Advances in Understanding the Genetic Basis of Fatty Acids Biosynthesis in Perilla: An Update

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Abstract: Perilla, also termed as purple mint, Chinese basil, or Perilla mint, is a flavoring herb widely used in East Asia. Both crude oil and essential oil are employed for consumption as well as industrial purposes. Fatty acids (FAs) biosynthesis and oil body assemblies in Perilla have been extensively investigated over the last three decades. Recent advances have been made in order to reveal the enzymes involved in the fatty acid biosynthesis in Perilla. Among those fatty acids, alpha-linolenic acid retained the attention of scientists mainly due to its medicinal and nutraceutical properties. Lipids synthesis in Perilla exhibited similarities with Arabidopsis thaliana lipids’ pathway. The homologous coding genes for polyunsaturated fatty acid desaturases, transcription factors, and major acyl-related enzymes have been found in Perilla via de novo transcriptome profiling, genome-wide association study, and in silico whole-genome screening. The identified genes covered de novo fatty acid synthesis, acyl-CoA dependent Kennedy pathway, acyl-CoA independent pathway, Triacylglycerols (TAGs) assembly, and acyl editing of phosphatidylcholine. In addition to the enzymes, transcription factors including WRINKLED, FUSCA3, LEAFY COTYLEDON1, and ABSCISIC ACID INSENSITIVE3 have been suggested. Meanwhile, the epigenome aspect impacting the transcriptional regulation of FAs is still unclear and might require more attention from the scientific community. This review mainly outlines the identification of the key gene master players involved in Perilla FAs biosynthesis and TAGs assembly that have been identified in recent years. With the recent advances in genomics resources regarding this orphan crop, we provided an updated overview of the recent contributions into the comprehension of the genetic background of fatty acid biosynthesis. The provided resources can be useful for further usage in oil-bioengineering and the design of alpha-linolenic acid-boosted Perilla genotypes in the future.

Keywords: fatty acid biosynthesis; Perilla; transcription factor; oil crop; genomics; fatty acid desaturase; triacylglycerol biosynthesis; transcriptomics

1. Introduction

Perilla frutescens var. frutescens is an oil crop from the mint family that is widely distributed in East Asia including India, Vietnam, China, and Korea [1]. The Perilla genetic resource encompasses the oil crop type P. frutescens var. frutescens, the weedy/wild type P. frutescens, and wild species Perilla setoyensis, Perilla hirtella, and Perilla citriodora [2]. While P. citriodora is known as one of the diploid progenitors [3] of tetraploid P. frutescens, the second diploid donor has not yet been elucidated. In Korean dietary habits, P. frutescens var. frutescens is used for its oil and as leafy vegetable. The fresh leaves can serve as a wrap for meat and boiled rice and are also prepared in a pickled form [2]. In China,
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where it originated [1,2]. Perilla is used secularly as a traditional herbal medicine and fragrance [2]. The health-promoting properties of this plant are attributable to its wide panel of phytochemical compounds [4]. Among them, fatty acids including omega-3, -6, and -9 have been reported as anti-cancer agents [5–7], coronary heart-disease protectants [8], anti-diabetic agents [9], insulin-resistant [10], anti-cardiovascular disease agents [11], and anti-depressive agents [12–14]. In addition, preclinical tests revealed the positive effect of Perilla for mitigating moderate dementia [15]. However, further investigations are required to confirm its role before a recommendation for its use as an antioxidative complement for patients with dementia [4,15]. In addition, Perilla is also used as a supplement in animal feeding [16,17]. Due to the numerous applications of fatty acids from Perilla in the health industry, the oil industry, and for animal breeding, a comprehensive background underpins fatty acid biosynthesis as a fundamental prerequisite for proper utilization in the biomedical, bioengineering, and animal industries.

Recently, Perilla entered into the genomics era with the sequencing of tetraploid P. frutescens and one diploid donor P. citriodora [3], laying a foundation for unraveling the genetic basis of its multiple health and nutraceutical benefits. In the present review, we will examine recent breakthroughs on the genetic basis of fatty acid biosynthesis in Perilla.

2. Earlier Identification and Cloning of Fatty Acid Encoding Gene in Perilla

The genetic characterization interest for Perilla as an oil crop with numerous health beneficial attributes started as early as the 1900s. Several fatty acid genes have been cloned and functionally characterized. Lee et al. (https://www.ncbi.nlm.nih.gov/nuccore/U59477.1/, accessed on 12 February 2021) first characterized a ω-3 fatty acid desaturase PfrFAD7 (Genbank accession: U59477.1) extracted from a Korean cultivar “Okdong” seedling. Subsequently, a cloning of a second gene PrFAD3 was conducted by Chung et al. [18]. PrFAD3 exhibited a seed-specific expression when compared to other organs including the leaf, stem, and root, suggesting a preferential accumulation of alpha-linolenic acid (ALA) in the seed.

Hwang et al. [19,20] also reported four 3-ketoacyl-acyl carrier protein synthases (KAS) encoding genes, PfKAS3a (KAS III) and PfKAS3b (KAS III), PfFAB1 (KAS I), and PfFAB24 (KAS II/IV), which were responsible in the high accumulation of alpha-linolenic synthesis in P. frutescens seeds. Another alpha-linolenic acid-related gene, the microsomal oleate 12-desaturase (PfFAD2) gene, was functionally characterized for the first time in P. frutescens var. frutescens seed [21] in later studies. In addition to the previously identified FAD3 and FAD7 type genes, Xue et al. [22] isolated two FAD8 alpha-linoleic-related genes (PrFAD8a and PrFAD8b) harboring two pyrimidine stretches. Interestingly, the expression of PrFAD8 genes was predominantly observed in the Perilla bud while its accumulation increased under injury, Methyl jasmonate (MeJA), Salicylic Acid (SA), and Abscisic acid (ABA) effects; highlighting their implications in plant defense, growth, and development.

3. Transcriptomics Sheds Lights into Key Master Player Enzymes of Perilla Fatty Acid Biosynthesis

Although some genes have been investigated earlier, the fully resolved biosynthesis pathway of fatty acids in Perilla was still unclear. To fill this gap, the RNA sequencing approach has been extensively used because it helps in uncovering expressed genes related to a biological process. By deciphering the transcriptome of Perilla using diverse organs, scientists were able to identify key genes related to fatty acid biosynthesis via de novo transcripts assembly and functional gene prediction. Thus, extensive transcriptome studies have been initiated using different materials, including P. frutescens var. frutescens, Perilla frutescens var. crispa f. purpurea (red Perilla), and P. frutescens var. crispa f. viridis (green Perilla) [23–26]. The uncovered key genes involved in fatty acid biosynthesis in Perilla have been summarized in Figure 1. Briefly, based on Perilla’s fatty acid desaturase subcellular localization prediction [27] and the well-studied Arabidopsis fatty acid biosynthesis model [28], most fatty acids, including palmitic acid (C16:0), stearic acid (C18:0),


and oleic acid (C18:1), were exclusively synthesized in plastids and conveyed into the cytoplasm where they entered into an acyl-CoA pool for the esterification process at sn-2 position resulting in phosphatidylcholine under the acyl-CoA:lysophosphatidylcholine acyltransferase (LPCAT) enzyme effect.

![Diagram of fatty acid biosynthetic pathway in *Perilla* and triacylglycerols (TAGs) assembly.](image)

**Figure 1.** A simplified putative diagram view of fatty acids biosynthetic pathway in *Perilla* and triacylglycerols (TAGs) assembly. The schematic view involved biochemical interactions occurring in plastid, cytoplasm, and endoplasmic reticulum, respectively. The resulting TAGs are indicated in yellow. Purple circles indicate transcription factors, including WRINKLED (WRII), FUSCA3 (FUS3), LEAFY COTYLEDON1 (LEC1, LCE2), and ABSCISIC ACID INSENSITIVE3 (ABI3). The transcriptional regulation of FUS3, LCE1, LCE2, and ABI3 with PfFAD3.1 is not yet uncovered.

**Abbreviations:** PDHC: plastidial pyruvate dehydrogenase complex; ACCase: acetyl-CoA carboxylase; MCMT: malonyl-CoA ACP transacylase; KASIII: ketoacyl-ACP synthase type III; KAR: 3-ketoacyl-ACP reductase; HAD: 3-hydroxyacyl-ACP dehydratase; EAR: 2-enoyl-ACP reductase; KASII: ketoacyl-ACP synthase type II; KASI: ketoacyl-ACP synthase type I; SAD: stearoyl-acyl carrier protein desaturase; FATB: acyl-ACP thioesterase B; FATA: acyl-ACP thioesterase A; MGDG: monogalactosyldiacylglycerol; PfFAD: *Perilla frutescens* fatty acid desaturase; PC Pool: phosphatidylcholines pool; PCH: palmitoyl-CoA hydrolase; LACS: long-chain acyl-CoA synthetase; PDCT: phosphatidylcholineacylglycerol cholinephosphotransferase; FAX: fatty acid export; LPCAT: lysophosphatidylcholine acyltransferase; PDAT: phospholipid diacylglycerol acyltransferase; DGAT: diacylglycerol acyltransferase; GPAT: glycerol-3-phosphate acyltransferase; LPAT: 1-acylglycerol-3-phosphate acyltransferase; DHAP: dihydroxyacetone phosphate; PAH: phosphatidic acid phosphatase; OLEO: Oleosin.
Oleic acid was then desaturated in the endoplasmic reticulum (ER) to become consecutively linoleic acid (LA) and alpha-linolenic acid (ALA) under FAD2 and FAD3 genes, respectively. The resulting polyunsaturated fatty acids were transacylated onto the sn-3 position of diacylglycerol by phospholipid:diacylglycerol acyltransferase (PDAT) or returned to the acyl-CoA pool via LPCAT to be incorporated into TAG through the Kennedy pathway, inducing the production of triacylglycerols (TAGs) [29].

Using Perilla as a plant model, numerous fatty acid-related genes have been identified. From a time-course seed transcriptome analysis, Kim et al. [25] identified 43 acyl-lipid related genes in P. frutescens var. frutescens cv. Dayudeulkkae (Table 1). The identified genes via Arabidopsis orthologs detection covered the de novo fatty acid biosynthetic key enzymes present in the plastid, endoplasmic reticulum desaturases, oil body proteins, acyl-CoA-, and phosphatidylcholine-mediated TAG synthesis.

**Table 1. Summary of Identified Major Genes Involved in Fatty Acid and Triacylglycerols Biosynthesis in Perilla.**

| Enzyme ID | Enzyme Name | GeneID | Homologous | Pathways Involved | Field of Study | References |
|-----------|-------------|--------|------------|-------------------|---------------|------------|
| PDH(E1α) | Pyruvate Dehydrogenase E1 Subunit Alpha 1 | Locus_2112 | AT1G01090.1 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| PDH(E1β) | Pyruvate Dehydrogenase E1 Subunit beta 1 | Locus_25208 | AT2G34590.1 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| EMB3003(E2) | Pyruvate dehydrogenase e2 component (dihydrolipoamide acetyltransferase) | Locus_33306 | AT1G34430.1 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| LTA2 (E2) | Plastid E2 Subunit of Pyruvate Decarboxylase, PLE2 | Locus_5104 | AT3G25860.1 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| LPD1 (E3) | Lipoamide dehydrogenase | Locus_7407 | AT3G16950.1 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| α-CTa | Alpha-carboxyltransferase Isoform a | Locus_8492 | AT2G38040.1 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| α-CTb | Alpha-carboxyltransferase Isoform b | Locus_2178 | AT2G38040.1 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| β-CT | Beta-carboxyltransferase | Locus_53041 | ATCG00500.1 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| BC | Biotin carboxylase | Locus_22078 | AT5G35360.1 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| BCCP1 | Biotin carboxyl carrier protein of acetyl-CoA carboxylase 1 | Locus_29162 | AT5G16390.1 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| BCCP2 | Biotin carboxyl carrier protein of acetyl-CoA carboxylase 2 | Locus_17340 | AT5G15530.1 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| MCMT | Malonyl-CoA ACP transacylase | Locus_14579 | AT2G30200.1 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| Enzyme ID | Enzyme Name                          | GeneID            | Homologous Pathways Involved | Field of Study          | References |
|----------|-------------------------------------|-------------------|-----------------------------|-------------------------|------------|
| PF40     | 3-Ketoacyl-ACP synthase             | Locus_10821       | A. Thaliana                 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| KASIII   | 3-Ketoacyl-ACP reductase            | Locus_1445        | A. Thaliana                 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| HAD      | 3-hydroxyacyl-ACP dehydratase       | Locus_19332       | A. Thaliana                 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| EAR      | 2-ensoyl-ACP reductase              | Locus_25443       | A. Thaliana                 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| FATA     | Fatty acyl-ACP thioesterase A       | Locus_29919       | A. Thaliana                 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| FATB     | Fatty acyl-ACP thioesterase B       | Locus_6603        | A. Thaliana                 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| FAB2     | Fatty acid biosynthesis 2           | Locus_13564       | A. Thaliana                 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| DE56     | Stearyl-acyl carrier protein desaturase | Locus_9486     | A. Thaliana                 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| KASI     | Ketoacyl-ACP Synthase I             | Locus_26341       | A. Thaliana                 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| KASII    | Ketoacyl-ACP Synthase II            | Locus_1373        | A. Thaliana                 | FA de novo biosynthesis and export from plastid | Transcriptomics [25] |
| LACS8    | Long-chain acyl-CoA synthetase 8    | chr07_36292788    | A. Thaliana                 | Genome Assembly, Transcriptomics | [3,25]     |
|          |                                     | chr07_36292788    | A. Thaliana                 | Genome Assembly, Transcriptomics | [3,25]     |
|          |                                     | chr19_22302145    | A. Thaliana                 | Genome Assembly, Transcriptomics | [3,25]     |
|          |                                     | chr02424545       | A. Thaliana                 | Genome Assembly, Transcriptomics | [3,25]     |
| LACS9    | Long-chain acyl-CoA synthetase 9    | chr03_70622879    | A. Thaliana                 | Genome Assembly, Transcriptomics | [3,25]     |
|          |                                     | chr09_58853241    | A. Thaliana                 | Genome Assembly, Transcriptomics | [3,25]     |
|          |                                     | chr19_22302145    | A. Thaliana                 | Genome Assembly, Transcriptomics | [3,25]     |
| FAX1     | Fatty acid export 1                 | chr05_24242740    | A. Thaliana                 | FA de novo biosynthesis and export from plastid | [25]       |
|          |                                     | chr01_71691539    | A. Thaliana                 | FA de novo biosynthesis and export from plastid | [25]       |
| FAX2     | Fatty acid export 2                 | chr07_10626150    | A. Thaliana                 | FA de novo biosynthesis and export from plastid | [25]       |
|          |                                     | chr01_71691539    | A. Thaliana                 | FA de novo biosynthesis and export from plastid | [25]       |
| FAX3     | Fatty acid export 3                 | chr04_00857340    | A. Thaliana                 | FA de novo biosynthesis and export from plastid | [25]       |
|          |                                     | chr04_00857340    | A. Thaliana                 | FA de novo biosynthesis and export from plastid | [25]       |
| FAX5     | Fatty acid export 5                 | chr04_65529797    | A. Thaliana                 | FA de novo biosynthesis and export from plastid | [25]       |
|          |                                     | chr07_22334802    | A. Thaliana                 | FA de novo biosynthesis and export from plastid | [25]       |
|          |                                     | chr06_00746938    | A. Thaliana                 | FA de novo biosynthesis and export from plastid | [25]       |
|          |                                     | chr01_010733560   | A. Thaliana                 | FA de novo biosynthesis and export from plastid | [25]       |
Table 1. Cont.

| Enzyme ID | Enzyme Name                                      | GeneID          | Homologous Pathways                    | Field of Study           | References |
|-----------|-------------------------------------------------|-----------------|----------------------------------------|--------------------------|------------|
| FAD2      | Omega-6 fatty acid desaturase                   | chrl2, 56932398, 56934446 chrl1, 05992061, 05993208 chrl1, 05979254, 05976393 | chr08_55538081, 55539229 AT3G12120.1 | Acyl editing of phosphatidyl-choline | Genome Assembly, Transcriptomics | [3,25] |
|           |                                                 | chrl2, 569484107 56949167 | chrl1, 5419412, 54197265 | chr08_55558209, 55559348 | Genome Assembly, Transcriptomics | [3,25] |
| FAD3      | Omega-3 fatty acid desaturase                   | chrl2, 04645208, 04647776 chrl1, 72114246, 72119454 | chr11_05592060, 05593208 chr11_05575254, 05576393 | AT2G29980.1 | Acyl editing of phosphatidyl-choline | Genome Assembly, Transcriptomics | [3,25] |
| FAD8      | Omega-8 fatty acid desaturase                   | chr12, 569484107 56949167 | chr11_05592060, 05593208 chr11_05575254, 05576393 | chr08_55558209, 55559348 | Acyl editing of phosphatidyl-choline | Genome Assembly, Transcriptomics | [3,25] |
| GPAT9     | Glycerol-3-phosphate acyltransferase 9          | chr12, 33733527, 33737991 chrl1, 26255533, 26259681 | chr08_33038421, 33042132 | AT5G05580.2 | Acyl editing of phosphatidyl-choline | Transcriptomics | [25] |
| LPAT2     | 1-acyl-sn-glycerol-3-phosphate acyltransferase 2 | chr05_2358386, 2358893 chrl05, 34440913, 34444444 chrl01, 72114246, 72119454 | chr02_43313059, 43318262 chr02_32585727, 32589258 | AT3G57650.1 | Acyl-CoA-dependent TAG synthesis in Kennedy pathway | Genome Assembly, Transcriptomics | [3,25] |
| PAH1      | Phenylalanine hydrolase 1                       | chr01, 61567423, 6157065 chrl4, 08979719, 08980206 chrl5, 37130964, 3718907 chrl3, 61656532, 61661875 chrl8, 09154357, 09159306 chrl7, 34575710, 34580644 chrl09, 5034045, 5034956 | chr01_11516392, 11522733 | Acyl-CoA-dependent TAG synthesis in Kennedy pathway | Genome Assembly, Transcriptomics | [3,25] |
| DGAT1     | Diacylglycerol O-acyltransferase 1              | chr01_09730655, 09741367 chrl01_48275733, 48286173 | chr05_08797620, 08800333 | AT2G19450.1 | Acyl-CoA-dependent TAG synthesis in Kennedy pathway | Genome Assembly, Transcriptomics | [3,25] |
| DGAT2     | Diacylglycerol O-acyltransferase 2              | chr14_26782964, 2678941 chrl18, 25811826, 25816791 | chr10_25785382, 25790335 | AT3G51520.1 | Acyl-CoA-dependent TAG synthesis in Kennedy pathway | Genome Assembly, Transcriptomics | [3,25] |
| DGAT3     | Diacylglycerol O-acyltransferase 3              | chr01_06996630, 0701595 chrl01_56678891, 56685081 chrl01_03079195, 03084058 chrl07_53028425, 53045367 chrl01_43224061, 43229071 chrl02_66141068, 66147271 chrl02_04634020, 04638976 chrl19, 35211932, 35217537 | chr08_11516392, 11522733 | Acyl-CoA-dependent TAG synthesis in Kennedy pathway | Transcriptomics | [25] |
| LPCAT     | Lysophosphatidylcholine acyltransferase        | chr01, 06996630, 0701595 chrl01_56678891, 56685081 chrl01_03079195, 03084058 chrl07_53028425, 53045367 chrl01_43224061, 43229071 chrl02_66141068, 66147271 chrl02_04634020, 04638976 chrl19, 35211932, 35217537 | chr05_03187620, 03190829 chrl05_03187620, 03190829 chrl06_54113419, 54119561 | AT1G12640.1 | PC-mediated TAG synthesis | Transcriptomics | [3,25] |
### Table 1. Cont.

| Enzyme ID | Enzyme Name | GeneID | Homologous Pathways Involved | Field of Study | References |
|-----------|-------------|--------|-----------------------------|---------------|------------|
| **CPT1** | Diacylglycerol cholinephosphotransferase | PF40* | AT1G13560.1 | PC-mediated TAG synthesis | Transcriptomics [25] |
| **CPT2** | Diacylglycerol cholinephosphotransferase | Dayudeulkkae** | AT3G25585.1 | PC-mediated TAG synthesis | Transcriptomics [25] |
| **PDA1** | Phospholipid: diacylglycerol acyltransferase 1 | chr05:44104376-44108847 | AT5G13640.1 | Acyl-CoA independent pathway | Transcriptomics [3,25] |
|          |             | Locus_7821 AT1G13560.1 | PC-mediated TAG synthesis | Transcriptomics [25] |
|          |             | Locus_22567 AT3G25585.1 | PC-mediated TAG synthesis | Transcriptomics [25] |
|          |             | chr05:44104376-44108847 | AT5G13640.1 | Acyl-CoA independent pathway | Transcriptomics [3,25] |
|          |             | chr02:52135886 AT4G25140.1 | TAG assembly Transcriptomics [3,25] |
|          |             | chr09:00376677_00380564 | Genome Assembly, Transcriptomics [3,25] |
| **PDA2** | Phospholipid: diacylglycerol acyltransferase 2 | chr05:38922115-38924735 | AT3G44830.1 | Acyl-CoA independent pathway | Transcriptomics [3,25] |
|          |             | Locus_29208 AT2G30526 | PC-mediated TAG synthesis | Transcriptomics [25] |
|          |             | chr02:5992086_45994691 | Genome Assembly, Transcriptomics [3,25] |
| **PDCT** | Phosphatidylcholine: diacylglycerol cholinephosphotransferase | chr03:46291224-46295449 | AT3G15820.1 | Acyl-CoA independent pathway | Transcriptomics [3,25] |
|          |             | chr09:37050943_37053194 | Transcriptomics [3,25] |
|          |             | Locus_15867 chr01:27228085_27230144 | Transcriptomics [3,25] |
| **OLEO2** | Oleosin2 | chr15:52133834-52134256 | AT5G40420.1 | TAG assembly Transcriptomics [3,25] |
|          |             | Locus_31790 | Transcriptomics [3,25] |
|          |             | chr17:50355018_50355440 | Transcriptomics [3,25] |
| **OLEO** | Oleosin | chr18:08871500_08871970 | AT3G18570.1 | TAG assembly Transcriptomics [3,25] |
|          |             | chr03:05196095_05196523 | Transcriptomics [3,25] |
| **OLEO1** | Oleosin1 | chr01:30156121_30156549 | AT4G25140.1 | TAG assembly Transcriptomics [3,25] |
|          |             | Locus_29266 AT4G25140.1 | Transcriptomics [3,25] |
| **OLEO5** | Oleosin5 | chr05:59989345_59989911 | AT3G01570.1 | TAG assembly Transcriptomics [3,25] |
|          |             | Locus_29276 AT5G0420.1 | Transcriptomics [3,25] |

* Perilla frutescens var. frutescens cv. PF40; ** Perilla frutescens var. frutescens cv. Dayudeulkkae; *** Perilla citriodora.

The mentioned genes have been identified through de novo transcriptome mining coupled with Arabidopsis homologous sequences prediction.

Transcriptome mining revealed five sub-unit genes (α-PDH, β-PDH, EMB3003, LTA2, and LPD1) of the precursor enzyme plastidial pyruvate dehydrogenase complex (PDHC) involved in the synthesis of acetyl-CoA from pyruvate. Afterward, acetyl-CoA carboxylase (ACCase) transformed acetyl-CoA into malonyl-CoA [30]. The ACCase in Perilla encompassed two ACCases subunits alpha (α-CTa and α-CTb), one ACCase subunit beta (β-CT), two isoforms of biotin carboxyl-carrier protein (BCCP1 and BCCP2), and one biotin carboxylase (BC).

Furthermore, the malonyl-CoA ACP transacylase, an acyl carrier protein transacylase, catalyzed malonyl-CoA to form malonyl-ACP, paving the way for fatty acid elongation under the action of acyl-chain enzymes, i.e., 3-keto-acyl-ACP synthase (KAS), 3-ketoacyl-ACP reductase (KAR), 3-hydroxylacyl-ACP dehydratase (HAD), and Trans-Δ2-enoyl-ACP
reductase (EAR), respectively [23,24,31]. It is worth mentioning that WR1 is well conserved in plant species. For instance, homologous genes have been identified in *Brachypodium distachyon* [32], *Camelina sativa* [33], *Solanum tuberosum* [34], *Cocos nucifera* [35], *Brassica napus* [36], *Elaeis guineensis* [37], and *Jatropha curcas* [38]. In *A. thaliana*, through the promoter binding element AW-box, WR1 targets upstream genes encoding for malonyl-CoA:ACP malonyl transferase, enoyl-ACP reductase, pyruvate dehydrogenase, oleoyl-ACP thioesterase, biotin carboxyl carrier protein 2, ketoacyl-ACP synthase, and hydroxyacyl-ACP dehydrase [39–46]. The homologous sequence of WR1 has been demonstrated in augmentation from 10 to 40% of seed oil in transgenic maize [47] and *Brassica napus* [36], suggesting that *Perilla’s WR1* gene might be a promising candidate for oil-oriented bioengineering in *Perilla*.

Through carbon chain elongation, palmitoyl-ACP (C16:0) is converted into stearoyl-ACP (C18:0). The latter is transformed into oleic acid (C18:1)-ACP under the catalysis of stearoyl-acyl carrier protein desaturase (*SAD*). In *Perilla*, two *SAD* genes have been identified, including *PfFAB2* and *PfDES6* [25]. Using a red *Perilla* (*Perilla frutescens var. crispa F. purpurea*) seed transcriptome, Liao et al. [23] identified fatty acid desaturases PfFAD6 and PfFAD7/8 that act on the vector glycerolipid, i.e., monogalactosyldiacylglycerol (MGDG), in order to process (C18:1) into (C18:2) and (C18:2) to (C18:3), respectively (Figure 1).

To terminate fatty acids synthesis in *Perilla* plastids, fatty acyl-ACP thioesterase (*FATA*), palmitoyl/stearoyl-acyl carrier protein thioesterase (*FATB*), and palmitoyl-CoA hydrolase (*PCH*) were solicitated. *PCH* specifically induced C18:1- and C18:2-synthesis, while *FATA* was a C18:1-exclusive catalyst. Meanwhile, *FATB* transformed only C16:0-ACP or C18:0-ACP to C16:0 or C18:0, respectively (Figure 1). Representative gene coding for these enzyme has been pinpointed by de novo transcriptome analysis and comparative transcripts with regard to the well characterized *A. thaliana* fatty acid-related gene [23,24]. Free FAs were then moved into the cytoplasm where they were esterified to form an Acyl-CoA pool under the action of long-chain acyl-CoA synthesis (LACS). Liao et al. [23] reported the important expression of LACS genes in *Perilla* seeds ten days after flowering, indicating an initiation of TAGs synthesis pathway in the endoplasmic reticulum (ER).

In the ER, esterified fatty acids are translated into phosphatidylcholines via lysophosphatidylcholine acyltransferase (*LPCAT*). Based on the Arabidopsis plant model, mainly two fatty acid desaturases have been identified in the ER: an *FAD2* that converts PC-C18:1 into PC-18:2 and an *FAD3* that catalyzes PC-C18:2 into PC-C18:3 [48–50]. Homologous sequences in *Perilla* seed (*PfFAD2* and *PfFAD3*) transcriptome [23–25] have also been identified (Table 1).

Recently, the transcriptome assessment of Chinese cultivar PF40 highlighted 33 candidate genes involved in TAG biosynthesis-covering transcription factors (Supplementary Table S1), and fatty acids were exported from plastid, acyl editing of phosphatidylcholine, acyl-CoA dependent Kennedy pathway, acyl-CoA independent pathway, and TAGs assembly into oil bodies (Table 1). The identified genes corroborated with previous findings [23–25], except for the first identification of fatty export1 (*FAX1*) as an additional enzyme to long-chain acyl-CoA synthetase (LACS) that mediated plastid fatty acid export.

In the absence of a whole genome representative resources, the detection of potential genes isoforms and the full FADs gene repertoire is difficult to predict, and diverse gene targets for functional validation and bio-engineering purposes are not provided. Due to the fact that *Perilla* has entered into the genomics era, the next section covers genomics-based advances in the detection of fatty acids in *Perilla* via genome-wide identification and genome-wide association study strategies.

### 4. Whole-Genome-Driven Fatty Acid Genes Discovery

With the advent of long-reads and chromosome conformation capture technologies, a high-quality chromosome scale genome of tetraploid *P. frutescens var. frutescens* has recently been assembled [3]. The genome spanned 1.203 Gb, along with 20 chromosomes with an N50 of 62.64 Mb and a total of 38,941 predicted gene models.
From a panel of 191 accessions, a genome-wide association study for seed alpha-linolenic acid content enabled the identification of an LPCAT encoding region located in chromosome 2. This finding corroborates previous observations, suggesting the role of LPCAT in FAs and TAGs synthesis in B. napus [51] and A. thaliana [52]. Interestingly, a deletion of this gene was noted in some individuals of the studied panel corresponding to a loss of around 6% of seed oil ALA content. This suggests that the transcriptional regulation of LPCAT might be responsible for ALA content variations in Perilla.

Taking advantage of the PF40-generated high-quality genome, in silico genome-wide analysis identified a repertoire of 42 fatty acid desaturases clustered into five families including omega-3 desaturase, Δ7/Δ9 desaturase, FAD4 desaturase, Δ12 desaturase, and front-end desaturase [27]. The heterologous validation of candidate fatty acid desaturase genes using A. thaliana revealed a positive impact (increase of 18–37% alpha-linolenic acid content) of the PfFAD3.1 gene.

Furthermore, the upregulation of WRINKLED (WRI1), FUSCA3 (FUS3), LEAFY COTYLEDON1 (LEC1 and LCE2), and ABSICIC ACID INSENSITIVE3 (ABI3) transcription factors was noted in PfFAD3.1 Arabidopsis transgenic lines [3] and Perilla seed expression profiles [23], suggesting their regulation roles in the Perilla FAs synthesis pathway.

5. Concluding Remarks and Outlook

Fatty acids play an important role in the lipid supply of plants and have valuable medicinal properties for humans. Here, we summarized the breakthroughs that shed light into the genetic and molecular determinants of FA and TAG synthesis in Perilla. Transcriptomics and genomics studies revealed the key master player enzymes responsible for FAs synthesis in Perilla, including polyunsaturated fatty acids desaturases, acyl-related enzymes, and transcription factors. However, the evidence of their role is still elusive since strong functional validation has not yet been provided.

The mechanism of the regulation of FA synthesis by TFs in Perilla is still elusive. Meanwhile, the recent work from Moreno-Perez et al. [53] suggested histone methylation (H3K4me3) implication into fatty acid biosynthesis in sunflowers with interactions with TFs. Moreover, acetyl-CoA, which is involved in fatty acid synthesis in plants, has been found to be correlated with histone acetylation and DNA methylation in A. thaliana through the beta-oxidation process [54]. Therefore, an in-depth investigation of identified TFs, such as ABI3, FUS3, LEC1, and LEC2, and the epigenome landmark of Perilla will pave a new avenue in deciphering the full landscape of fatty-acid biosynthesis in Perilla.

Functional validation using Perilla as a material instead of A. thaliana would drastically shape the validation efficiency of the identified genes. For this purpose, Agrobacterium-based protocols [55,56] have been tested and can serve as further functional validation. Moreover, in the current era of gene and genome editing with applicable cases in plants [57–60], designing appropriate gene editing strategies that fit into the Perilla system will surely expedite the production of enriched alpha-linolenic acid-Perilla genotypes. Furthermore, considering the species diversity within the Perilla genus, systematic fatty acid content evaluation within the Perilla species will help reveal potential alpha-linolenic acid-enriched species donors and characterize their respective biosynthetic pathways.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants11091207/s1, Table S1: Identified transcription factors from Perilla through transcriptome, whole genome, and in silico co-expression analyses.

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