental conditions. This has slowed down the traditional breeding techniques, and is constrained by the limits of the genetic variety and heritability of traits. To overcome these constraints, new genetic technologies based on molecular biology and genomics have been created, allowing specific modifications that are difficult to obtain with older approaches.

Recent advances include methods to alter genomes, e.g., CRISPR/Cas9, TALENs and ZFNs and to modulate gene expression using RNA interference (RNAi). Desired genotypes can be identified by molecular markers and marker assisted selection (MAS) and new alleles can be introduced by transgenic technology. These advancements are transforming crop development programmes with novel strategies to boost oil content, fatty acid profiles and tolerance to conditions limiting seed quality.

2. Oil Biosynthesis and Quality Determinants in Oilseed and

Legume Crops

Seed oil synthesis is a result of a cascade of metabolic events that convert carbon assimilates into fatty acids and triacylglycerol's (TAGs), the main storage form of oil. Plastids are the main site of fatty acid synthesis. Acetyl-CoA carboxylase (ACCase), fatty acid synthase (FAS) and desaturases are principally responsible for chain elongation and desaturation activities. TAG assembly occurs in the endoplasmic reticulum and is catalyzed by acyltransferases, such as diacylglycerol acyltransferase (DGAT). The quantity and the nutritional quality of oil seed depends on the overall efficiency of these routes and the degree of saturation and unsaturation of the fatty acids.

The fat content was frequently the quality criterion. Oleic acid (C18:1) is known to have excellent features of oxidative stability and health benefits. High saturated fatty acid levels (e.g. palmitic acid) are linked to bad health impacts. Linoleic (C18:2) and linolenic (C18:3) acids are essential fatty acids and can diminish shelf life by oxidation. One of the most important strategies to change the fatty acid balance and to achieve a better oil quality has been the modification of desaturase enzymes like FAD2 and FAD3.

Oil yield and composition are complex quantitative features, suggesting that genetic determinants, metabolic control and environmental influences are interplaying. Therefore, effective attempts at

improvement must have tools capable of striking a myriad of genes and pathways at one and the same time – the capabilities of modern genetic technology.

3. Molecular Markers and Marker-Assisted Selection

Molecular markers are specific DNA sequences that might be associated with features of interest, such as behavioural traits. Breeding programs have exploited the application of tools such as SSRs (simple sequence repeats), SNPs (single nucleotide polymorphisms) and DArT (Diversity Arrays Technology) to follow genetic variants linked with oil content, fatty acid composition and stress tolerance. Marker assisted selection shortens the breeding process since the individuals with favorable alleles can be identified early in the crop cycle. For instance, markers associated to quantitative trait loci (QTLs) controlling oil content in soybean have been used to produce genotypes with increased oil content in the seed. Similarly, MAS has been used in groundnut to introgress genes for high oleic to linoleic acid ratio, which results in oils with improved stability and nutritional qualities. Genetic variants linked with oil content, fatty acid composition and stress tolerance have been tracked by breeders with SSRs (simple sequence repeats), SNPs (single nucleotide polymorphisms) and DArT (Diversity Arrays Technology).

Marker assisted selection shortens the breeding cycle by allowing selection of individuals with favorable alleles at an early stage of the crop cycle. For example, markers associated with quantitative trait loci (QTLs) for oil content in soybean have been exploited to develop genotypes with enhanced levels of oil in the seed. MAS has also been employed in groundnut for the introgression of genes for high oleic to linoleic acid ratio to produce oils with improved durability and nutritional characteristics.

Table 1 below summarizes key QTLs and associated markers for oil-related traits identified in major oilseed and legume crops.

Table 1: Summary of QTLs and Molecular Markers Linked to Oil Traits

Crop	Trait	QTL/Marker	Effect	Reference
Soybean	Oil content	Satt159 (SNP)	Increased oil content in seeds	Zhang et al., 2024
Groundnut	Oleic to Linoleic ratio	AhFAD2 (SSR)	Enhanced oleic acid content, improved oil stability	Kargozar et al., 2024
Canola (Rapeseed)	Fatty acid composition	Rf1 (SSR)	Altered fatty acid profile, higher oleic acid levels	Martin et al., 2014
Sunflower	Oil yield	Hal1-1 (SNP)	Higher seed oil content in hybrid cultivars	Liu et al., 2023

Camelina	Oil content	CAMSNP1 (SNP)	Elevated oil content and better quality oil	Chen et al., 2022
Cotton	Oil content	GhBHLH (DArT)	Increased oil yield in seeds	Singh et al., 2021
Groundnut	Stress tolerance	AhACD1 (SNP)	Improved drought resistance and oil stability	Xu et al., 2021

4. Transgenic Approaches in Oilseed Enhancement

Transgenic procedures involve the introduction of foreign or modified genes into the plant genome to produce desirable features. In oilseed crops, transgenics have been used to alter enzymes in the lipid biosynthesis pathway, to increase carbon flux towards oil accumulation and to improve stress tolerance, thereby affecting seed quality. A clear example is overexpression of genes producing ACCase or DGAT which may increase the rate of fatty acid synthesis and TAG assembly, respectively. In canola, transgenic lines with changed DGAT genes showed increased oil content as compared to wild-type lines. There has also been work to turn off the genes for lipases, enzymes that break down oil during seed development, as a way of keeping increased oil levels. Transgenic techniques have also been targeted to fatty acid desaturases. For example, RNAi inhibition of FAD2 genes in soybean and sunflower resulted in increased quantities of oleic acid, thereby enhancing the stability of the oil and its market value.

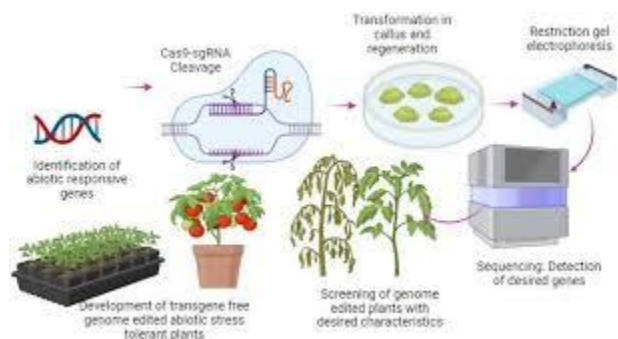
5. Genome editing: Tools for precise targeting of traits

Genome editing techniques are revolutionizing plant breeding by precise and heritable change of specific genomic areas. The most popular platform, CRISPR/Cas9, employs guide RNA sequences to lead the Cas9 nuclease to specified DNA loci, where the double-strand break is then repaired by endogenous processes, typically resulting in targeted changes.

CRISPR/Cas9 has been used to modify genes for fatty acid desaturation in oilseed crops. For instance, deliberate mutagenesis of FAD2 genes in soybean yielded lines with considerably increased oleic acid levels without the introduction of foreign DNA. Genome editing of numerous homologous genes in came Lina and rapeseed has also led to improved oil quality attributes.

In addition to fatty acid metabolism, genome editing may be used to adjust seed size regulators, lipid droplet production and transcription factors coordinating metabolic networks. These technologies offer the way to stacking numerous beneficial genes in elite cultivars in a fraction of the time of conventional breeding.

Figure 1 conceptually illustrates the CRISPR/Cas9 editing mechanism and its role in altering oil-related genes.



6. Integration of Omics Technologies

Advances in genomics, transcriptomics, proteomics, and metabolomics provide comprehensive insights into the complex biological networks governing oil biosynthesis. High-throughput sequencing technologies have enabled the identification of key gene families involved in lipid metabolism, while transcriptome profiling reveals temporal expression patterns during seed development.

Integration of metabolomics with genomic data allows researchers to correlate specific metabolites with genetic loci, pinpointing regulatory nodes that influence oil composition. Such systems-level understanding guides the prioritization of targets for genetic modification and predictive modeling of trait outcomes.

For instance, metabolomics analyses in soybean have identified metabolic bottlenecks in the TAG assembly pathway, suggesting gene targets that, when edited, could enhance oil accumulation. Similarly, proteomic studies in canola seeds have highlighted differential protein expression linked with fatty acid desaturation, offering new avenues for breeding interventions.

7. Case Studies in Major Oilseed and Legume Crops

Advances in genomes, transcriptomics, proteomics and metabolomics provide holistic views of the complex biological networks that regulate oil production. High-throughput sequencing technologies have identified major gene families involved in lipid metabolism. Transcriptome analysis revealed temporal expression patterns throughout seed development.

Combining metabolomics with genomic data enables researchers to link individual metabolites to genetic loci and to identify regulatory nodes controlling oil composition. A systems-level understanding enables the prioritization of targets for genetic manipulation and the predictive modelling of trait consequences.

For example, metabolomics analysis in soybean has discovered metabolic bottlenecks in the assembly process of TAG, identifying gene targets that could be altered to improve oil accumulation. Proteomic investigations in canola seeds have also demonstrated altered protein expression associated with fatty acid desaturation, providing additional pathways for breeding treatments.

Major Legume and Oilseed Crops: Case Studies Soja

Soybean is the most frequently farmed soybean in the world and is valued for its oil and protein. Numerous QTLs related to seed oil % and fatty acid composition have been found through genetic investigations. Successful application of transgenic and genome editing techniques to target FAD2 and FAD3 boosted oleic acid and decreased linolenic acid content, increasing the nutritional profile and oxidative stability of the oil. And MAS has enabled breeders to combine high oil content with agronomic features such as yield and disease resistance.

Canola oil

Canola oil is valued for its low saturated fat and high monounsaturated fat levels. Transgenic production of rate limiting enzymes and targeted editing of fatty acid desaturase genes have been employed to increase oil yield. Simultaneous enhancement of numerous features like as oil content and drought tolerance has also been assisted using genomics selection based on dense marker panels.

Peanut

Groundnut is an important legume oilseed with significant genetic variation for oil quality properties. Molecular breeding has targeted at enhancing the oleic to linoleic acid ratio and MAS has provided the opportunity of selecting high oleic genotypes. Genome editing promises to further improve these features and improve resistance to aflatoxin contamination, a major constraint on seed quality.

Crop	Soybean (<i>Glycine max</i>)	Canola/Rapeseed (<i>Brassica napus</i>)	Sunflower (<i>Helianthus annuus</i>)	Groundnut/Peanut (<i>Arachis hypogaea</i>)	Camelina (<i>Camelina sativa</i>)	Chickpea (<i>Cicer arietinum</i>)
 Key Trait Targeted	Increase oleic acid content and total oil yield	Improve oil quality (↑ oleic acid, ↓ erucic acid and glucosinolates)	Enhance oil content and oleic/linoleic acid balance	Increase oil content and improve fatty acid profile	Improve oil quality (↑ omega-3, ↓ oleic acid) and yield	Enhance seed size, oil content and stress tolerance
 Genetic Tool(s) Used	CRISPR/Cas9 Genome Editing	CRISPR/Cas9, Transgenics, Marker-Assisted Selection (MAS)	Conventional Breeding + MAS, Genome Editing (ongoing)	QTL Mapping, Marker-Assisted Selection, Transgenics (in study)	CRISPR/Cas9, RNAi, MAS	QTL Mapping, MAS, Genomics Selection
 Target Gene(s)/ QTL(s)	FAD2A, FAD2B (GmFAD2-1A, GmFAD2-1B)	FAD2, FAE1, GSL genes, BnAnac071 (seed size)	HaFAD2, HaFAD3, OilQTLs on LG5, LG15	ANDGAT1, AHFAD2, QTLs for oil content (A02, B03)	CsFAD2, CsFAD3, WR1, LEC2	QTLs on CaLG04, CaLG06 (seed size & oil content), DREB2A (stress)
 Approach/Strategy	Knockout of FAD2 genes to reduce linoleic acid and increase oleic acid	Multi-gene editing + low erucic acid trait introgression using MAS	Marker-assisted breeding for high oil QTLs, genome editing to modify FAD genes	Pyramiding favorable QTLs for oil content and modifying DGAT genes	Editing desaturase genes and regulators to improve oil quality	MAS for major QTLs and genomic selection for complex traits
 Key Outcome(s)	Up to 80% reduction in linoleic acid and increase in oleic acid to >70%	Low erucic acid (<2%), improved oleic acid (>60%) and reduced glucosinolates	High-oil lines with improved oleic acid (>80%) and yield stability	10-30% increase in oil content in elite lines; improved oleic/linoleic ratio	Increased omega-3 (ALA) up to 40% of total fatty acids; improved yield	Improved seed size (10-15%), higher oil content and better drought tolerance
 Reference (Examples)	Haun et al., 2014; Zhang et al., 2017; Jiang et al., 2020	Raman et al., 2016; Kagale et al., 2020; Lu et al., 2021	Borra et al., 2014; Debaeke et al., 2017; Adl et al., 2020	Pandey et al., 2014; Chu et al., 2016; Varshney et al., 2021	Zubir, 2019; Hajduch et al., 2020; Liu et al., 2022	Varshney et al., 2013; Nayak et al., 2017; Gaur et al., 2021

Overall Impact of Modern Genetic Tools

 Precise gene
modification
and selection

 Development
of elite varieties

 Higher oil yield
and improved
quality

 Better nutrition
and health
benefits

 Sustainable agriculture
and food security

Legend of Abbreviations

FAD2/FAD3: Fatty acid desaturase genes
DGAT1: Diacylglycerol acyltransferase 1
QTL: Quantitative Trait Loci
MAS: Marker-Assisted Selection
RNAi: RNA interference
ALA: Alpha-linolenic acid (omega-3)

8. Challenges and Limitations

Notwithstanding promising improvements, significant obstacles to the widespread use of genetic technology in agricultural development remain. The regulatory frameworks for genetically modified

organisms (GMOs) and genome-edited crops vary by country and affect their commercial acceptance and timing of deployment. Public opinion and socio-economic considerations are crucial for acceptance of genetically modified crops especially in the developing countries. Careful consideration of environmental challenges include gene flow to wild cousins and unintended ecological effects. In addition, oil related traits have complicated genetic architecture with multi-genes and gene by environment interactions, thereby needing sophisticated breeding schemes and comprehensive field evaluations to confirm trait performance in different contexts.

9. Future prospects

Genetic technology combined with artificial intelligence, high throughput phenotyping and predictive modelling will accelerate development of crops even more. Machine learning techniques can be applied to analyse big data of multi-omics to find hidden patterns and improve breeding decisions. Precision breeding technologies using genome editing and speed breeding techniques can drastically shorten breeding cycles and deliver superior cultivars at a much faster rate.

The ethical, legal and societal consequences will have to be tackled by partnership between public research institutions, private sector breeders and regulatory organizations while guaranteeing fair access to the technical benefits. We are learning more about genetics, and our tools are improving, so the prospects for generating persistent gains in the yield and quality of oil from oilseed and legume crops continue to grow.

10. Conclusion

Modern genetic tools have revolutionized the field of oilseed and legume crop modification and offer precise and economical means to boost oil yield and quality. Researchers have made great strides in understanding and modifying complex trait networks from molecular markers and MAS to genome editing and transgenic technology. Regulatory and public perception and environmental stewardship aside, continued investigation and judicious application of these technologies appears to have potential for meeting global demands for edible oils and healthful legumes. Integration of biotechnological discoveries with conventional breeding schemes will be vital for production of high yielding and durable cultivars required to sustain future generations.

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