PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(sp4): 01–16
https://doi.org/10.14719/pst.5602

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REVIEW ARTICLE

Molecular insights driving oleic acid improvement in groundnut: A review

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OPEN ACCESS

ARTICLE HISTORY
Received: 07 October 2024
Accepted: 07 November 2024
Available online
Version 1.0 : 28 December 2024

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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CITE THIS ARTICLE
Ellandula A, Kalaiyarasi R, Sasikala R, Rajagopal B, Amirtham D, Senthivelu M. Molecular insights driving oleic acid improvement in groundnut: A review. Plant Science Today.2024;11(sp4):01-16. https:/doi.org/10.14719/pst.5602

Abstract

Groundnut (*Arachis hypogea* L.) plays a prominent role in the global food and oil industries. Its nutritional value and shelf life are significantly influenced by oleic acid which is the primary constituent of groundnut oil. Given the industrial applications and health benefits, increasing its levels in groundnut has become a central breeding objective. The genetics of oleic acid content in groundnuts involves intricate quantitative trait loci (QTL) and multiple genes governing fatty acid biosynthesis. Breakthroughs with high-throughput sequencing and genotyping techniques have made it easier to identify and characterize key genes and regulatory elements that affect oleic acid synthesis. These insights underscore the importance of molecular approaches in enhancing oleic acid content in groundnuts, offering prospects for improved nutritional quality and industrial utility. By targeting crucial enzymes like fatty acid desaturase (FAD) and stearoyl-ACP desaturase (SAD), genetic manipulation is employed to enhance oleic acid levels. Techniques, notably, CRISPR-Cas9 gene editing and transgenic methods offer precisely increasing oleic acid content with minimal off-target effects. Transcriptomics, proteomics and metabolomics collectively referred to as integromics, provide a comprehensive understanding of groundnut molecular responses to increased oleic acid levels. Advancements in raising oleic acid levels in groundnuts, driven by molecular breakthroughs in genetic research, biochemical investigations and omics technologies, are sustainably meeting the demand for healthier, higher-quality groundnut oil. This review summarizes the importance of oleic acid and in-depth overview of the molecular advancements driving the enhancement of oleic acid content in groundnut, with a focus on key genetic and breeding strategies, omics insights and their implications for developing high-oleic peanut cultivars.

Keywords

CRISPR Cas; groundnut; integromics; molecular approaches; oleic acid

Introduction

Peanut is an allotetraploid with an AABB genome type, 2n = 4x = 40 (1). It arose from a hybridization followed by one tetraploidization event (2, 3). Cytogenetic and molecular evidence shows that *Arachis duranensis* and *Arachis ipaensis* are the A and B sub genome parents of peanuts, respectively (2, 4-10). These two species diverged 2-3 million years ago (10,11). The hybridization along with spontaneous polyploidy (5,000-10,000 years ago) of *A. duranensis* and *A. ipaensis*, led to the modern crop (3,11,12). The initial genome duplication event isolated the new allopolyploid from other diploid Arachis species. DNA evidence suggests a narrow polyploid origin that

resulted in a genetic bottleneck, leading to reduced genetic diversity. This limited diversity-imposed challenges for breeding efforts, as it restricts the availability of beneficial alleles to enhance quality traits and other traits like disease resistance and stress tolerance (3,13). Despite this, peanuts have evolved diverse growth habits, architecture and morphological forms, as well as varied testa colors, seed numbers per pod and flower patterns. Two subspecies of *A. hypogaea* are recognized: *hypogaea* and *fastigiata*, which show partial sexual incompatibility *i.e.,* they can cross but often with reduced fertility or success rates, limiting gene flow between them. The two subspecies have two (*hypogaea* and *hirsuta*) and four (*fastigiata, vulgaris, aequatoriana* and *peruviana*) botanical varieties, respectively (14). Several diploid Arachis species were cultivated before the tetraploid, but only the latter developed a full domestication syndrome and became a global crop (3).

This suggests that polyploidy played a key role in the crop's success by enhancing phenotypic plasticity and environmental adaptability. Induced allotetraploids generated from peanut's progenitor species to replicate the genetic events following the origin of *A. hypogaea* and its wild counterpart, the allotetraploid *A. monticola*. Both progenitor species are still extant and were recently sequenced (11). The B genome donor, *A. ipaensis*, is represented today by a single accession, K 30076. Biogeography indicates it was moved by humans into the range of the A subgenome donor, facilitating the formation of the allotetraploid species. DNA identity (99.98%) between *A. ipaensis* and the B subgenome of peanut suggests descent from the same population that gave rise to peanut (11,13). The A subgenome donor, *A. duranensis* V 14167, also shows high similarity to the A sub genome of cultivated peanuts (99.75%) (12). These close genetic relationships provide unique opportunities to "replay" the origin of *A. hypogaea*.

A. hypogaea, a tetraploid species (2n = 4x = 40), is distinctive among cultivated plants for being positively geotropic, with flowers blooming above ground and fruits developing below (14). It holds significant global importance as a key legume crop owing to its valuable contributions in terms of edible oil and high protein content. With cultivation extending approximately 28 million hectares worldwide, it yields a total production of 50 million tons (AtlasBig, 2024). The leading contributors to global peanut production include China, India, Nigeria and the USA, collectively responsible for nearly 70% of the global peanut output (AtlasBig, 2024). Globally, the entire peanut production is utilized for edible oil in around 53% of cases, with 32% heading towards baked goods and confections and 15% embarking feed for animals in addition to seed production (15). Peanut cultivation exhibits geographical variations, impacting the quality attributes of the harvested produce. In regions such as India and China, along with other Asian nations, 50% of the peanut yield undergoes crushing for oil extraction, while the remaining portion is utilized for confectionary and other food intents. In contrast, America alongside other European nations allocates almost three -quarters of its groundnut produce for sweets, deserts and other food intents, reserving the leftover one-third for oil extraction. Notably, preferences for low-oil-content peanuts are evident in regions where these varieties are favoured for use on tables and in the preparation of foods that are low in calorific values. The development of intriguing groundnut varieties through various breeding techniques has been spurred over the past several years by commercial requirements, mainly concerning their dietary value (16).

Peanut oil possesses a composition of eight main fatty acids (17) *viz.,* behenic acid (22:0), lignoceric acid (24:0), arachidic acid (20:0), eicosenoic acid (20:1), oleic acid (18:1), linoleic acid (18:2) and palmitic acid (16:0). Eighty percent of the fatty acids in peanut oil are reported to be unsaturated oleic and linoleic fatty acids (18). The rest 20% is saturated fatty acids, among which 10% of the total oil content is palmitic acid (19) affecting its oil quality. Besides broadening the shelf life of peanut commodities, an elevated proportion of oleic and linoleic acid (O/L ratio) lowers the risk of cardiovascular disease (CVD) due to the presence of MUFA (Mono Unsaturated Fatty Acids) and encompasses additional advantages on human health (20). Oleic acid has been shown to reverse the inhibitory effect of the inflammatory cytokine TNF-α on insulin production. This finding, derived from studies conducted both in vitro and in vivo, highlights the potential therapeutic benefits of oleic acid for improving insulin secretion in conditions associated with chronic inflammation, such as obesity and type 2 diabetes. A diet rich in oleic acid, found in foods like peanuts and olive oil, may thus provide significant health benefits (20). The content of oleic acid in common varieties of groundnut ranges between 48-54%, while in high-oleic variants, spiking to 80% (21). Linoleic acid is essential in small quantities, but a spike in its percentage can lead to undesirable qualities such as off-flavours, rancidity and limited shelf life for both the oil and its products, making high concentrations less suitable for dietary use. Oleic acid enhances the oxidative stability of groundnut oil, meaning it is more resistant to rancidity and degradation when exposed to heat, light, or air. This stability extends the shelf life of the oil, making it ideal for both cooking and long-term storage (22). Groundnut oil with a high oleic acid content has a higher smoke point, making it suitable for high-temperature cooking methods such as frying and sautéing. This property is highly valued in both domestic kitchens and the food industry (23). Major food manufacturers prefer high-oleic oils because they don’t need hydrogenation (a process that creates harmful trans fats). As countries and regions enforce stricter regulations on trans fats, the demand for high-oleic oils increases. Oleic acid-rich groundnut oil has a mild flavour, making it versatile for use in a variety of culinary applications without overpowering the taste of the food. This versatility enhances its marketability and appeal. There is increasing consumer demand for healthier oil options and groundnut oil high in oleic acid is positioned as a premium product. This demand drives its value in the oil industry, encouraging producers to cultivate high oleic acid groundnut varieties. Beyond cooking, oleic acid is valuable in non-food industries like cosmetics and pharmaceuticals, where it serves as an emollient, enhancing skin moisture and as an emulsifier, stabilizing mixtures of oil and water. Its preferred status over other fatty acids stems from its stability, skin compatibility and ability to improve product texture. Groundnut oil, being a natural source of oleic acid, meets the needs of these markets, highlighting the significance of peanuts in global agriculture and trade (24). The present review highlights the significance of oleic acid and provides a comprehensive overview of the molecular advancements that contribute to enhancing oleic acid content in

groundnuts. This review emphasizes key genetic and breeding approaches, explores omics findings and discusses their implications for the development of high-oleic peanut cultivars.

Genetic basis of oleic acid biosynthesis

The process of fatty acid synthesis involves enzymatically regulated desaturation and elongation of the carbon chain (25) as depicted in Fig 1. According to (26), enzymes called fatty acid desaturases add double bonds to the hydrocarbon chains of fatty acids. They are crucial to maintaining biological membranes and the metabolism of fatty acids. Acetyl-CoA is the precursor molecule for fatty acid biosynthesis. It is produced from the breakdown of carbohydrates in plant cells during the process of oxidative decarboxylation, the pyruvic acid is converted into acetyl–CoA by the action of pyruvate dehydrogenase (27). In the fatty-acid biosynthesis pathway**,** the mechanism of acetyl-CoA carboxylation into malonyl-CoA is mediated by acetyl-CoA carboxylase (ACC), comprising the first concerted phase where multiple enzymatic activities and substrates collaborate efficiently to facilitate the transformation. This phase is crucial for ensuring a coordinated and effective biosynthetic process. Fatty Acid Synthase plays a significant role in various organisms, from bacteria to mammals, influencing metabolic regulation by controlling the synthesis of fatty acids, which are essential for membrane formation and energy storage. Additionally, its activity is tightly regulated in response to nutritional status, impacting overall lipid metabolism and homeostasis. This fatty acid synthase (FAS) complex is involved in the sequential addition of two-carbon units (malonyl-CoA) to a growing fatty acid chain. This process involves multiple steps of condensation, reduction, dehydration and a final reduction to form a saturated fatty acid, typically palmitic acid (16:0). The conversion of palmitic acid (16:0) to stearic acid (18:0) involves the action of stearoyl-CoA desaturase (SCD). The enzyme oleate desaturase further introduces a double bond between carbon atoms 9 and 10 of stearic acid, resulting in the formation of oleic acid (18:1) (28). Linoleic acid (18:2) is synthesized from oleic acid (18:1) through the action of another enzyme, omega-6 fatty acid desaturase or delta-12 desaturase by introducing another double bond between 12C and 13C of the oleic acid chain, converting it into linoleic acid.

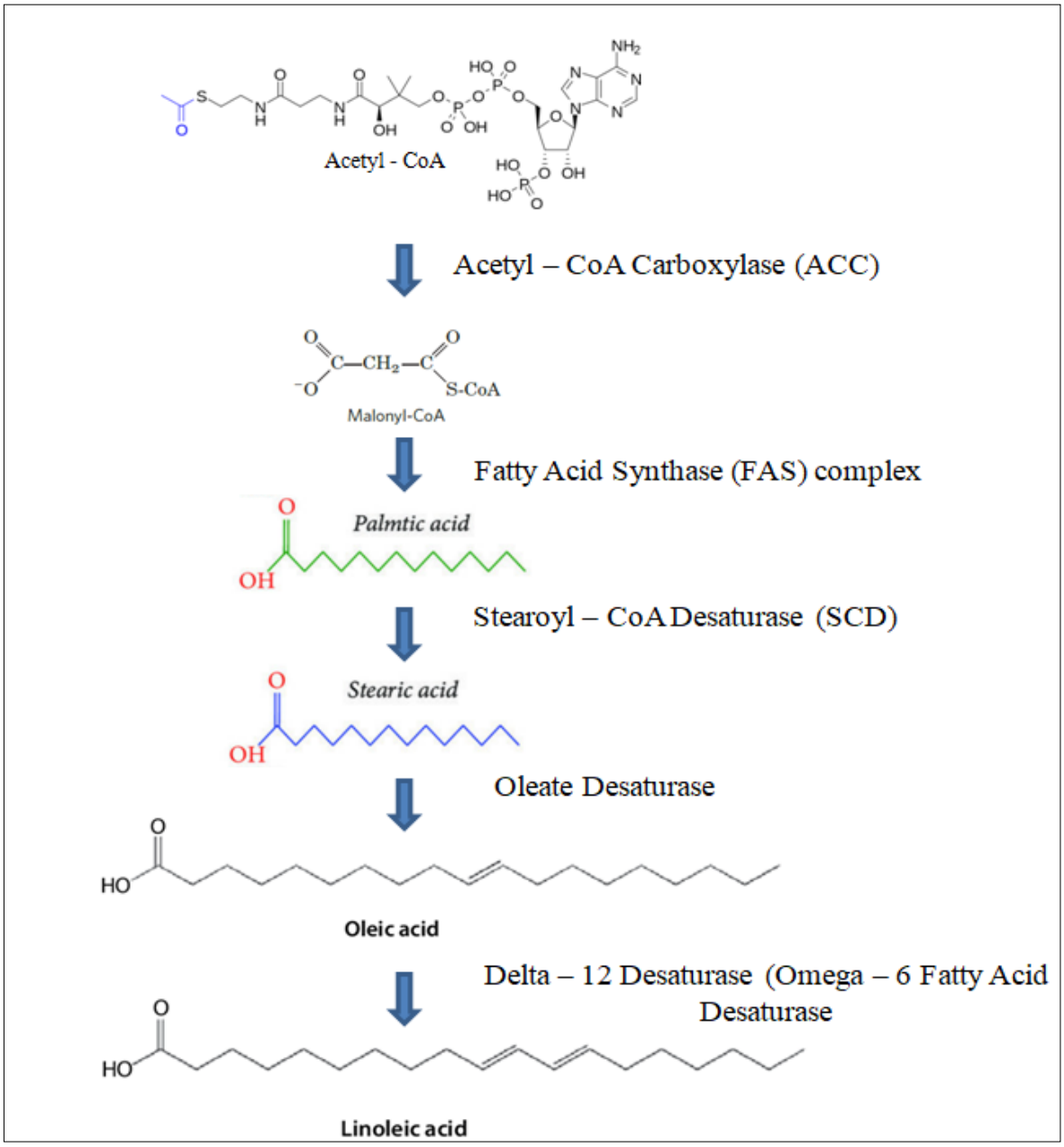

Fig. 1. Flow chart representing oleic acid biosynthetic pathway

Identification of genes

SunOleic 95R, the initial oleate groundnut variety, evolved through conventional breeding with pedigree F435-2-3-B2-l-b4-B-3-b3-l-B×Sunrunner in the United States as a breach to enhance the standards of peanut oil. This is followed by chemical mutagenesis, a technique used to induce mutations in the peanut genome, which resulted in the generation of high-oleic-acid mutants such as C458 (ethyl methane sulfonate - treated 'Florunner) and M2-225 (diethyl sulfate - treated AT-108). By exposing seeds to these chemical agents, specific genetic changes are introduced, leading to variations in oleic acid content and enabling the selection of desirable traits in subsequent generations (29, 30). The key enzymes that add a double bond to oleic acid and modify it into linoleic acid are encoded by the fatty acid desaturase gene *AhFAD2* (31, 32). The two genes namely *AhFAD2A*, AA & *AhFAD2B*, BB corresponding to the A and B subgenomes are identified in the cultivated peanut genome. The "F435" peanut mutant is the first naturally developed high-oleic-acid variety comprising 80% oleic and 2% linoleic acid (21). This mutant possesses an early stop codon in the B subgenome due to the mutated *AhFAD2B* (*bb*) gene's 'A' insertion (A: T) and mutated *AhFAD2A* (*aa*) gene's substitution (G: C to A: T) in the A subgenome (Fig. 2) (32, 33). The presence of these dual mutations (*aabb*) resulted in an increased content of oleic acid and a decreased linoleic acid in "F435" in contrast to the wild type.

Molecular tools for identifying ahFAD2 mutations

Understanding the genetics and molecular mechanisms associated with the ahFAD2 gene has led to the development of various assays for detecting FAD2 gene mutations. These include Allele Specific PCR (AS-PCR), Cleaved Amplified Polymorphic Sequences (CAPS), Real-Time PCR (RT-PCR) and Kompetitive Allele-Specific PCR (KASP) (Table 1). (34) presented initial documentation of CAPS markers for the high oleic as well as low linoleic acid trait in groundnuts. Despite this milestone, the assay had not been incorporated into breeding programs due to its scanty profile. Subsequently, a CAPS technique was devised by (35) for detecting the 448G > A mutation (Substitution) in the *ahFAD2A* gene and 441_442insA (Insertion at 441_442 position) mutation in the *ahFAD2B* gene and confirmed later (36). These techniques are proven to be precise and robust, earning widespread utilization by researchers globally for discerning heterozygotes (37-40).

A rapid yet cost-intensive genotyping technique, Real-time Polymerase Chain Reaction (RT-PCR), has been devised for the *ahFAD2A* gene by (41) and the *ahFAD2B* gene by (42). Furthermore, the researcher explored the genetic impact of the *ahFAD2* gene on fatty acid (FA) profiles in segregating populations using RT-PCR (43). Earlier (33) introduced a straightforward Allele-Specific Polymerase Chain Reaction (AS-PCR) assay capable of identifying mutant alleles on both the A genome and B genome and this can be considered as an economic alternative. Conversely, this technique faced limitations in distinguishing between heterozygotes and homozygotes. To address this challenge, another AS-PCR assay is crafted by (44) for *ahFAD2B* and by (45) for *ahFAD2A* and *ahFAD2B* alleles. These assays involve distinct reactions for unveiling mutant and non-mutant alleles, providing a solution to the differentiation issue. In a recent study, (46) introduced a KASP assay, employing competitive binding of AS forward primers in a unified reaction for performing bi-allelic identification. The research findings to identify the ahFAD2 mutations in groundnuts employing various molecular tools were presented in Table 1.

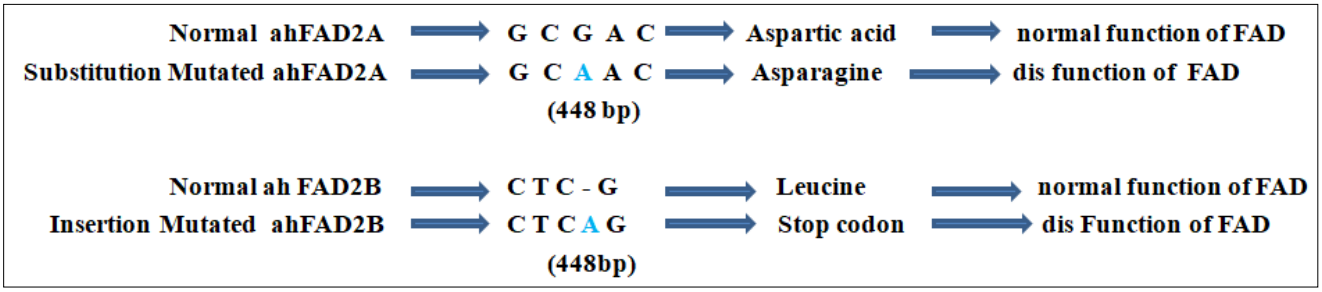

Fig. 2. Type of *FAD2* gene mutations in groundnut

Table 1. Molecular tools for identifying and exploiting ahFAD2 mutations.

Tool	Allele	Population	Application	Reference
AS-PCR assay	*ahFAD2A& ahFAD2B*	F_2 population and some HO lines	Establishment and Validation of assay for *ahFAD2* Alleles in Peanut Genotypes	(33)
Real time PCR assay	*ahFAD2*	Back cross population	HO trait transferred to South African cultivars	(47)
RT-PCR assay	*ahFAD2*	539 F2 progenies from 06 crosses	high oleate trait is not exclusively governed by dominant gene action	(43;47)
CAPS & AS-PCR assay	*ahFAD2*	MABC of three elite genotypes	469 introgression lines (ILs) for HO trait.	(48)
CAPS & AS-PCR assay	*ahFAD2*	174 germplasm accessions	AhFAD2A mutation was detected in 46% of the accessions, whereas none exhibited ahFAD2B mutation.	(40)
KASP assay	*ahFAD2*	179 BC4F2 plants	Identified multiple alleles of ahFAD2 genes in one reaction.	(46)
Kompetitive allele-specific PCR (KASP)	*FAD2*	BC_4F_6	Transferred HO trait into huayu 22	(49)
Allele-specific marker	*ahFAD2A & ahFAD2A*	F_6	Increased oleic acid content to 83.3%	(50)

QTL Mapping

Quantitative Trait Loci (QTL) mapping is a pivotal technique in genetic improvement programs aimed at enhancing desirable traits in plants. Enhancing traits like oil content and disease resistance is crucial, as they significantly impact crop yield and quality, thereby contributing to food security and agricultural sustainability. By linking these traits to particular genetic markers, QTL mapping allows breeders to more accurately select individuals with desirable traits for breeding programs. This accelerates the process of genetic improvement by enabling marker-assisted selection (MAS), where selection decisions are based on genetic information rather than solely on observable characteristics. Besides, it serves as a standard approach for pinpointing the location of genes that govern desired traits (51). As mentioned in Fig. 3 effective QTL mapping necessitates (a) developing a suitable mapping population and precise phenotypic trait assessment (b) choosing appropriate molecular markers and generating molecular data containing a sufficient quantity of evenly distributed polymorphic markers (c) developing genetic linkage maps using statistical software programs for locating QTL precisely. The abundance of genomic materials, such as molecular markers, genetic and physical maps, has significantly streamlined the QTL gene mapping process (52, 53).

Numerous QTL associated with traits related to pod, seed, fresh seed/seed dormancy, nutritional quality characteristics, resilience to disease and drought have been documented (54, 55). These QTLs are reported to be valuable resources for enhancing peanut traits. Despite the wealth of identified QTLs, only a limited number are actively employed in peanut enhancement initiatives targeting the development of superior cultivars.

QTL mapping has uncovered noteworthy discoveries concerning oil content as well as protein content, alongside fatty acid makeup, in peanuts (17). Additionally, QTL associated with unsaturated fatty acids and the O/L ratio have been documented (56), along with QTL concerning saturated fatty acid composition (57). A mapping population consisting of 146 RILs derived from the cross TG26 x GPBD4 was utilized for identifying QTL related to protein, oil, oleic and linoleic acid measure, as well as the O/L ratio (58). The study revealed 17 QTLs spread across four genomic sites, encompassing two major QTLs associated with protein content. From this study, we infer that the QTLs identified may further be validated for their utilization in the breeding programme for enhanced protein, oil and oleic acid content. (52) developed a RIL population from the cross (Sun Oleic 97R x NC94022 and Tifrunner x GT - CT20). This study displayed 78 main effect QTLs and 10 epistatic QTLs for oil content and oil quality traits. Alongside, two consistent QTLs were identified for oleic acid and linoleic acid. These QTLs can further be validated for their deployment in genomics-assisted breeding for superior cultivars with increased oleic acid.

In a similar study conducted by (17), two maps were constructed to identify the genomic regions for oil content and oil quality traits. QTL analysis revealed the presence of 21 QTLs for fatty acids, among which 20 QTLs were major. Besides, two mutant alleles, *ahFAD2B* and *ahFAD2A* were found to show phenotypic variance explained for palmitic acid, oleic acid and linoleic acid. This study can further be investigated for candidate gene identification and thereby marker discovery and can be exploited for marker-assisted selection. Furthermore, a detailed genetic map comprising 2334 SNP markers revealed 29 significant QTL located on chromosomes A03 and A09/B09, elucidating between 10 to 57.6% of the phenotypic variance for linoleic and oleic acid content, as well as the O/L ratio (59). From this study, we infer that the QTLs identified can further be introgressed into cultivars/elite cultivars for enhanced oleic acid and linoleic acid content. Similar findings of different QTLs for oleic acid are mentioned in Table 2.

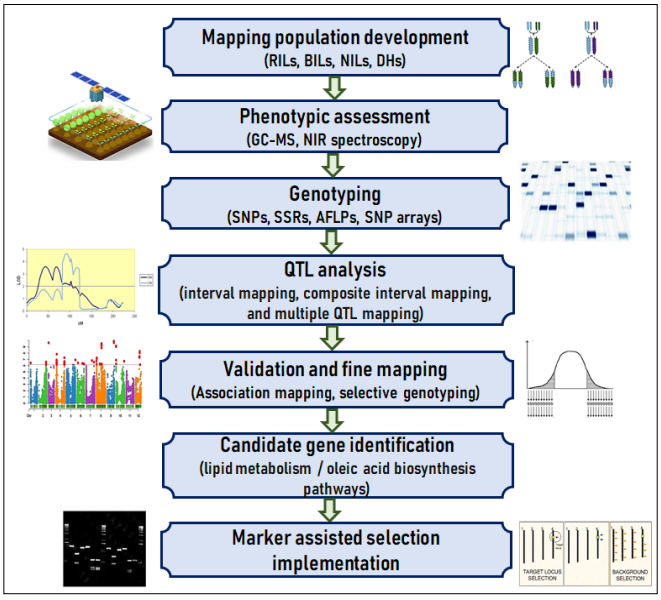

Fig 3. Steps involved in QTL mapping

Table 2. QTL identified for Oleic acid in Groundnut

QTL Identified	Population	Chromosome	Marker interval	Phenotypic variance explained (%)	References
03	RIL	LG - 01	AHGS2353 - AHGS1981	5.077	(60)
		LG - 05	UPN027 - UPN071	4.6046	
		LG - 14	UPN100 - UPN024	3.859	
03	RIL	B02	AHGS2238 - Ai02B4283	9.28	(61)
		B06	Ai06B8308 - AGGS1205	21.53	
		B06	AGGS2573 - AhTE0569	12.67	
02	RIL	A09	IPAHM372 - *ahFAD2B*	3.42	(52)
		B09	GM1840 - *ahFAD2B*	42.33	
02	RIL	LG- 05	bin1601 - bin1602	5.41	(62)
		LG - 05	bin185 - bin1853	3.14	
03	RIL	A03	IPAHM_103- Marker4016934	3.98	(56)
		A09	Marker4391589- Marker4463600	8.60	
		B09	Marker2575339- Marker2379598	57.54	

Marker-Assisted Selection (MAS)

The advancement of molecular marker technology has established MAS as a significant breeding technique due to its ability to enhance precision and efficiency in selecting desirable traits. MAS allows breeders to identify and select for traits such as disease resistance and improved yield early in the breeding process, reducing the time and resources required to develop superior crop varieties (63). Being a pivotal approach for enhancing selection effectiveness, genetic markers hold promising potential for enhancing oil content and quality through MAS (64). Traditional backcross program facilitates transferring the favourable trait to a preferred genetic background *i.e* elite cultivar (65). Marker-assisted backcrossing can introgress a gene into the background of an elite cultivar within just two to three backcross generations. This process also facilitates the recovery of the recurrent parent genome, significantly shortening the breeding duration (66,67). Main objectives typically revolve around enhancing both the quantity and quality of seed oil in groundnut breeding initiatives (52). Lately, growing awareness of utilizing ground oil is characterized by high oleic and low linoleic acid concentrations. Nevertheless, the production of high-oleic-acid peanut cultivars primarily involves hybridization and pedigree selection, processes that are challenging to execute on a commercial scale due to the complexities of managing genetic variability and ensuring consistent trait expression across generations. These methods require extensive resources and time to achieve the desired outcomes, complicating large-scale implementation. For enhancing the oleic acid level, while maintaining yield along with other quality characters, significant effort has been focused on investigating MABC strategies that incorporate ample genetic variability, which includes diverse alleles and genetic resources from both wild relatives and existing cultivars. This variability is crucial for improving the chances of successful trait introgression and achieving desirable phenotypic outcomes (57, 68).

(50) followed MAS for developing high-oleic lines along with pod yield using high-yielding line NC-7 and a high-oleic line, HOG. They selected two lines (NH-2 and NH-3) showing the highest oleic acid levels were forwarded to the next generations. At last, 21 advanced inbred lines in addition to high pod yields along with the highest levels of oleic acid content ranging at 83.3% were obtained. These advanced lines with combined high oleic content and desired agronomic traits can meet the market demand for high-quality oil. Similarly, by using KASP assay and SNP arrays, an oleic acid gene from KN176 was introgressed into high-yielding cultivar huayu22 by (49). Across all the superior lines, a single line “YH61” with high oleic acid along with pod yield was found. This study suggests that a marker-assisted backcross strategy utilizing cost-effective KASP assays and SNP arrays for detecting mutations in the fad2 gene and evaluating genetic backgrounds can enhance peanut breeding programs. By improving oil quality and increasing yield, these methods could lead to more productive and sustainable peanut cultivation. A high-yielding groundnut cultivar GJG33 crossed with high oleic line Girnar4 (69) resulted in the generation of homozygous lines for high oleic acid in the BC_2F_2 population. This study serves as a guide in the identification of primers to be used in designing the backcrossing programme for developing cultivars with high oleic acid. Some of the work done in MAS for oleic acid improvement is mentioned in Table 3.

Genome editing techniques

Gene silencing, mega nucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and CRISPR/Cas are the genome editing tools used to modify double-stranded DNA at specific chromosomal sites in plants as presented in Fig. 4 (73). Subsequently, the plant’s natural DNA repair mechanisms mend these breaks, leading to mutations at the intended locus. Even though the basic mechanisms of the above-stated genome editing platforms share similarities, CRISPR/Cas has risen as the favoured tool because of its user-friendly design, cost-effectiveness, remarkable flexibility and capability to aim multiple genes at a time (74).

Gene silencing

Gene silencing through RNA interference (RNAi) is a modern technique that results in targeted gene suppression (75). It offers an innovative approach to reverse genetics and plays a significant role in improving the qualitative characteristics of the plant for economic purposes (76). In plant metabolism, RNAi can be employed to modify metabolic pathways by inhibiting the function of specific enzymes, achieving objectives like modifying proteins, starch, fatty acids and other macromolecules' structure and components (77, 78). RNAi techniques can be employed to target oleate desaturase, a crucial enzyme responsible for catalyzing the conversion of oleic acid to linoleic acid. While these approaches have been successfully applied to inhibit linoleic acid biosynthesis in various plant species such as *Arabidopsis thaliana*, rape, cotton and soybean (77), there is a gap in reported applications for peanuts. Future research could explore optimizing RNAi strategies for peanuts, investigating other genetic pathways that influence oil composition and evaluating the combined effects of RNAi with traditional breeding methods to develop high-oleic acid cultivars. Reverse-repeating specific-expression vectors were created in a work by (79) using a conserved region from the FAD2 gene. Through Agrobacterium-mediated genetic transformation of peanuts, they obtained 11 transgenic plants. Fatty acid analysis revealed a significant rise in oleic acid levels in four transgenic plants having low or no expression. It was inferred from this study that this sheds light on molecular methodologies in the downregulation

Table 3. Marker Assisted Selection using different breeding techniques in different populations

Method	Population	Findings	References
MAS & MABC	ICGV 06110, ICGV 06142 and ICGV 06420	Rise in Oleic acid level by 0.5-1.1 folds	(48)
MAS	ICGV 05141	Increased oleic acid up to 40%	(70)
MABC	SunOleic95R, ICGV06100	97% increase in oleic acid	(71)
MABC	GJG 9, GG 20 & GJGHPS 1	Resistant to rust in addition to leaf spot and also nearly 80% of Oleic acid	(72)
MABC	huayu22 x KN176	Increased oleic content	(49)
MAS	NC-7 x HOG	Increased oleic content up to 83.3%	(50)

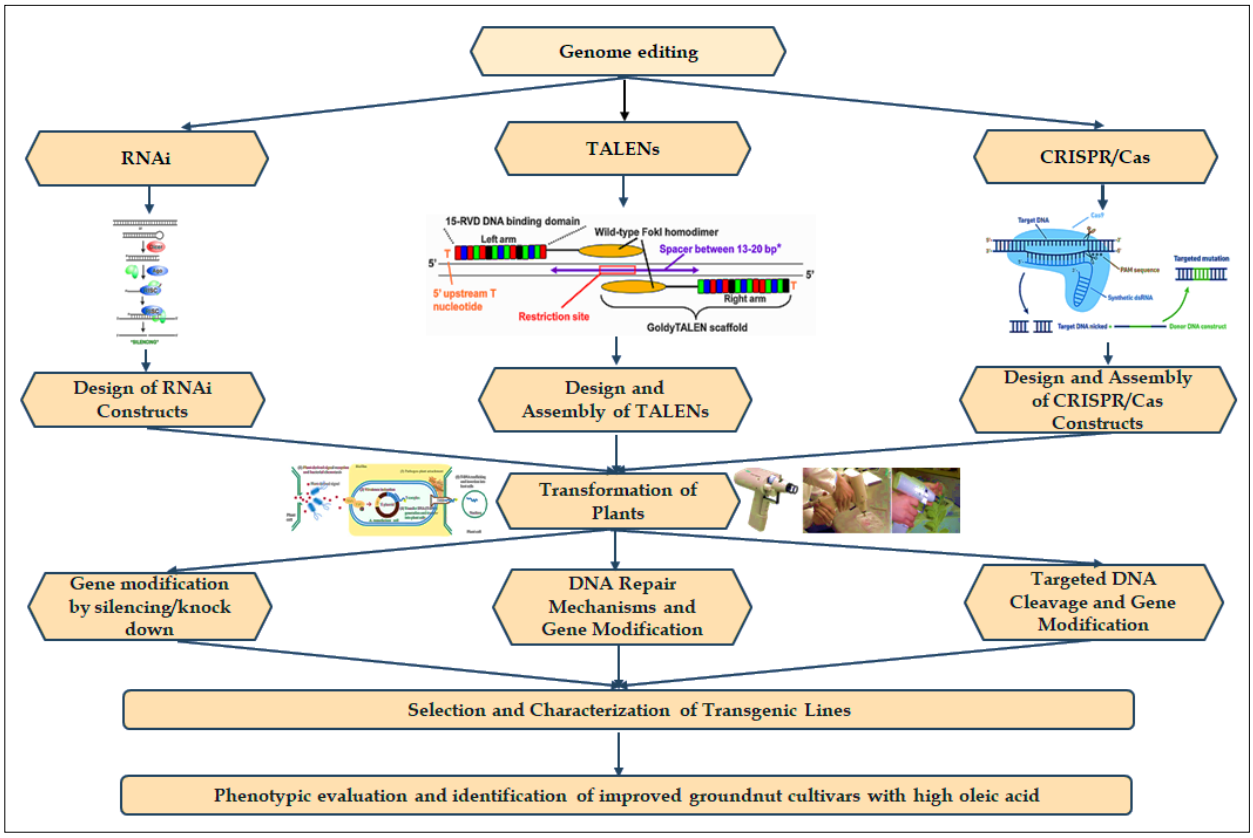

Fig 4. Different genome editing techniques utilized for oleic acid improvement in groundnut

of *FAD2*gene resulting in a spike in oleic acid content, thereby genetically modifying the seed quality.

Nevertheless, the predominant focus of studies aiming for increased 18:1 content has often been concerned with down-regulating or knocking off the *FAD2* gene. This enzyme drives the conversion of 18:1 to 18:2, resulting in the amassing of 18:1 while reducing the levels of 18:2 and 18:3. An illustration of this method can be seen in the development of "super high" 18:1 safflower, which attains levels of up to 94% 18:1 in its seed oil, surpassing the approximately 80% noticed in other high oleic lines. This was achieved by knockdown of *FAD2.2* and *FATB* genes at a time using RNAi technique (80). In a similar study conducted by (81), a 390-bp conserved sequence from GmFAD2-1B was used to induce RNAi-mediated gene knockdown in soybeans. Expression analysis revealed a significant down-regulation of GmFAD2-1B in seeds through RNAi-induced gene suppression directed by the seed-specific promoter. The transgenic seeds exhibited a substantial increase in oleic acid content from 20% to approximately 80%, accompanied by a decline in linoleic and linolenic acid levels compared to wild types. From this, we infer that RNAi using seed-specific promoter serves as a competitive tool compared to other genome editing techniques for breeders in targeted gene silencing without altering the other traits. Apart from fatty acids, RNAi is also successfully implemented in developing disease-resistant lines. The first successful application of RNAi mediated approach was demonstrated in peas by targeting the immunodominant allergen *Ara h2*, which reduced the growth of *A. flavus* in seeds (82). (83) conducted a case study for silencing *Ara h2* through RNAi mechanism in peanut. In another study, *Ara h2* and *Ara h6* genes were silenced by introducing the RNAi construct targeting homologous coding sequence and this resulted in the reduction of *A. flavus* growth in peanuts (84).

Transcription Activator Like Effector Nucleases (TALENs)

TALENs, enabling precise DNA editing, have been successfully created and employed in genome engineering across various organisms (85). However, their utilization in higher plants and crop enhancement, particularly in allopolyploid plants is constrained. In a study by (86), TALENs were employed to achieve targeted mutagenesis in peanuts. Precisely, TALENs were employed to introduce desired mutations into the *AhFAD2* coding sequence. DNA sequencing confirmed the genetic stability of *AhFAD2* mutations up to 9.52% and 4.11% of the regenerated plants at two distinct locations. The frequency of mutation correlated significantly with oleic acid increase. Individuals inheriting genetic stability from beneficial mutant lines exhibited a 0.5-2-fold rise in oleic acid content when contrasted with non-transgenic counterparts.

Lately, genome editing techniques have been employed to accurately modify FAD2 genes to increase the 18:1 level in the oil.

Notably, 18:1 enhanced soybean oil obtained through TALEN is formerly available in the United States. The effective manipulation of FAD2 genes by TALEN-mediated means has been proven in camelina (87) and soybean (88). These efforts resulted in seed oil with elevated 18:1 content, ranging from 50% to 83%, in comparison with the 10-25% in wild genotypes. Additionally, these modifications were accompanied by concurrent reductions in 18:2 and 18:3 levels. In a similar study by (89), DNA sequences that are conserved in both genes are recognized and cleaved by TALENs. DNA isolated from leaf tissue displayed mutations in *FAD2-1A* and *FAD2-1B* among the four of the 19 transgenic soybean lines encoding the TALENs; three out of four lines passed on heritable *FAD2-1* mutations to subsequent generation. In plants with homozygous mutations in *FAD2-1A* and *FAD2-1B*, the fatty acid profile of the seed was significantly altered: oleic acid raised from 20% to 80% and linoleic acid reduced from 50% to less than 4%.

From the aforementioned reports, we infer that the TALEN-mediated gene editing offers a distinct advantage over conventional techniques as it significantly accelerates the genome modifications in the groundnut. While TALENs are effective for precise genome editing, they face challenges like regulatory hurdles and public skepticism, particularly in terms of the acceptance of genetically modified crops. Regulatory frameworks for TALENs can be complex and public perception varies across regions, potentially affecting adoption in agriculture. TALENs are precise genome-editing tools that cut DNA at specific sites. However, like other genome-editing methods, they can cause off-target effects, where the nuclease cuts DNA at locations other than the intended target, though typically fewer than earlier techniques. These off-target effects may lead to unwanted genetic alterations which may result in the disruption of essential genes or deactivation of biological pathways. In comparison, CRISPR is often preferred due to its higher efficiency, ease of use and lower cost. CRISPR also allows for more rapid and scalable genome editing, which is why it has become a more popular choice for many applications, though TALENs remain useful in certain precise editing scenarios (90).

CRISPR - CAS9

The microbial CRISPR-Cas mechanism is the source of the RNA-guided Cas9 nucleases, which have become a powerful genome editing approach across various organisms (91, 92). Modified from adaptive immunity mechanisms found in bacteria or archaea, CRISPR/Cas functions by incorporating viral DNA fragments into its genome. These short RNA pieces, which are transcribed into recognition signals, stop further viral attacks by causing homologous viral DNA to be cleaved by Cas proteins (93). For targeted genome editing, the CRISPR/Cas system involves a Cas nuclease, inducing double-stranded breaks (DSB) and a small noncoding single-guide RNA (sgRNA) of approximately 20 nucleotides. This sgRNA directs Cas to the specific genomic site, typically formed by a chimeric gRNA and trans-activating CRISPR -RNA (tracrRNA; essential for maturation of crRNA). In the majority of Cas systems, the sgRNA must be crafted to anneal directly upstream of a protospacer adjacent motif (PAM). For Cas9 derived from *Streptomyces pyogenes*, which is extensively utilized for genome editing, the PAM sequence comprises -NGG-. Cleavage typically occurs about 3 nucleotides upstream from this region (94).

Genome editing tools, including CRISPR/Cas, rely on the plant's inherent repair mechanisms, homology-directed repair (HDR), or non-homologous end joining (NHEJ) to amend double-stranded breaks (DSBs). The main DNA repair process in higher organisms is NHEJ (95), which is the primary and simpler pathway in genome modification, often resulting in error-prone repairs such as insertions and deletions that modify the targeted gene. The application of CRISPR/Cas9 is challenging in polyploidy species, particularly those with closely related sub genomes like peanuts because the target genes exist in numerous copies.

Furthermore, the accumulation of oleic acid traits may be influenced by combinatorial mutations in different sub genomes. Recent assessments conducted in the hexaploid species, *Camelina sativa* examined the impacts of mutant allele combinations resulting from gene editing at the FAD2 loci (96). Their results illustrated that different combinations of mutant alleles led to varying levels of oleic acid from 10% - 62%. Nevertheless, the complete depletion of FAD2 function resulted in notable developmental abnormalities, underscoring the significance of polyunsaturated fatty acids in plant physiology. It is essential to design multiple sgRNAs targeting various regions within the coding sequence of both homologous ahFAD2 genes. Furthermore, a systematic analysis of ahFAD2 genes is essential for enhancing our comprehension of ahFAD2 gene expression. A study conducted by (97), utilized the CRISPR/Cas9-based method for precise genome modification, targeting downregulation of the expression of two homologous FAD2 genes specifically in seeds. It has been determined that two cis-regulatory regions in the 5′UTR and related intron of FAD2 genes, the RY repeat domain and the 2S seed protein motif, may be essential for controlling seed-specific gene expression. Differential editing efficiencies were found at both cis-regulatory elements (CREs) when single guide RNAs (gRNAs) targeting two distinct sgRNA scaffolds were applied by hairy root and stable germ line transformation. Editing efficiencies varied when both gRNAs were expressed concurrently. Moreover, seeds from stably transformed plants exhibited a rise in oleic acid levels in varying proportions when compared to wild type. The aforementioned reports provide new insights to investigate the impact of mutation combinations resulting from gene editing on oleic acid content and to offer novel sources for the high oleate character in groundnut breeding. The success of CRISPR/Cas9 in different oilseed crops is mentioned in Table 4. CRISPR's potential for germline editing, where genetic changes are passed on to future generations, raises significant ethical concerns. One of the most debated issues is whether such modifications should be allowed, especially considering the potential for unintended consequences, such as "designer babies" or altering traits beyond disease prevention. The famous case in 2018 of genetically edited embryos in China highlights the global unease surrounding heritable gene editing. Ethical discussions need to weigh the benefits of disease eradication against societal and moral implications. CRISPR technologies are advancing rapidly, but regulatory frameworks have struggled to keep pace. Different countries have adopted varying stances, with some opting for strict regulation while others are more lenient. The lack of international consensus makes commercialization and clinical applications challenging, as legal and safety standards can differ widely. Public acceptance also remains a key issue, often shaped by misinformation and concerns about the long-term implications of gene editing (98,99).

Omics

Comparative advantage of all the omics studies such as genomics, transcriptomics, proteomics and lipidomics will provide valuable information starting from gene to metabolome about oleic acid improvement (Fig. 5). Transcriptomics, a well-established technique in the post-genomic era, encompasses the study of the entirety of RNA transcripts within a particular cell or tissue, considering various RNA types like mRNA, tRNA, rRNA and ncRNAs (105). It explores gene expression at the RNA level, offering comprehensive insights into the structure and function of genes across the genome. This approach aids in unraveling the molecular mechanisms underlying specific biological processes. In oilseed crops transcriptomics has been used to identify key genes regulating the fatty acid composition. For example, the *FAD2* gene, which is involved in converting oleic acid to linoleic

Table 4. Genome Editing achievements in different Oilseed crops

Sl No.	Crop	Gene	Tool	Findings	Reference
1	Peanut	*FAD2*	CRISPR/Cas9	Three mutations were identified - G448A in *ahfad2a*, 441_442insand G451T in *ahfad2b*	(98)
2	Soyabean	*FAD2*	CRISPR/Cpf1	-	(100)
3	Soyabean	*GmFAD21A and GmFAD2-1B*	CRISPR/Cas9	In the fad2-1a and fad2-1b mutants, the oleic acid content rose from 11% to 40-50% and reached 85% in the fad2-1a/fad2-1b mutants.	(101)
4	Soyabean	*FAD2-1A and FAD2-1B*	CRISPR/Cas9	Mutation in FAD2-2	(102)
5	Camelina	*FAD2 gene*	CRISPR/Cas9	The oleic acid content rose from 16% to surpass 50% of the fatty acid proportion.	(87)
6	Peanut	*FAD2*	TALEN	0.5-2-fold rise in the oleic acid level	(86)
7	Camelina	*FAD2*	TALEN	50-83% increase in oleic acid	(87)
8	Soyabean	*FAD2*	TALEN	Enhanced levels of 18:1 in seed oils	(101)
9	Soyabean	*FAD2-1A and FAD2-1B*	TALEN	20%-80% increase in oleic acid	(89)
10	Flax	*FAD2*	RNAi	Increased oleic acid level to 80%	(55)
11	Canola	*PDCT*	RNAi	Increase in 18:1	(103)
12	Mustard	*BnaFAD2*	RNAi	Increased oleic acid level up to 85%	(104)

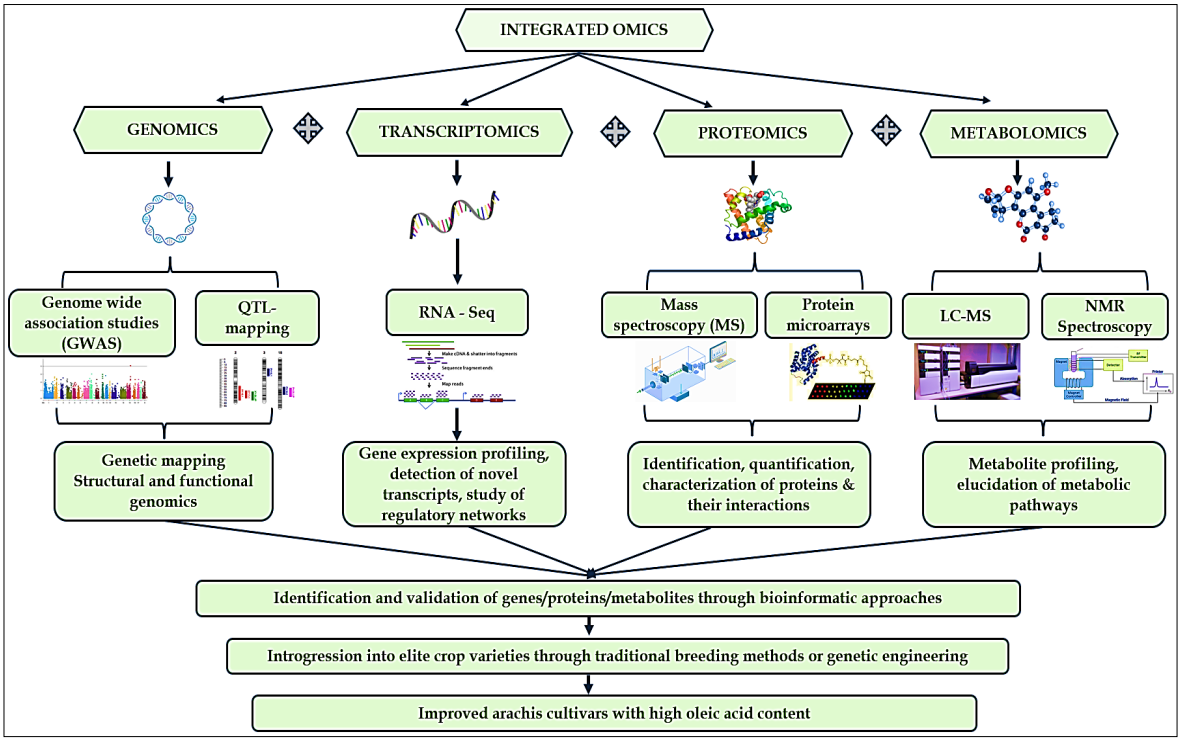

Fig 5. Integrated Omics approach for improving oleic acid in groundnut

acid, was identified as a critical target. Mutations or downregulation of this gene, unveiled through transcriptomic studies, have been applied in marker-assisted selection to breed high-oleic varieties with improved oil quality. Transcriptomic analyses in groundnuts have identified genes involved in resistance to *Aspergillus flavus*, a fungus responsible for aflatoxin contamination. By focusing on differentially expressed genes in resistant and susceptible varieties, breeders can prioritize these genes in selection programs targeting reduced aflatoxin contamination in groundnuts. Comparative transcriptomics utilized by (61) to assess the gene expression of normal and high oleic groundnut cultivars at different stages of seed development. The outcome revealed that the occurrence of early translation termination at a time in the *FAD2A* and *FAD2B* coding sequence and the cultivar H176 can be utilized as a potential source for future groundnut breeding for high oleic acid. Differential processing events of mRNA were identified for most of the peanut genes and found that 15.8% and 18.0% of the unigenes were expressed differentially between high and low oil varieties at 30 and 50 days after flowering, respectively (106). From this study, we infer that this unigene resource helps in the development of new markers and their exploitation in oil breeding programmes.

Lipidomics, a nascent methodology, centers on elucidating the control of lipid metabolism in diverse essential functions. In peanut breeding programs, integrating lipidomic data into traditional breeding strategies allows for a more precise selection of varieties with improved oil composition. By using lipidomic signatures as biomarkers, breeders can accelerate the selection process for high-oleic varieties and ensure consistency in fatty acid profiles. This approach also helps in breeding varieties with better resistance to oxidative rancidity, extending the shelf life of peanut oil and products. The combined use of lipidomics with genomics and transcriptomics presents a more holistic view of fatty acid metabolism, offering a powerful tool for peanut breeders aiming to enhance oil quality traits. Recent years have highlighted the potential applications of lipid analysis in plant studies. High-throughput profiling of lipids offers insights into particular species and phospholipid (PL) equilibrium during seed growth in *Arabidopsis* (107). Proteomics, a branch of

molecular biology, systematically investigates different proteins, their structure and their function in a biological system. These tools enable scientists to unravel the complexity of cellular processes and gain insights into the mechanisms underlying diseases. In agriculture, proteomics unravels the molecular foundations of traits, thereby contributing to advancements in crop improvement (108). Through the use of mass spectrometry, 547 lipid characteristics are detected in high-oleic and normal-oleic peanut seeds. During the development of high-oleic acid (OA) seeds, lipids that are differentially expressed due to *fad2* induction exhibited a polar distribution at both early and maturation stages. Following this, the amalgamation of proteomic data previously published and lipidomic data unveiled that 21 proteins and 149 differentially expressed lipids (DELs) were annotated within the triacylglycerol assembly map. Among them, nine enzymes and 31 lipid species exhibited comparable variation trends. Moreover, the fluctuation patterns of 17 acyl fatty acids were outlined within a theoretical biosynthetic pathway. Proteomic studies have identified proteins involved in oxidative stress responses, such as superoxide dismutase (SOD) and catalase (CAT), which play crucial roles in scavenging reactive oxygen species (ROS) under drought conditions. These proteins are upregulated in drought-tolerant varieties, suggesting their potential as molecular markers for selecting drought-resistant genotypes (109). By targeting these proteins, breeders can select varieties that better cope with water deficit conditions. In groundnuts, seed storage proteins like arachin and conarachin have been studied in relation to nutritional quality and allergenicity. Proteomic analyses have shown that variations in the expression levels of these proteins can influence the nutritional content of seeds, particularly the levels of essential amino acids (110). By identifying specific isoforms of these proteins, breeders can select varieties with improved nutritional profiles or reduced allergenicity. Proteomic studies in crops like groundnut have uncovered proteins associated with pathogen defense, such as pathogenesis-related (PR) proteins, which are upregulated in response to fungal infections like *Aspergillus flavus*. These proteins are part of the plant's innate immune system and are essential for resistance against aflatoxin contamination (111). Their identification through proteomics can guide breeding programs to enhance disease resistance in groundnuts. This integrated proteomic-lipidomic study provides a foundation for high oleic peanut breeding to develop superior cultivars.

From the aforementioned techniques, the specificity of each method was chosen based on its ability to target key genes regulating fatty acid biosynthesis. RNAi was employed for its capacity to silence specific gene expressions (75), while TALENs and CRISPR/Cas9 were selected for their precision in genome editing (83, 87). The choice of these tools was driven by the need for high specificity and efficiency in modifying oleic acid-related genes like *FAD2*. Additionally, omics approaches, including transcriptomics and lipidomics, were used to identify molecular markers and lipid profiles associated with high-oleic traits. The selection of these methods was guided by their proven effectiveness in previous studies and their adaptability to peanut breeding programs, ensuring targeted improvement of oleic acid content. The experimental design involved target gene selection where the genes related to the fatty acid desaturation pathway were identified using prior studies, omics data and functional genomics approaches. Following construct development for RNAi, specific constructs were designed to silence target genes, while TALENs and CRISPR/Cas9 were used to create gene knockouts or edits. The guide RNAs (for CRISPR) and target sequences (for TALENs) were selected based on sequence conservation and functional significance. Groundnut plants were transformed using *Agrobacterium*-mediated or other transformation techniques and successful gene modifications were confirmed through molecular assays such as PCR, sequencing and expression analysis. Edited plants were evaluated for oleic acid levels through gas chromatography and lipidomic profiling to confirm the impact of genetic modifications. Selection was based on the most promising lines exhibiting enhanced oleic acid content and stable inheritance of the trait (112). Though conventional breeding succeeded in developing groundnut cultivars with high oleic acid, it is destined to few constraints *i.e.,* more time, less precision and linkage drag. These constraints can be overcome by the use of biotechnological advancements like QTL mapping, marker-assisted selection and genome editing techniques (RNAi, TALENs, CRISPR- Cas). These techniques reveal their applications in uncovering the gene locations, introgressing genes at specific locations with more precision in less time and gene stacking without linkage drag. Additionally, omics technologies provide the profiling of particular traits from gene level to metabolite level.

Association of oleic acid with other traits

In groundnut, oleic acid content can significantly influence various agronomic traits. Groundnut is a significant oilseed crop, particularly, with oleic acid content and is an essential consideration for both agronomic performance and market value emphasizing its effects on agronomic traits in groundnut. Many studies have been conducted to determine the association between the oleic acid trait with other traits in groundnuts. There was no notable correlation observed between pod yield and oil quality characteristics such as oleic acid, linoleic acid and O/L ratio. This suggests that increases in pod yield can be achieved without impacting the oleic acid content (113). (114) reported that no correlation was detected between pod yield and O/L ratio among the tested genotypes, also reported the non-significant correlation between pod yield and oleic acid, so the accessions possessing high oleic acid will be selected directly which doesn't show any impact on yield. Oleic acid had a significant and positive correlation towards Total Monounsaturated fatty acids (TUMS), Total Unsaturated Fatty acids (TUS) and O/L ratio (115). Besides, oleic acid displayed a significant negative correlation with both linoleic and palmitic acids, implying a potential elevation in oleic acid content concurrent with reductions in linoleic and palmitic acid levels. Other biochemical traits like linoleic acid, palmitic acid, steric acid and oil content are negatively correlated with oleic acid content (116). Notably at lower temperatures (16˚C and 14˚C), the germination of peanuts diminished as the O/L and unsaturated/saturated ratios rose (70). From the above-mentioned reports, we infer that increasing the level of TUMS, TUS and O/L ratio significantly increases oleic acid content and hence molecular techniques could be deployed for elevating their levels and developing elite cultivars.

Future Prospects and Conclusion

Currently, the majority of existing high-oleic peanut cultivars originate from a handful of high-oleic parental lines. Hence, to mitigate inbreeding and broaden the genetic diversity among these lines, it is imperative to identify new natural high-oleic genetic resources from the current gene pool or to develop them through mutation breeding programs (117). Expediting the development of high-oleic peanut varieties can be achieved by rapidly resequencing individual progenies within a peanut breeding population using high-throughput techniques. Moreover, leveraging genome-wide association studies (GWAS) to differentiate peanut genotypes could help breeders pinpoint lines with the desired mutations in the *ahFAD2A* and *ahFAD2B* genes at an early stage, thus eliminating the need for field phenotype validation. The advancement of genomics technologies such as NGS and SNPs in peanuts has resulted in vast amounts of sequence data (118).

Moreover, with the reference genome available for diploid progenitors and the upcoming availability of the tetraploid genome, identifying nucleotide-level variations, even among closely related parental lines, will become more accessible. This will greatly enhance the utilization of these variations for improving the oleate trait (53). In the foreseeable future, the adoption of designer nucleases for gene editing, knockouts and replacements in peanuts is expected to significantly accelerate, leading to the creation of high-oleic peanut varieties.

Along with the above techniques omics studies namely transcriptomics will be used to find the differential gene expressions which uncover the genes that are favouring the improvement of oleic acid. This will provide supplementary information for other techniques like MAS and Genome editing. The advancement of Next-Gen high-throughput sequencing has significantly enhanced transcriptome profiling, contributing to a more comprehensive understanding of RNA-based gene regulatory networks (77).

The expression levels of proteins are influenced not only by transcript levels but also by translational efficiency and regulated degradation, as highlighted by (119) and (120). This knowledge is crucial for devising targeted breeding programs and genetic engineering strategies to boost oleic acid levels in groundnut seeds. Through proteomic analyses, researchers can identify crucial enzymes, pathways and regulatory proteins linked to oleic acid synthesis (61). Moreover, proteomics aids in exploring environmental and physiological factors influencing oleic acid accumulation. Fractionation before LC-MS/MS analysis led to a significant enhancement in the number of identified proteins and the coverage of individual proteins, as demonstrated by (121), in comparison to conventional methods by deciphering the proteomic responses to diverse stressors or growth conditions, researchers can refine agronomic practices to optimize oleic acid production in groundnut crops.

Lipid compounds play crucial roles in various physiological functions. Despite insights gained from model plants, a detailed understanding of the mechanisms underlying the production of lipids and metabolism in peanuts remains essential (122). Notably, no research has yet defined the lipidomic profile of peanuts, particularly the chemical constitution and fluid alterations in lipid compounds in high-oleic acid peanut seeds. Various lipidomic approaches, including gas chromatography (GC), have been employed to investigate the physiological changes induced by *FAD2* mutations. *FAD2*-mediated membrane lipid polyunsaturation is linked to endoplasmic reticulum stress tolerance in *Arabidopsis* (123). MALDI-MSI profiling aided in identifying *FAD2* alleles via extensive screening of high-oleic acid accessions in *Gossypium* (124). Despite these advancements, lipidomics has not been extensively utilized to examine the composition of lipids in developing peanut seeds, especially in high-oleic acid germplasm resources. Hence making use of lipidomics will pave a new way for oleic acid improvement in groundnut. Over the past decade, Paint Omics, a web server designed for combined evaluation and depiction of diverse omics data, has undergone continuous development. This platform enables researchers to explore their multi-omics datasets, encompassing gene expression, protein quantification and metabolite identification (125, 126). Speed breeding is a cutting-edge technique that accelerates plant growth and generation turnover through controlled environmental conditions. It can be combined with molecular breeding techniques like MAS and genomic selection to develop high-oleic varieties faster. The combination of traditional breeding methods with cutting-edge molecular tools, gene-editing technologies, epigenetic modifications, synthetic biology and AI-driven predictive models provides a powerful toolkit for improving oleic acid content in groundnut. These advanced techniques are accelerating the development of high-oleic peanut varieties, meeting consumer demand for healthier, more stable oils while improving agricultural efficiency and sustainability.

Consequently, breeding groundnut accessions for high oleic content and low linoleic acid has become a crucial goal in recent breeding efforts. Research on the biochemistry and fundamental genetics of HO content has resulted in the creation of genetic tools in peanut breeding initiatives to produce high oleic lines. Their numerous health benefits, including improved heart health and reduced inflammation, position them as a superior alternative to conventional peanuts. The extended shelf life makes them highly attractive to the food industry, contributing to reduced waste and cost efficiency in product manufacturing. Additionally, the potential to command premium prices and align with consumer preferences for health-conscious, sustainable and functional foods makes high-oleic peanuts a driving force in the global food market. This combination of scientific advancements and market potential reinforces the importance of continued efforts in developing high -oleic varieties through advanced breeding techniques. As the demand for healthier, more sustainable food products rises, the future of high-oleic peanuts looks bright both from a consumer and agricultural perspective. In the recent past over a decade, there has been a noticeable increase in the production of high oleic dietary oils with improved stability, aiming to replace oils rich in saturated fats and trans-fats. Earlier many cultivars have traditionally resulted from breeding efforts that were focused mainly on yield, the advent of reliable molecular assays has made it easier to attain high oleic peanut through MAS and MABC which is achieved by deploying trait-specific major QTLs and genetic markers which are associated to that trait. Successful alteration of a *FAD2* gene in peanuts by employing different

genome editing techniques has produced an increased oleic content in the resulting transgenic lines. In addition to the aforementioned techniques, considering the comparative advantage of transcriptomics in deciphering the differential gene expression patterns and proteomics for quantification of different proteins with metabolomics unveiling the primary and secondary metabolites, integromics opens new avenues to achieve the objective of increasing oleic acid in groundnut.

Acknowledgements

We acknowledge the Tamil Nadu Agricultural University for providing technical support in the manuscript preparation.

Authors' contributions

All authors contributed to the preparation of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare

Ethical issues: None

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