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Bioinformatics Characterization of the AhFAD2 Gene Associated with Fatty Acid Biosynthesis in Peanut (*Arachis hypogaea* L.)

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Abstract

Peanut (*Arachis hypogaea* L.) is an important legume crop widely cultivated as a source of edible oil and plant-based protein. The quality of peanut oil is largely determined by its fatty acid composition, particularly the oleic to linoleic acid ratio, which is regulated by the fatty acid desaturase gene AhFAD2. This gene catalyzes the conversion of oleic acid into linoleic acid, and natural mutations within AhFAD2 are associated with the high-oleic trait that improves oxidative stability and storage quality of peanut oil. This study aimed to characterize the AhFAD2 gene using a bioinformatics approach to elucidate its molecular structure and functional properties. Gene sequences were retrieved from the NCBI GenBank database and analyzed using multiple sequence alignment to identify genetic variations such as SNPs and insertions/deletions. Protein domain analysis was performed using InterProScan, while functional annotation was supported by UniProtKB. Amino acid sequences were translated using the ExPASy Translate Tool and further examined for conserved motifs and structural features. The results revealed a high level of sequence conservation across accessions, with limited nucleotide variation. The AhFAD2 protein contains a conserved omega-6 fatty acid desaturase domain, histidine-rich catalytic motifs, and transmembrane helices typical of endoplasmic reticulum membrane proteins. Structural prediction indicated stable conformational regions that support its catalytic role in fatty acid desaturation. Overall, these findings provide comprehensive bioinformatics insights into AhFAD2 and support its potential application in peanut breeding programs aimed at improving oil quality.

Keywords: *Peanut (Arachis hypogaea L.); AhFAD2 gene; Bioinformatics; Fatty acid desaturase*

Abstrak

Kacang tanah (*Arachis hypogaea* L.) merupakan tanaman legum penting sebagai sumber minyak nabati dan protein. Kualitas minyaknya ditentukan oleh komposisi asam lemak, terutama rasio asam oleat dan linoleat yang dikendalikan oleh gen desaturase AhFAD2. Gen ini berperan dalam konversi asam oleat menjadi asam linoleat dan mutasinya berkaitan dengan sifat high-oleic yang meningkatkan stabilitas oksidatif serta kualitas penyimpanan minyak. Penelitian ini bertujuan untuk mengkarakterisasi gen AhFAD2 melalui pendekatan bioinformatika guna memperoleh informasi struktur dan fungsi molekuler. Sekuens gen diperoleh dari basis data NCBI GenBank dan dianalisis menggunakan multiple sequence alignment untuk mengidentifikasi variasi genetik seperti SNP dan indel. Analisis domain protein dilakukan menggunakan InterProScan, sedangkan anotasi fungsi diverifikasi melalui UniProtKB. Sekuens protein ditranslasi menggunakan ExPASy Translate Tool dan dianalisis lebih lanjut untuk identifikasi motif konservatif serta karakteristik struktural. Hasil menunjukkan bahwa AhFAD2 memiliki tingkat konservasi tinggi dengan variasi nukleotida yang terbatas antar aksesi. Protein AhFAD2 memiliki domain omega-6 desaturase dengan motif histidin konservatif dan segmen transmembran khas protein membran retikulum endoplasma. Prediksi struktur menunjukkan stabilitas konformasi pada inti protein yang mendukung aktivitas katalitiknya. Secara keseluruhan, hasil ini memperkuat

pemahaman mengenai karakteristik molekuler AhFAD2 dan potensinya dalam mendukung program pemuliaan kacang tanah untuk peningkatan kualitas minyak.
Kata Kunci: *Kacang tanah (Arachis hypogaea L.); Gen AhFAD2; Bioinformatika; Asam lemak desaturase*

1. INTRODUCTION

Peanut (*Arachis hypogaea* L.) is an economically important legume crop cultivated extensively as a source of edible oil and plant-based protein (Çiftçi & Suna, 2022). Peanut seeds generally contain high levels of lipids, with oil content reaching approximately 45–50%, making this crop highly valuable for food and industrial applications (Özçinar, 2022). The nutritional and commercial quality of peanut oil is largely influenced by its fatty acid composition, particularly the proportion of oleic and linoleic acids (Lim *et al.*, 2017). Peanut varieties with elevated oleic acid levels are preferred because they exhibit greater oxidative stability, prolonged shelf life, and improved nutritional properties compared with conventional cultivars (Nawade *et al.*, 2018; Derbyshire, 2014). Consequently, the improvement of fatty acid composition has become one of the major objectives in peanut breeding programs worldwide (Barkley *et al.*, 2013).

The biosynthesis and modification of fatty acids in peanut are regulated by several desaturase enzymes, among which fatty acid desaturase-2 (FAD2) plays a critical role (Zhao *et al.*, 2022). The AhFAD2 gene encodes the microsomal oleoyl-phosphatidylcholine desaturase enzyme that catalyzes the conversion of oleic acid (C18:1) into linoleic acid (C18:2). Variations occurring within the ahFAD2A and ahFAD2B alleles have been reported to significantly affect the oleic-to-linoleic acid ratio in peanut sedes (Pandey *et al.*, 2014). Mutations in these genes are strongly associated with the high-oleic trait, which is widely utilized in modern peanut improvement programs due to its positive contribution to oil quality and storage stability (Barkley *et al.*, 2013).

Advances in molecular genetics and genomic technologies have further emphasized the significance of FAD-related genes in regulating lipid metabolism in peanut. Genome-wide studies identified multiple fatty acid desaturase gene families distributed across the peanut genome, indicating the complexity of fatty acid biosynthesis pathways in this species (Gai *et al.*, 2022). Several studies also demonstrated that genes involved in lipid metabolism contribute not only to seed oil composition but also to plant growth, seed development, and responses to environmental stress. In addition, gene-editing approaches such as CRISPR/Cas9 have successfully targeted FAD-associated genes to improve fatty acid profiles in peanut, highlighting their potential application in molecular breeding strategies (Yuan *et al.*, 2019; Peng *et al.*, 2020).

Along with the rapid expansion of genomic databases, bioinformatics approaches have become increasingly important for studying gene structure and predicting molecular function (Harrow *et al.*, 2009). Computational analyses enable researchers to investigate nucleotide and amino acid sequences, conserved motifs, physicochemical characteristics, phylogenetic relationships, and protein structures efficiently using publicly available resources. These analyses are particularly useful for understanding the evolutionary conservation and functional properties of genes involved in essential metabolic pathways, including fatty acid biosynthesis (Loewenstein *et al.*, 2009; Sjölander, 2010). Although numerous studies have investigated the functional role of AhFAD2 in relation to oil quality, comprehensive bioinformatics characterization of this gene remains relatively limited, especially regarding its molecular features and predicted protein properties (Peng *et al.*, 2020).

Therefore, this study aimed to characterize the AhFAD2 gene associated with fatty acid biosynthesis in peanut using a bioinformatics approach. The analysis included sequence retrieval from public databases, multiple sequence alignment, identification of genetic variations, conserved domain analysis, and prediction of protein structure and function. The findings of this study are expected to provide fundamental molecular information supporting future peanut breeding and genetic improvement programs.

2. METHOD

2.1. Genomic Data Collection and Processing

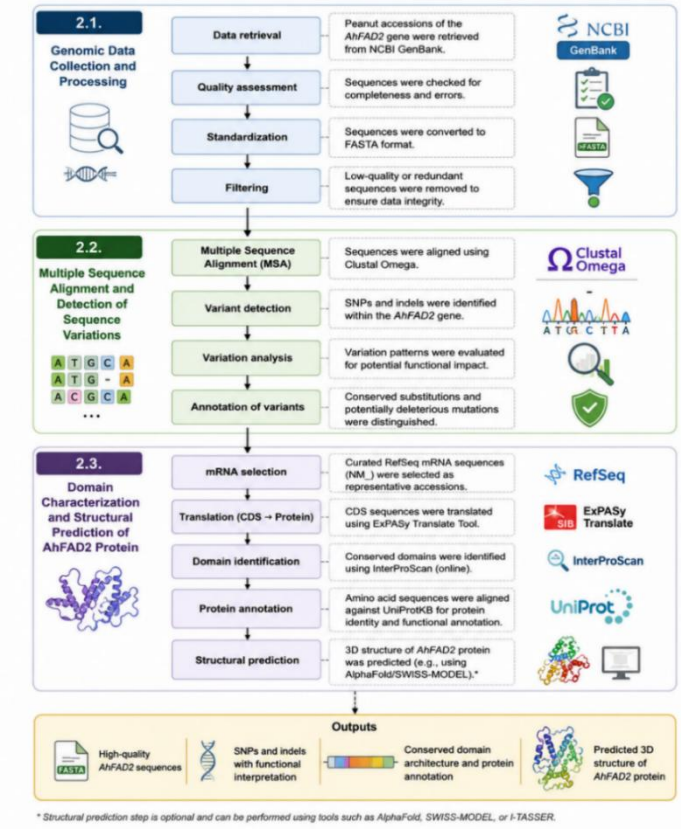
Representative peanut accessions of the AhFAD2 Gene were obtained from publicly accessible repositories, including the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/). Prior to analysis, the retrieved sequences underwent quality assessment and were standardized into FASTA format, followed by filtering steps to verify sequence integrity (Jayhoon & Kumar, 2024).

2.2. Multiple Sequence Alignment and Detection of Sequence Variations

After obtaining the sequence datasets, multiple sequence alignment (MSA) was carried out using Clustal Omega (https://www.ebi.ac.uk/jdispatcher/) to systematically detect genetic polymorphisms, including single nucleotide polymorphisms (SNPs) as well as insertion and deletion (indel) events within the AhFAD2 gene. Subsequently, the identified variation patterns were further evaluated to assess their potential functional significance, with particular attention given to distinguishing conserved substitutions from mutations that may influence enzymatic activity (Jayhoon & Kumar, 2024).

2.3. Domain Characterization and Structural Prediction of AhFAD2 Protein

Domain analysis and three-dimensional structural modeling were conducted based on curated RefSeq mRNA sequences (NM_), which were selected as representative accessions following multiple sequence alignment. The AhFAD2 protein sequences obtained from the previous curation step were translated from their coding DNA sequences (CDS) using the ExPASy Translate Tool (https://web.expasy.org/translate/). Conserved domain identification was performed using the online InterProScan platform (https://www.ebi.ac.uk/interpro/search/sequence/). The translated amino acid sequences were subsequently compared against reference protein entries in the UniProtKB database to confirm protein identity and to provide preliminary functional annotation (https://www.uniprot.org/).



(**Figure 1.** Schematic Overview of the Bioinformatics Pipeline for AhFAD2 Gene Analysis) (Personal doc.)

3. RESULT AND DISCUSSION

3.1. Genomic Data Collection and Processing

Table 1. List of AhFAD2 gene sequences of peanut (*Arachis hypogaea* L.) used in the in silico análisis

No	Gene ID NCBI	mRNA Transcript	Protein	CDs Lenght (nt)	Protein Length (aa)	Status
1	LOC112776164	XM_025820199.3	XP_025675984.1	1,604	379	Komplit
2	LOC112776164	XM_072227673.1	XP_072083774.1	1,505	379	Komplit
3	LOC112776164	XM_025820200.3	XP_025675985.1	2,316	379	Komplit
4	LOC112776164	XM_025820202.3	XP_025675987.1	1,614	379	Komplit
5	LOC112776164	XM_025820198.3	XP_025675983.1	1,538	394	Komplit
6	LOC112776164	XM_025820197.3	XP_025675982.1	1,516	396	Komplit
7	LOC112712140	XM_025764945.3	XP_025620730.1	1,569	379	Komplit
8	LOC112712140	XM_025764948.1	XP_025620733.1	1,472	379	Komplit
9	LOC112712140	XM_025764942.3	XP_025620727.1	1,620	421	Komplit
10	LOC112712140	XM_072203559.1	XP_072059660.1	1,606	405	Komplit
11	LOC112712140	XM_025764944.2	XP_025620729.1	1,566	394	Komplit
12	LOC112696114	XM_025748718.3	XP_025604503.1	1,898	383	Komplit

The AhFAD2 gene sequences of peanut (*Arachis hypogaea* L.) were retrieved from the NCBI GenBank database through an in silico approach using keywords related to FAD2 genes in peanut. Sequence selection was conducted based on the completeness of transcript, coding sequence (CDS), and protein information. A total of 12 complete AhFAD2 sequences were successfully collected and used for further analysis. The obtained sequences originated from several distinct loci, namely LOC112776164, LOC112712140, and LOC112696114. The presence of multiple loci indicates that FAD2 belongs to a multigene family in peanut, which is closely associated with genome duplication events in allotetraploid species. Such genomic complexity is common in *Arachis hypogaea* L., which evolved from hybridization between ancestral diploid species (Chi *et al.*, 2027).

The CDS lengths of the collected sequences ranged from 1,472 to 2,316 bp, whereas the predicted protein lengths varied between 379 and 421 amino acids. Most sequences encoded proteins consisting of 379 amino acids, suggesting that the AhFAD2 protein structure is relatively conserved despite transcript variation. Differences in CDS and protein length may reflect transcript variants or alternative isoforms generated through post-transcriptional regulation. Previous studies have similarly reported structural diversity within the peanut FAD2 gene family (Wang *et al.*, 2015).

AhFAD2 proteins function as ω-6 fatty acid desaturases that catalyze the conversion of oleic acid into linoleic acid during unsaturated fatty acid biosynthesis. Therefore, the conservation observed in most protein sequences indicates the preservation of essential catalytic domains required for desaturase activity. Variations identified in several accessions may be associated with evolutionary adaptation and differential regulation of lipid metabolism in peanut sedes (Nawade *et al.*, 2016).

3.2. Multiple Sequence Alignment and Detection of Sequence Variations

Multiple sequence alignment (MSA) of AhFAD2 coding sequences (CDS) was conducted using Clustal Omega to evaluate sequence conservation and detect nucleotide variations among peanut (*Arachis hypogaea* L.) accessions. The alignment results showed a high level of sequence similarity, indicated by the dominance of conserved nucleotide positions marked with asterisks (*) in the alignment output (Figure 2). This finding suggests that the AhFAD2 gene possesses conserved coding regions that are important for maintaining its biological function in fatty acid biosynthesis.

```
ref|XM_025748718.3|     AGTGGGACTGGTTGAGGGGAGCATTGGCGACCGTGGACAGAGACTATGGAATCCTGAACA   1187
ref|XM_025820199.3|     AATGGGACTGGTTAAGAGGAGCATTGGCAACAGTGGACAGAGATTATGGGATACTGAATA   1147
ref|XM_072203559.1|     AATGGGACTGGTTAAGAGGAGCATTGGCAACAGTGGACAGAGATTATGGGATACTGAATA   1152
ref|XM_025764944.2|     AATGGGACTGGTTAAGAGGAGCATTGGCAACAGTGGACAGAGATTATGGGATACTGAATA   1112
ref|XM_025764942.3|     AATGGGACTGGTTAAGAGGAGCATTGGCAACAGTGGACAGAGATTATGGGATACTGAATA   1166
ref|XM_025820197.3|     AATGGGACTGGTTAAGAGGAGCATTGGCAACAGTGGACAGAGATTATGGGATACTGAATA   1059
ref|XM_025820198.3|     AATGGGACTGGTTAAGAGGAGCATTGGCAACAGTGGACAGAGATTATGGGATACTGAATA   1081
ref|XM_025820200.3|     AATGGGACTGGTTAAGAGGAGCATTGGCAACAGTGGACAGAGATTATGGGATACTGAATA   1859
ref|XM_025820202.3|     AATGGGACTGGTTAAGAGGAGCATTGGCAACAGTGGACAGAGATTATGGGATACTGAATA   1157
ref|XM_025764945.3|     AATGGGACTGGTTAAGAGGAGCATTGGCAACAGTGGACAGAGATTATGGGATACTGAATA   1115
ref|XM_072227673.1|     AATGGGACTGGTTAAGAGGAGCATTGGCAACAGTGGACAGAGATTATGGGATACTGAATA   1048
ref|XM_025764948.1|     AATGGGACTGGTTAAGAGGAGCATTGGCAACAGTGGACAGAGATTATGGGATACTGAATA   1018
                        * *********** ** *********** ** *********** ***** ** ***** *
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(**Figure 2.** Multiple sequence alignment (MSA) of AhFAD2 coding sequences (CDS) from peanut (*Arachis hypogaea* L.) generated using Clustal Omega)
(source: https://www.ebi.ac.uk/jdispatcher/)

Several nucleotide polymorphisms were identified at specific alignment positions, indicating the presence of sequence variation among the analyzed accessions. These variations may represent *single* nucleotide polymorphisms (SNPs) or minor insertion–deletion events resulting from the evolutionary diversification of the FAD2 gene family. Previous studies reported that sequence variation in AhFAD2 genes can affect fatty acid composition, particularly oleic and linoleic acid content in peanut sedes (Wang *et al.*, 2015).

The conserved regions observed in the alignment are likely associated with essential catalytic *domains* of ω-6 fatty acid desaturase proteins. Preservation of these regions indicates that the encoded proteins probably retain their functional role in catalyzing the conversion of oleic acid into linoleic acid. Similar conservation patterns have also been reported in other members of the plant FAD2 gene family (Wu *et al.*, 2018).

3.3. Domain Analysis and Protein Structure Prediction of AhFAD2

5'3' Frame 2

```
GDGEQI-GREWQGGIPRPCPAPLTSLAAGSDSII-KPNVSETTT-HIT-NLKRGCEIHHRRSTHFSSLCWNCFHGLHYVPFIIKLSL
FLCSALLLLLIVFFYIIHANRSKKLNSCCWFLLVSISITSTCMLG-LLILIFYNNLGFSEF-SKGW-SFLPCYVYWISDSALNNINE
NADKFLLSSDTQFCYLSWFHSRPFFIFLLFIYLFFGISEDSLFSLNSFLEMTPIIYIFTYMT-KLSYRNRNIS-TIRLFCCKMFSSP
HHFFFLIFLFSGSKSKQ-LMGPFLLFHG--IYK-GM-CNTKTPQLLFLEYSCNFFYP-SFP-G-YSFLICLVND-IIFDFIFSVSMN
Y-TRNPLPFPLVV-FILVEEKN-KKIFAYIKALVIASGPSLIYDFVIVYLCLVYDSFCMSTSHVY-YK-TSESCPLQLYI-CEINVS
VHVSCESVTLLPPSKIQTVVCVVVVNPWHTLQALLLCPSL-KMHAFLSNEGCKTSSLILVTWHDKLNCDI-MPHVFGNWCLLC-AFS
HPFFSFLYFYRIPKA--NFK-LVVISEILSLLFFWSWFKSLSSAMTIIHSFS-ISEPLAL--CKVLTLSFYIGNRSFNNTTMGAGGR
VTKIEAQKKPLSRVPHSNPPFSVGQLKKAIPPHCFERSLFISFSYVVYDLLMAYLLFYIATTYFHKLPYPFSFLAWPIYWAIQGCIL
TGVWVIAHECGHHAFSKYQLVDDMVGLTLHSCLLVPYFSWKISHRRHHSNTGSLDRDEVFVPKPKSKVSWYNKYMNNPPGRAISLFI
TLTLGWPLYLAFNVSGRPYDRFASHYDPYAPIYSNRERLLIYVSDSSVFAVTYLLYHIATLKGLGWVVCVYGVPLLIVNGFLVTITY
LQHTHASLPHYDSSEWDWLRGALATVDRDYGILNKAFHHITDTHVAHHLFSTMPHYHAMEATNAIKPILGDYYQFDGTPVYKALWRE
AKECLYVEPDDGASQKGVYWYKNKF-CIVRVGNVCVKLET-VL--LVMVINKNKECVCGFAM-CTTTTSTTSFCEILAWCDL-S-IY
LMQYYYCAKHFE
```

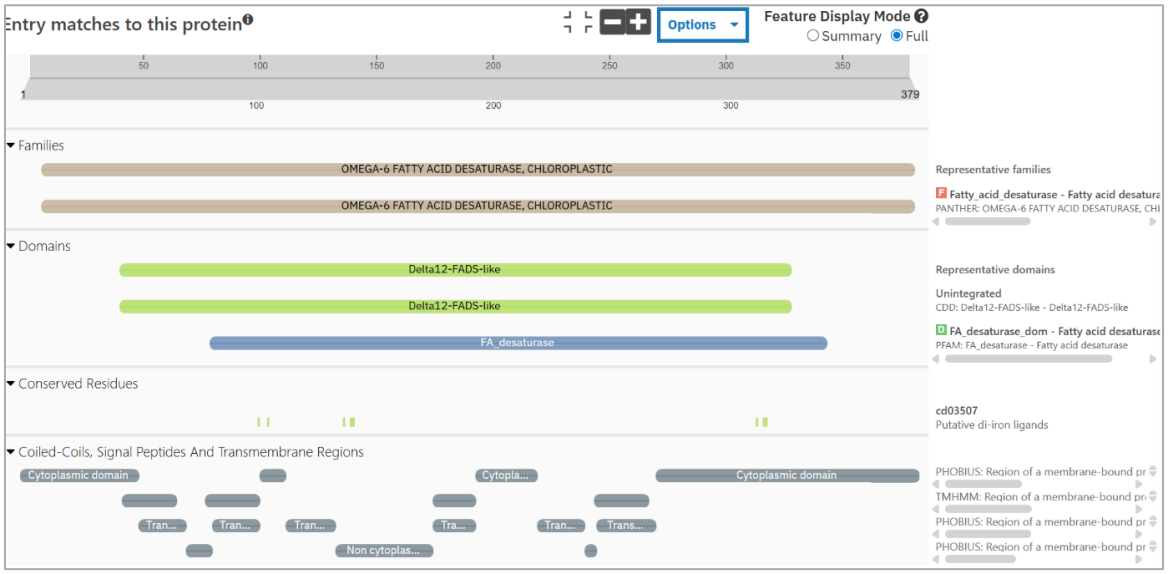
(**Figure 3.** Translation result of the representative mRNA CDS sequence NC_092054.1 of AhFAD2 generated using ExPASy Translate Tool)
(Source: https://web.expasy.org/translate/)

The *representative* AhFAD2 coding sequence was translated into its corresponding amino acid sequence using the ExPASy Translate Tool (Figure 3). The translation results revealed a long open reading frame (ORF) encoding a putative membrane-associated fatty acid desaturase protein. The obtained amino acid sequence was dominated by hydrophobic residues, particularly leucine (L), valine (V), isoleucine (I), phenylalanine (F), and alanine (A),

indicating that AhFAD2 is likely an integral membrane protein localized in the endoplasmic reticulum membrane.

The translated protein sequence also showed several highly conserved regions characteristic of ω-6 fatty acid desaturases. Conserved histidine-rich motifs, which are essential catalytic *components* of FAD2 enzymes, were identified within the amino acid sequence. These motifs are known to function as iron-binding sites required for desaturation activity during the conversion of oleic acid to linoleic acid. The conservation of these motifs suggests that the translated AhFAD2 protein retains its functional role in unsaturated fatty acid biosynthesis. Similar conserved catalytic regions have been widely reported in plant FAD2 proteins, including peanut and other oilseed crops (Wu *et al.*, 2018).

In addition, the abundance of hydrophobic amino acids observed throughout the sequence indicates the presence of multiple transmembrane domains. Membrane-bound desaturases such as FAD2 *typically* contain several transmembrane helices that anchor the protein to the endoplasmic reticulum, where fatty acid desaturation occurs. This structural characteristic is consistent with previous reports describing FAD2 proteins as microsomal membrane-associated enzymes involved in lipid metabolism (Zhao *et al.*, 2022).



(**Figure 4.** InterProScan analysis of the representative AhFAD2)
(Source: https://www.ebi.ac.uk/interpro/search/sequence/)

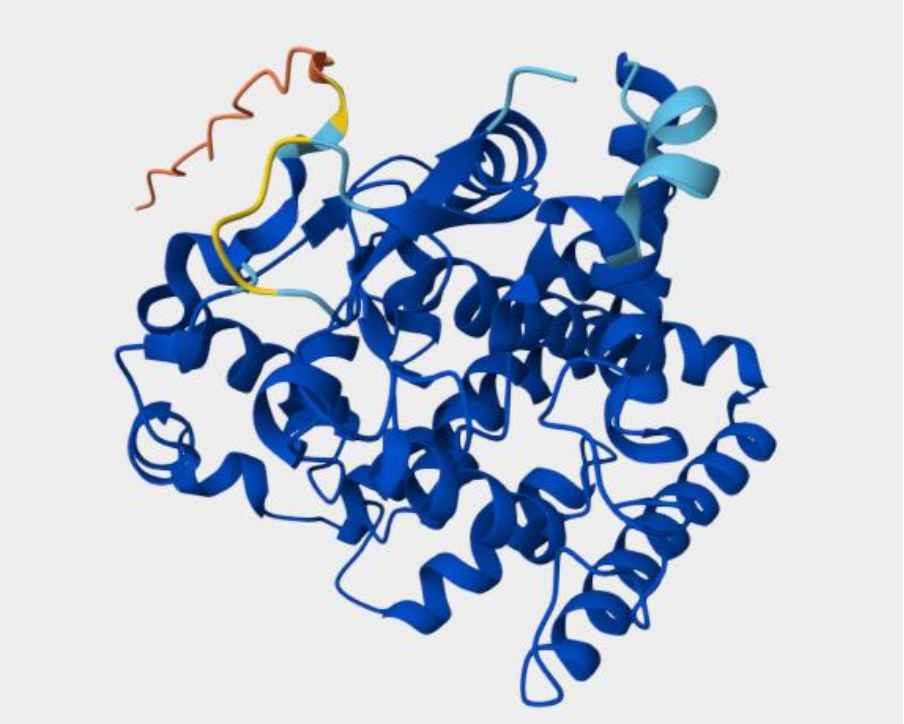
InterProScan analysis of the representative AhFAD2 protein revealed that the translated sequence belongs to the omega-6 fatty acid desaturase family (Figure 3). The protein showed strong similarity to membrane-bound fatty acid desaturases and was classified within the Omega-6 Fatty Acid *Desaturase* superfamily. The identified domains included Delta12-FADS-like and FA_desaturase domains distributed across most regions of the protein sequence, indicating that AhFAD2 possesses the conserved structural characteristics required for fatty acid desaturation activity.

The detected Delta12-FADS-like domain is a characteristic feature of ω-6 desaturase enzymes that *catalyze* the conversion of oleic acid (C18:1) into linoleic acid (C18:2) through the introduction of a double bond at the Δ12 position. This reaction represents an essential step in the biosynthesis of polyunsaturated fatty acids in plants. The presence of this conserved domain confirms that AhFAD2 plays an important role in lipid metabolism and oil quality determination in peanut seeds. Similar domain organization has been reported in FAD2 proteins from peanut and other oilseed crops (Chu *et al.*, 2009; Zhao *et al.*, 2022).

Several conserved residues identified by InterProScan were associated with iron-binding catalytic regions *typical* of membrane-bound desaturases. These conserved histidine-rich residues are essential for enzymatic activity because they coordinate iron ions required

during electron transfer and desaturation reactions. Preservation of these catalytic residues suggests that the analyzed AhFAD2 protein retains functional desaturase activity. Previous studies demonstrated that mutations occurring within conserved catalytic motifs of FAD2 proteins can significantly alter fatty acid composition in peanut (Dar *et al.*, 2017; Wang *et al.*, 2015; Mullen *et al.*, 2004).

The InterProScan results also predicted multiple transmembrane regions distributed throughout the protein sequence. These hydrophobic transmembrane helices indicate that AhFAD2 is an integral membrane protein localized in the endoplasmic reticulum membrane, which is the primary site of fatty acid desaturation in plant cells. The presence of cytoplasmic and non-cytoplasmic *regions* further supports the structural organization commonly observed in microsomal fatty acid desaturases. Similar membrane topology has been widely reported for plant FAD2 proteins (Wu *et al.*, 2018; Wallis *et al.*, 2022).



(**Figure 5.** Protein Structure Analysis of AhFAD2 Based on UniProt and AlphaFold Prediction)
(Source: https://www.uniprot.org/)

Analysis of the representative AhFAD2 protein using UniProt and AlphaFold structural prediction demonstrated that the protein belongs to the omega-6 fatty acid desaturase family, a group of membrane-bound enzymes involved in unsaturated fatty acid biosynthesis. The predicted protein structure exhibited a predominantly α-helical conformation with several folded transmembrane-associated regions, which is characteristic of microsomal fatty acid desaturases found in higher plants.

The AlphaFold confidence model showed that most regions of the protein possessed high to very high pLDDT scores (>70–90), indicated by the dominance of blue-colored regions in the predicted structure. These results suggest that the core structure of AhFAD2 was predicted with relatively high confidence and likely reflects a stable functional conformation. In contrast, several terminal and loop regions displayed lower confidence values, represented by yellow to orange colors, indicating flexible or intrinsically disordered regions that may exhibit structural mobility under physiological conditions (Jumper *et al.*, 2021).

Structural annotation from UniProt and conserved domain analysis indicated that AhFAD2 functions as an endoplasmic reticulum (ER)-associated omega-6 fatty acid desaturase. This enzyme catalyzes the desaturation of oleic acid (18:1) into linoleic acid

(18:2), an essential step in plant lipid metabolism. Similar functions have been reported for FAD2 proteins in peanut, soybean, and Arabidopsis (Dar *et al*., 2017).

4. CONCLUSION

This study demonstrated that the AhFAD2 gene family in peanut (*Arachis hypogaea* L.) exhibits a high degree of sequence and structural conservation despite the presence of several nucleotide and protein variations among accessions. Genomic analysis identified multiple AhFAD2 loci with conserved coding regions and relatively stable protein lengths, indicating the preservation of essential functional characteristics associated with fatty acid desaturation. Multiple sequence alignment further confirmed the existence of conserved nucleotide regions together with limited polymorphic sites that may contribute to differences in fatty acid composition in peanut seeds. Protein characterization revealed that AhFAD2 encodes a membrane-associated omega-6 fatty acid desaturase containing conserved histidine-rich catalytic motifs, transmembrane helices, and characteristic Delta12-FADS-like domains required for oleic acid desaturation. InterProScan, UniProt, and AlphaFold analyses consistently supported the classification of AhFAD2 as an endoplasmic reticulum-localized desaturase involved in unsaturated fatty acid biosynthesis. The predicted protein structure also showed high-confidence conformational stability in most core regions, suggesting the preservation of functional catalytic activity.

Overall, the findings provide important bioinformatics evidence regarding the structural and functional properties of AhFAD2 and highlight its potential role in regulating peanut oil quality through fatty acid metabolism. These results may serve as a valuable foundation for future molecular breeding and functional genomics studies aimed at improving fatty acid composition in peanut cultivars.

5. ACKNOWLEDGMENT

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6. REFERENCES

Barkley, N.A., Isleib, T.G., Wang, M.L. et al. (2013). Genotypic effect of ahFAD2 on fatty acid profiles in six segregating peanut (*Arachis hypogaea* L) populations. *BMC Genet 14*, 62. https://doi.org/10.1186/1471-2156-14-62

Chi X, Zhang Z, Chen N, Zhang X, Wang M, Chen M, et al. (2017) Isolation and functional analysis of fatty acid desaturase genes from peanut (*Arachis hypogaea* L.). *PLoS ONE 12*(12): e0189759. https://doi.org/10.1371/journal.pone.0189759

Chu, Y.; Holbrook, C.C.; Ozias-Akins, P. (2009). Two alleles of ahFAD2B control the high oleic acid trait in cultivated peanut. *Crop. Sci*, 49, 2029–2036. https://doi.org/10.2135/cropsci2009.01.0021

Çiftçi, S., & Suna, G. (2022). Functional components of peanuts (*Arachis hypogaea* L.) and health benefits: A review. *Future Foods*, 5, 100140. https://doi.org/10.1016/j.fufo.2022.100140

Dar AA, Choudhury AR, Kancharla PK and Arumugam N (2017) The FAD2 Gene in Plants: Occurrence, Regulation, and Role. *Front. Plant Sci*. 8:1789. https://doi.org/10.3389/fpls.2017.01789

Derbyshire, E. J. (2014). A review of the nutritional composition, organoleptic characteristics and biological effects of the high oleic peanut. International Journal of Food Sciences and Nutrition, 65(7), 781–790. https://doi.org/10.3109/09637486.2014.937799

Gai, W., Sun, H., Hu, Y., Liu, C., Zhang, Y., Gai, S., & Yuan, Y. (2022). Genome-Wide Identification of

Membrane-Bound Fatty Acid Desaturase Genes in Three Peanut Species and Their Expression in *Arachis hypogaea* during Drought Stress. *Genes*, 13(10), 1718. https://doi.org/10.3390/genes13101718

Harrow, J., Nagy, A., Reymond, A. et al. (2009). Identifying protein-coding genes in genomic sequences. *Genome Biol* 10, 201. https://doi.org/10.1186/gb-2009-10-1-201

Jayhoon, A.S., Kumar, P. (2024). In-silico characterization and expression analysis of the Waxy gene in rice (Oryza sativa L.). *agriRxiv*. https://doi.org/10.31220/agriRxiv.2024.00260

Jumper, J., Evans, R., Pritzel, A. et al. (2021). Highly accurate protein structure prediction with AlphaFold. *Nature* 596, 583–589. https://doi.org/10.1038/s41586-021-03819-2

Lim, HJ., Kim, DS., Pan, J.H. et al. (2017). Characterization of physicochemical and sensory attributes of a novel high-oleic peanut oil cultivar (*Arachis hypogaea* ssp. Fastigiata L.). *Appl Biol Chem* 60, 653–657. https://doi.org/10.1007/s13765-017-0324-6

Loewenstein, Y., Raimondo, D., Redfern, O.C. et al. (2009). Protein function annotation by homology-based inference. *Genome Biol* 10, 207. https://doi.org/10.1186/gb-2009-10-2-207

Mullen, R., McNew, J., Andrews, D., Kim, P., Dhanoa, P., Dyer, J., & McCartney, A. (2004). Membrane-bound fatty acid desaturases are inserted co-translationally into the ER and contain different ER retrieval motifs at their carboxy termini. *Plant Journal*, 37(2), 156–173. https://doi.org/10.1111/j.1365-313x.2004.01949.x

Nawade B, Bosamia TC, Thankappan R, Rathnakumar AL, Kumar A, Dobaria JR, Kundu R and Mishra GP. (2016). Insights into the Indian Peanut Genotypes for ahFAD2 Gene Polymorphism Regulating Its Oleic and Linoleic Acid Fluxes. *Front. Plant Sci*. 7:1271. https://doi.org/10.3389/fpls.2016.01271

Nawade, B., Mishra, G.P., Radhakrishnan, T., et al. (2018). High oleic peanut breeding: Achievements, perspectives, and prospects. *Trends in Food Science & Technology*, 78, 107–119. https://doi.org/10.1016/j.tifs.2018.05.022

Özçinar, A. B. (2022). Food Grade Oil Quality of Peanut (*Arachis hypogaea* L.). *MAS Journal of Applied Sciences*, 7(1), 81–87. https://doi.org/10.52520/masjaps.207

Pandey, M.K., Wang, M.L., Qiao, L. et al. (2014). Identification of QTLs associated with oil content and mapping FAD2 genes and their relative contribution to oil quality in peanut (*Arachis hypogaea* L.). *BMC Genet* 15, 133 (2014). https://doi.org/10.1186/s12863-014-0133-4

Peng, Z., Ruan, J., Tian, H. et al. (2020). The Family of Peanut Fatty Acid Desaturase Genes and a Functional Analysis of Four ω-3 AhFAD3 Members. *Plant Mol Biol Rep* 38, 209–221. https://doi.org/10.1007/s11105-019-01191-0

Sjölander, K (2010) Getting Started in Structural Phylogenomics. *PLoS Comput Biol* 6(1): e1000621. https://doi.org/10.1371/journal.pcbi.1000621

Wallis JG, Bengtsson JD and Browse J (2022) Molecular Approaches Reduce Saturates and Eliminate trans Fats in Food Oils. *Front. Plant Sci*. 13:908608. https://doi.org/10.3389/fpls.2022.908608

Wang Y, Zhang X, Zhao Y, Prakash CS, He G, Yin D. (2015). Insights into the novel members of the FAD2 gene family involved in high-oleate fluxes in peanut. *Genome*. 58(8):375-83. https://doi.org/10.1139/gen-2015-0008. *Epub* 2015 Jul 23. PMID: 26332746.

Wu P, Zhang L, Feng T, Lu W, Zhao H, Li J, Lü S. (2018). A Conserved Glycine Is Identified to be Essential for Desaturase Activity of IpFAD2s by Analyzing Natural Variants from Idesia polycarpa. *Int J Mol Sci.* 19(12):3932. https://doi.org/10.3390/ijms19123932.

Yuan, M., Zhu, J., Gong, L. et al. (2019). Mutagenesis of FAD2 genes in peanut with CRISPR/Cas9 based gene editing. *BMC Biotechnol* 19, 24. https://doi.org/10.1186/s12896-019-0516-8

Zhao, S., Sun, J., Sun, J., Zhang, X., Zhao, C., Pan, J., Hou, L., Tian, R., & Wang, X. (2022). Insights into the Novel FAD2 Gene Regulating Oleic Acid Accumulation in Peanut Seeds with Different Maturity. *Genes*, 13(11), 2076. https://doi.org/10.3390/genes13112076