Gene expression profiles of oleic acid in Brassica napus by microarray analysis

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Introduction

Rapeseed oil usually contains 55-60% oleic acid (18:1), 20% linoleic acid (18:2) and 10% linolenic acid (18: 3), with oleic acid being the main fatty acid. Therefore, a high content of oleic acid is one of the most important indicators of quality for edible vegetable oil. Vegetable oil with a high content of oleic acid is not sensitive to oxidative changes so it can be stored for a longer period. Furthermore, it is smokeless when heated to a high temperature. In the daily diet, using vegetable oil with a high content of oleic acid may help reduce the blood level of low-density lipoprotein and cholesterol and prevent arteriosclerosis. Additionally, it was reported that oleic acid can kill tumor cells such as breast cancer cells.

To further improve the quality of rapeseed oil, it is necessary to carry out research on high oleic acid rapeseed. Oleic acid is a monounsaturated fatty acid whose content in low erucic acid rapeseed is affected by desaturation of oleic acid into linoleic acid and desaturation of stearic acid. If the fatty acid desaturase (FAD) gene of rapeseed is inhibited or mutated, the content of oleic acid will be greatly increased. Previous studies showed that FAD2, an oleic acid desaturase, is located in the endoplasmic reticulum.

Materials and Methods

Plant Materials

High oleic acid (HO) material with an oleic acid content of 71.71% was mutated from Xiangyou 15 by treated with ethylmethanesulfonate (EMS, stock concentration was 10 M and the working concentration was 1.5%). The traits of oleic acid in HO material were faithfully inherited during six generations of self-crossing and selection. Low oleic acid (LO) material with an oleic acid content of 55.6%, an inbred line of Xiangyou 15, was used as a control. The above rapeseed strains were provided by the Research Institute of Oil Plants at Hunan Agricultural University. The oleic acid content was determined by gas chromatography.

Methods

(1) Extraction and purification of total RNA
(2) Fluorescent labeling of RNA samples
(3) Microarray hybridization and scanning
(4) Real-Time PCR

(5) Data Analysis (Functional annotation of differentially expressed genes)

Results

Information extraction from hybridized microarrays and screening of differentially expressed genes

The signals from the scanned microarray were processed into digital signals using LuxScan3.0 image analysis software (CapitalBio). These signals were the basis for the further data analysis (Lorkowski 2003; Li 2006). To correct any systematic error between Cy3- and Cy5-labeling, the data were normalized. The ratio values are shown after Lowness normalization. There were 31,200 (Ratio = H/L) “all data” obtained from HO and LO rapeseed. After omitting genes with weak fluorescence, positive controls, negative controls and external standards, 10,243 effective genes, presented as “Checked genes”, were obtained. In the present study, the method of multiplicity was used to screen differentially expressed genes. A fluorescence intensity ratio of experimental group to control group greater than 2 or less than 0.5 suggested that the gene was differentially expressed. If the ratio was greater than 2, the gene was up-regulated. If the ratio was less than 0.5, the gene was down-regulated. Our results indicated 562 differentially expressed genes, of which 194 genes were up-regulated and 368 genes were down-regulated. The distribution of up-regulated genes was as follows: there was one gene with a ratio of 14.595, 11.2852 or 9.728, 12 genes with a ratio ranging from 5-8, 36 genes with a ratio ranging from 3-4, and 139 genes with a ratio ranging from 2.0013-2.9595. The down-regulated genes displayed a ratio between 0.0713 and 0.4996.

Functional classification of differentially expressed genes

By Go classification, the functions of differentially expressed genes were classified into 23 categories.

Differentially expressed genes related to fatty acids metabolism

Go annotations were from three aspects: cellular process, cellular component and cellular function. The functions of differentially expressed genes related to fatty acids metabolism involved in the cellular process (including fatty acid biosynthesis, lipid transport, lipid metabolic biosynthesis, lipid transport, lipid metabolism and unsaturated fatty acid biosynthesis), cellular component (including plastid, endoplasmic reticulum and endomembrane system) and cellular function (including acyl carrier activity, oxidoreductase activity, lipid binding, triacylglycerol lipase activity, caboxylesterase activity, acetylglucosamine transferase activity and ω-3 fatty acid desaturase). The locations and distributions in the chromosome of the loci that play an important role in oleic acid metabolism were annotated by Go.

Biological pathway analysis of differentially expressed genes (KEGG pathway analysis)

At present, three public databases are used for pathway analysis, namely KEGG, Biocarta and GeMAPP. In the present study, the KEGG (Kyoto Encyclopedia of Genes and Genomes) database (http://www. Genome. jp/kegg/), currently the most well-known biological pathway database was used. There were 60 differentially expressed genes classified to relevant KEGG pathways, including

flavonoid biosynthesis, peptidoglycan biosynthesis, fructose and mannose metabolism, pyrimidine metabolism, lysine biosynthesis, coenzyme Q biosynthesis, ascorbate and aldarate metabolism, DNA polymerase and purine metabolism, coenzyme A (CoA) and pantothenate biosynthesis, carbon fixation, photosynthesis, cytochrome P450 metabolism and the metabolism of glyoxylic acid and ethanedioic acid. The glycolysis metabolic pathway is closely involved with lipid formation.

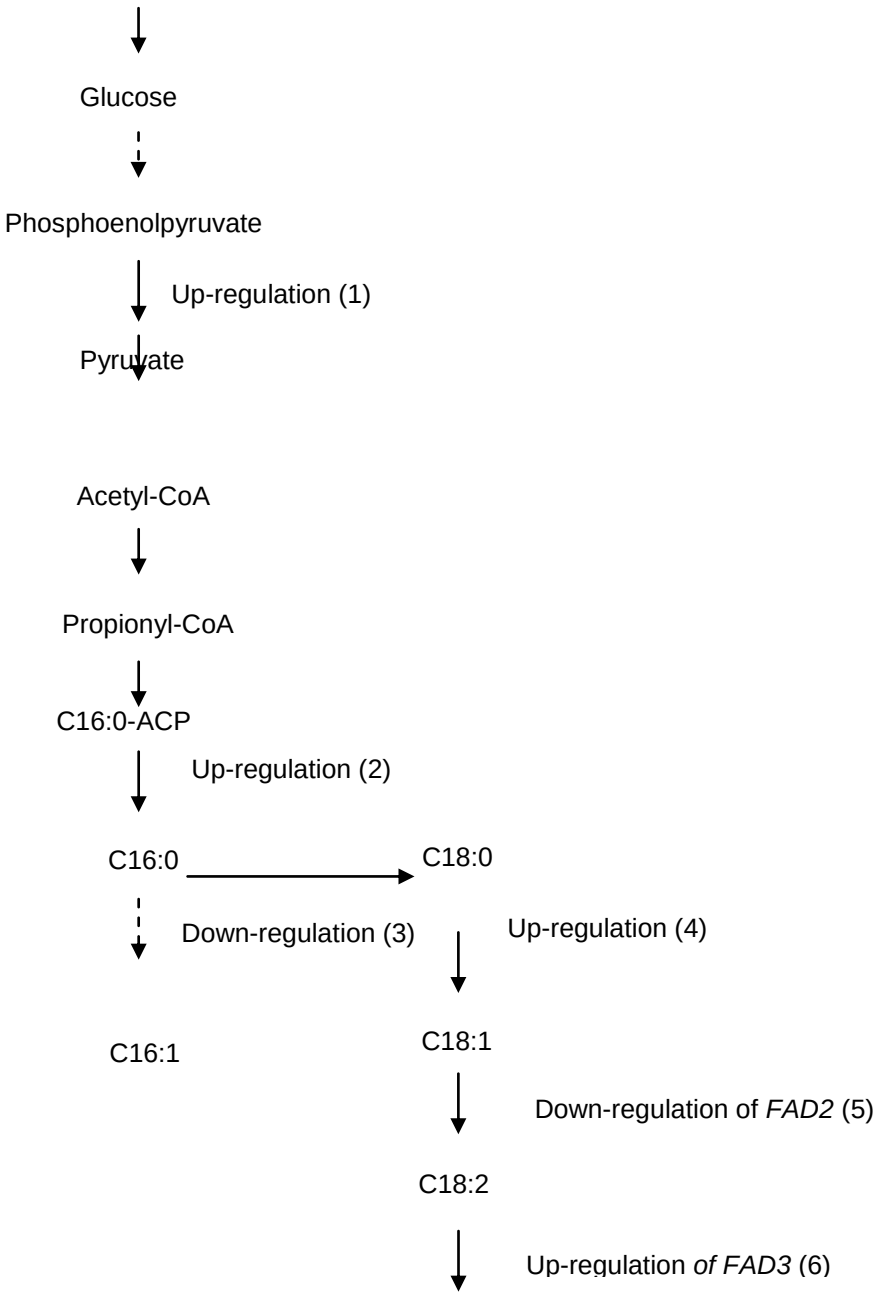
In the present study, pathway analysis was applied to one differentially expressed gene (NM_115439) of rapeseed. It was associated with 80 genes in the glycolysis pathway, of which pyruvate kinase (EC2, 7, 1, 40) is a key enzyme (Figure 3). The results of microarray experiments indicate that the gene encoding pyruvate kinase was significantly up-regulated with a ratio greater than 2.0421. In addition, this gene (NM_115439) was associated with 65 genes in the pyruvate metabolism pathway.

Verification by Real-Time PCR

The results of microarray analysis were verified by real-time PCR. An up-regulated gene (NM_100489) and a down-regulated gene (NM_130183) were randomly selected. The results of the up-regulated genes (NM_100489) were consistent with the results of the microarray.

Discussion

We found that five key genes involved in fatty acid metabolism played an important role in the increase of oleic acid. Of the five genes, there were three up-regulated genes, namely pyruvate kinase (NM_115439, ratio value: 2.0421), ∆9 stearoyl- acetyl carrier protein (ACP) desaturase (NM_100489, ratio value: 3.7227) and acyl-ACP thioesterase (NM_100724, ratio value: 4.8057) and two down-regulated genes, namely ∆9 acyl-lipid desaturase (NM_ 128693, ratio value: 0.4862) and ω-3 fatty acid desaturase (FAD3) (NM_128552, ratio value: 0.4865). Database queries confirmed that these five enzymes are key enzymes for fatty acid synthesis (Buchanan et al. 2000). Based on the above analysis, the relationship between gene regulation during the formation of oil with high oleic acid is as follows:



C18:3

Note: (1) pyruvate kinase, (2) acyl-ACP thioesterase, (3) △9 acyl-lipid desaturase (ADS2), (4) △9 stearoyl-ACP desaturase (ADS1), (5) fatty acid desaturase 2 (FAD2) and (6) fatty acid desaturase 3 (FAD3)