Research Article

FAD2-DGAT2 Genes Coexpressed in Endophytic Aspergillus fumigatus Derived from Tung Oilseeds

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Recent efforts to genetically engineer plants that contain fatty acid desaturases to produce valuable fatty acids have made only modest progress. Diacylglycerol acyltransferase 2 (DGAT2), which catalyzes the final step in triacylglycerol (TAG) assembly, might potentially regulate the biosynthesis of desired fatty acids in TAGs. To study the effects of tung tree (Vernicia fordii) vfDGAT2 in channeling the desired fatty acids into TAG, vfDGAT2 combined with the tung tree fatty acid desaturase-2 (vfFAD2) gene was co-introduced into Aspergillus fumigatus, an endophytic fungus isolated from healthy tung oilseed. Two transformants coexpressing vfFAD2 and vfDGAT2 showed a more than 6-fold increase in linoleic acid production compared to the original A. fumigatus strain, while a nearly 2-fold increase was found in the transformant expressing only vfFAD2. Our data suggest that vfDGAT2 plays a pivotal role in promoting linoleic acid accumulation in TAGs. This holds great promise for further genetic engineering aimed at producing valuable fatty acids.

1. Introduction

Various forms of Δ12-oleic acid desaturase (FAD2), which catalyzes the introduction of a cis-Δ12 double bond into oleic acid to produce linoleic acid, have recently been used in genetically engineered plants [1]. FAD2-like enzymes are able to produce fatty acids, but they are not effective at channeling fatty acid removal from phosphatidylcholine and their accumulation as triacylglycerols (TAGs) [2, 3]. Diacylglycerol acyltransferase 2, which catalyzes the final step of TAG biosynthesis in the classical Kennedy pathway, might mediate the accumulation of the desired fatty acids in TAGs [2]. For example, Ricinus communis fatty acid hydroxylase 12 (FAH12) produced nearly 30% more hydroxy fatty acid when coexpressed with R. communis type-2 acyl-coenzyme A:diacylglycerol acyltransferase (RcDGAT2) [4]. There is evidence that tung tree (Vernicia fordii) vfDGAT2 enhances trieleostearic biosynthesis in yeast [5]. We examined vfDGAT2 function in the incorporation of linoleic acid into TAGs in a transgenic fungus.

2. Materials and Methods

2.1. Materials. Nuts were collected from tung oil trees in Tianlin County, Guangxi Zhuang Autonomous Region, China. Healthy nuts were harvested during the developmental stage on 10 September, when tung oil accumulates during seed maturation [5]. The nuts were hand-shelled and the kernels were frozen immediately in liquid N2 and then stored at −80°C. The reagents used were purchased from Takara, Promega, and Invitrogen. Oligonucleotides were synthesized by Sangon Biotech. DNA was isolated and purified using a kit from Axygen. Plant clone vector pMD18-T was purchased from Takara. Plant expression vector pCAMBIA1301 was preserved in our lab.

2.2. Aspergillus fumigatus Derived from Tung Oilseeds. The endophytic fungus A. fumigatus was isolated from oilseeds of healthy tung trees and cultivated on yeast extract peptone dextrose agar (YPD). The strain was identified based on its morphological characteristics and molecular data (nuclear ribosomal internal transcribed spacer sequence).
2.3. Genes Isolated from Tung Oilseeds. Total RNA was extracted from tung kernels using an improved TRIzol method. cDNA was obtained by reverse transcription and used as the template for polymerase chain reaction (PCR). Specific primers were designed from sequences in GenBank (vfFAD2, accession number AF525534; vfDGA T2, accession number DQ356682) (Table 1).

2.4. Cointroduction of vfFAD2-vfDGA T2 into A. fumigatus

Using Agrobacterium tumefaciens-Mediated Transformation. The foot-and-mouth disease virus 2A (FMDV-2A) sequence, which cleaves proteases, was used to conjugate vfDGA T2 with vfFAD2. The three sequences were subcloned into expression vector pCAMBIA1301 using different restriction enzymes (Figure 1, Table 1). The recombinant expression vector contained a unique open reading frame (ORF) for the enzyme (Figure 1, Table 1). The recombinant expression vector was constructed successfully (Figures 1 and Figure 2(a)). Primers 7–10 were used to amplify vfFAD2 and vfDGA T2 to give PCR products of 150 and 190 bp, respectively.

2.5. Fatty Acid Analysis. The crude oil was obtained by Soxhlet extraction. To analyze the fatty acid composition, fatty acid methyl esters (FAMEs) were prepared using potassium methoxide [6]. The FAMEs were purified using an AccuBOND solid-phase extraction column and evaporated to dryness under a nitrogen stream. The residue was resuspended in 1 mL 10% isopropanol in n-hexane containing methyl heptadecanoate as an internal standard.

Gas chromatography was performed on a Hewlett-Packard 5890 series II gas chromatograph (Agilent) equipped with flame ionization detection (FID) and a Supelco SP-2380 column (Sigma-Aldrich). Helium was used as the carrier gas at a flow rate of 8 mL/min. The inlet and detector were held at 200 °C. The temperature of the column oven was programmed to increase from 110 °C to 160 °C at a rate of 17 °C/min, then to 175 °C at 5 °C/min, and finally to 200 °C at 3 °C/min, and held for 3 min. The data were collected and processed using a Hewlett-Packard 3365 series II ChemStation.

3. Results and Discussion

3.1. vfFAD2/vfDGA T2 Coexpressed in A. fumigatus. The ORF sequence of the tung linoleic acid desaturase gene (vfFAD2) was 1152 base pairs (bp) long and that of tung diacylglycerol acyltransferase gene (vfDGA T2) was 968 bp (Figure 2(a)). The vfFAD2-FMDV2A-vfDGA T2/pCAMBIA1301 recombinant vector was constructed successfully (Figures 1 and Figure 2(a)). The transformants of A. fumigatus/vfFAD2,

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Table 1: Primer sequences of vfFAD2, vfDGA T2, and FMDV-2A.

| Primers | Sequences 5′-3′ |
|---------|-----------------|
| 1       | vfFAD2 Forward (KpnI) |
| 2       | vfFAD2 Reverse (BamHI) |
| 3       | FMDV-2A Forward (BamHI) |
| 4       | FMDV-2A Reverse (XbaI) |
| 5       | vfDGA T2 Forward (XbaI) |
| 6       | vfDGA T2 Reverse (PstI) |
| 7       | vfFAD2 Forward’ |
| 8       | vfFAD2 Reverse’ |
| 9       | vfDGA T2 Forward’ |
| 10      | vfDGA T2 Reverse’ |

The nucleotides in lowercase letters indicate restriction sites. ATG and TCA in bold indicate the initiation and stop codon, respectively, for the open reading frame of vfFAD2-FMDV2A-vfDGA T2. Primers 7–10 were used to amplify vfFAD2 and vfDGA T2 to give PCR products of 150 and 190 bp, respectively.
Figure 2: PCR verification of the vfFAD2-FMDV2A-vfDGAT2/pCAMBIA1301 recombinant vectors and A. fumigatus/vfFAD2-FMDV2A-vfDGAT2 transformant. (a) PCR verification of the vfFAD2-FMDV2A-vfDGAT2/pCAMBIA1301 recombinant vector. (b) PCR amplification of the A. fumigatus transformant using special primers. (c–f) A. fumigatus transformants grew in PDA medium containing 100 µg/mL hygromycin B. 1, vfDGAT2; 2: vfFAD2; 3: vfFAD2-FMDV2A-vfDGAT2; 4: pCAMBIA1301 vector; 5: DNA Marker. (c) A. fumigatus/vfFAD2-FMDV2A-vfDGAT2; (d) A. fumigatus/vfFAD2; (e) A. fumigatus/vfDGAT2; (f) A. fumigatus.

Figure 3: The fatty acid profile of A. fumigatus transformants containing vfFAD2 and vfDGAT2. C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid. The two A. fumigatus transformants with vfFAD2-FMDV2A-vfDGAT2 showed a more than sixfold increase in linoleic acid production, while the transformant expressing vfFAD2 had about a twofold increase compared to the original A. fumigatus strain.
A. fumigatus/vfDGAT2, and A. fumigatus/vfFAD2-FDMV2A-vfDGAT2 are shown in Figures 2(b)–2(f). Specific primers for vfFAD2 and vfDGAT2 were used to identify the transformants using PCR (Table 1).

3.2. Fatty Acid Profile in the A. fumigatus Transformants. Fatty acid production in A. fumigatus was investigated using Gas chromatography. Five fatty acids were identified: palmic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3). Because the A. fumigatus transformant with vfDGAT2 grew so slowly, its fatty acids were not considered. The linoleic acid production in A. fumigatus expressing only vfFAD2 was 1.81 times that in A. fumigatus. In contrast, that in A. fumigatus transformed with vfFAD2-FDMV2A-vfDGAT2 was more than 6 times that of the control (Figure 3). Fatty acid desaturase 2 (vfFAD2) in tung oilseed catalyzes the conversion of oleic acid (C18:1) into linoleic acid (C18:2) [7]. Nevertheless, linoleic acid production increased only modestly in A. fumigatus expressing the vfFAD2 gene only. Diacylglycerol acyltransferase (vfDGAT2) catalyzes the final step in TAG biosynthesis, and it likely improves the fatty acid accumulation when coexpressed with vfFAD2.

Recent efforts to genetically engineer plants to produce unusual fatty acids have been met with only modest success, because the fatty acids flux into substrate pools instead of TAG [2]. This study used a seed-associated endophytic fungus as the transformation model. Consequently, the fatty acid desaturase was able to catalyze linoleic acid production, and vfDGAT2 seemed to play a role in prompting fatty acid flux into TAG in the transgenic fungus. Therefore, vfDGAT2 might be a candidate gene mediating fatty acid assembly into TAG, which holds promise for transgenic engineering aimed at producing valuable fatty acids.

Authors’ Contributions

Yi-Cun Chen and Yang-Dong Wang contribute equally to the paper.

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