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Genome-wide identification and expression analysis of the fatty acid desaturase genes in *Medicago truncatula*

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ABSTRACT

Fatty acid desaturases (FADs) are of great importance and play critical roles in regulating plant fatty acid (FA) compositions. But to date, no reports about characterization of the *FAD* genes have been reported in the model dicotyledonous grass species *Medicago truncatula.* In this study, using database searches, 20 full-length *FAD* genes were identified in *M. truncatula*. These *FAD* genes were unevenly distributed on six chromosomes except the chromosome 6 and 8. Phylogenetic analysis showed the *FAD* genes in *M. truncatula* were clustered into six subfamilies and had similar exon number and intron phase in the same subfamily. Moreover, expression analysis based on qRT-PCR indicated these *FAD* genes were extensively involved in cold and heat responses. This study would provide an important foundation for future cloning and functional studies of *FAD* genes in *M. truncatula* and other related legume species.

1. Introduction

Fatty acids are the major components of seed storage lipids and plant membrane lipids [1,2]. In plant cells, the *de novo* generation of polyunsaturated fatty acids (PUFAs) in the stroma of plastids has been documented by Browse and Somerville [3], which are catalysed by a series of desaturation and elongation pathways [4]. After their biosynthesize, these fatty acids are integrated into the glycerolipid-synthesizing steps either in endoplasmic reticulum (ER) or plastids, for assembly into sulfolipids, phospholipids and galactoglycerolipids [3].

A lot of fatty acid desaturation is a similar characteristic for cell membranes in plant [5]. Hydrocarbon chains of unsaturated fatty acids often have same unsaturated bonds [5]. The type and content of unsaturated fatty acids are critically important for nutritional traits of cooking oils [6]. Furthermore, the position and number of the unsaturated bonds of a fatty acid considerably affect its physiological and physical features [7,8]. The composition of the unsaturated fatty acids intrinsically controls the membranes function, and thus for the optimal plant growth and development [7]. Also, the degree of unsaturation is an important determinant of plant resistance to various environmental stresses, particularly adverse temperature [9]. Melting temperature of unsaturated fatty acids is relatively low compared to saturated fatty acids, and their accumulation helps to maintain the optimal fluidity and integrity of membranes thereby accelerating cold acclimation; thus, polyunsaturated fatty acid augmentation may exacerbate thermal damage [10].

These enzymes known as fatty acid desaturases (FADs) are involved in the desaturation of the fatty acids [5]. FADs act by introducing unsaturated bonds into the fatty acids hydrocarbon chain [11]. Considering the benefits of fatty acid desaturation in plant stress responses and development, the identification and characterization of *FAD* genes have been extensively performed in various plants. In *Arabidopsis*, the mutants defective in a particular step of desaturation have been used to investigate the properties of *FADs* [5]. Although FADs are coded by nuclear genes, but the process of desaturation occurs in diverse subcellular locations. For instance, FAD2 and FAD3 are localized in ER in which they regulated extra-chloroplast lipids desaturation, and other FADs, such as FAB2, FAD4, FAD5, FAD6, FAD7, and FAD8, are located in the chloroplasts and play important roles in regulating lipid desaturation [5]. These FADs are believed to be intrinsically substrate-specific. FAB2 appertains to the dissoluble fatty acid desaturase and controls the stearic acid (18:0) to oleic acid (18:1) desaturation in a form of acyl-

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carrier protein (ACP)-bound [12], and hence FAB2 is also called stearoyl-acyl carrier protein desaturase (SAD). The other FADs are thought to be membrane-bound fatty acid desaturase. FAD2 and FAD6 are ω-6 desaturases producers of the oleic acid (18:1) to linoleic acid (18:2) in the endoplasmic reticulum (FAD2) and plastids (FAD6), separately. The endoplasmic reticulum enzymes (FAD3) and within plastidial enzymes (FAD7 and FAD8) are sorts of omega-3 fatty acid desaturases that integrated the double bond into linoleic acid to produce linolenic acid, while *FAD8* gene is believed to encode a cold-inducible isomer of FAD7 [12]. FAD4 and FAD5 function on palmitic acid particularly to generate palmitoleic acid within the plastids [7]. Recently, *FAD* genes have been characterized in many plants, such as soybean (*Glycine max*) [14,15], cotton (*Gossypium raimondii*) [16,17], cacao (*Theobroma cacao*) [12], cucumber (*Cucumis sativus*) [18] and olive (*Olea europaea*) [6,19]. For example, 29 desaturase genes were identified in soybean [14]. In cotton, its membrane-bound FADs were encoded by 19 genes [17]. *FAD3A*, *FAD3B*, *FAD7-1* and *FAD7-2* genes were cloned from olive. Of them, *FAD3A* was mostly involved in the linolenic acid biosynthesis in the seeds, whilst *FAD7-1* and *FAD7-2* genes contributed mainly to the linolenic acid in fruits [6,19].

FADs are also crucial importantly sustain plants resilience to adverse environmental cues. In *Arabidopsis*, the expression of *FAD8* is remarkably induced under cold temperatures [13]. Far more, the activity of *FAD2* and *FAD6* is enhanced in seedlings exposed to salinity and osmotic stresses [20–22]. More Na^+ was accumulated in the root cells cytoplasm of an *FAD2*-devoid mutant, which was particularly sensitive to salinity in the course of early seedling growth [21]. Also, the *Arabidopsis FAD7* gene exhibited enhanced cold stress when it was overexpressed in the transgenic tobacco, while plants with its own *FAD7* gene silenced were more tolerant to heat injury than the wild-type [23–25]. Overexpression of *Arabidopsis FAD3/FAD8* gene resulted in increased tolerance to osmotic stress in transgenic tobacco lines [26]. In soybean, the *FAD3* and *FAD7* expression is firmly induced during low temperatures [15]. Also, in tomato, the *FAD7* expression is deterred by heat stress, and silencing the *FAD7* gene palliated high-temperature stress [27]. In the case of ω-3 *FAD* gene (*LeFAD3*) in tomato, its expression is triggered by salinity stress, and the salinity stress tolerance of tomato seedlings could be greatly improved when *LeFAD3* was overexpressed [28].

Because of its small genome size and high transformation efficiency, *Medicago truncatula* has been used as an excellent model organism to study functional genomics of legume plants. In this study, *FAD* genes in *M. truncatula* were systematically identified and characterized through phylogenetic analysis, structural diversification, and cold and heat stresses analysis. This work will serve to broaden our understanding on the structure and function of *FAD* gene family in *M. truncatula* and other related legume species such as alfalfa (*Medicago sativa*), and helpful for unraveling the candidate genes for potential modifications of fatty acid profiles in a bid to improve the plant vigor and herbage nutritional value.

2. Materials and methods

2.1. Sequence retrieval and data analysis

To identify all *FAD* genes in *M. truncatula*, the *M. truncatula* genome data were downloaded from the latest database of *M. truncatula* genome (JCVI, http://www.jcvi.org/medicago/). The identified plant FAD protein sequences (Table S1) were used as queries performing local BLAST with E-value set as 1E-005. To define the exon/intron organization for the *M. truncatula FAD* genes by loading its coding sequence (CDS) and corresponding genomic sequence into the Gene Structure Display Server 2.0 (GSDS2.0, http://gsds.cbi.pku.edu.cn/). The molecular weights (MW), isoelectric points (p*I*s) and grand average of hydropathicity (GRAVY) of the candidate MtFAD proteins were detected using ExPASy (http://web.expasy.org/protparam/).

2.2. Multiple sequence alignments and phylogenetic analysis

Multiple sequence alignments of the full-length FAD amino acid sequences were performed using Clustal Omega program (https://www.ebi.ac.uk/Tools/msa/clustalo/). A phylogenetic tree was constructed using MEGA 7 (https://www.megasoftware.net/) by the Neighbor-Joining (NJ) and bootstrap tests were performed using 1000 replicates to support the statistical reliability.

2.3. Estimating Ka/Ks ratios for duplicated gene pairs

Synonymous (Ks) and nonsynonymous (Ka) substitution rates were computed as described previously [29] and Ka/Ks ratio was estimated by online tools: Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) and PAL2NAL (http://www.bork.embl.de/pal2nal/).

Table 1
The related information of the *FAD* genes in *M. truncatula*.

No.	Gene name	Locus ID	Genomics position	Proteion length	Mw (KDa)	p*I*s	GRAVY	Strand
1	MtFAB2.1	Medtr1g029150.1	chr1:9950088..9952717	390	44219.6	5.79	−0.385	+
2	MtFAB2.2	Medtr3g089025.1	chr3:40742511..40744042	369	42457.7	5.92	−0.295	+
3	MtFAB2.3	Medtr3g089973.1	chr3:40901743..40902182	104	11935.9	8.6	−0.209	−
4	MtFAB2.4	Medtr3g089980.1	chr3:40909190..40909582	90	10516.1	6.58	−0.378	−
5	MtFAB2.5	Medtr3g090010.1	chr3:40914266..40915672	370	42335.4	5.42	−0.409	−
6	MtFAB2.6	Medtr3g090020.1	chr3:40920281..40921893	366	42022.2	6.09	−0.37	−
7	MtFAB2.7	Medtr3g090100.1	chr3:40945145..40946820	366	41707.9	6.37	−0.313	−
8	MtFAB2.8	Medtr3g090110.1	chr3:40949975..40950370	108	12021.9	8.39	−0.262	−
9	MtFAB2.9	Medtr4g087440.1	chr4:34328476..34331741	393	44638.9	5.75	−0.428	+
10	MtFAB2.10	Medtr5g024130.1	chr5:9667333..9670533	393	44657.9	5.87	−0.442	−
11	MtFAD2.1	Medtr1g054035.1	chr1:23029056..23031957	384	44277.9	8.36	−0.091	+
12	MtFAD2.2	Medtr1g111880.1	chr1:50591248..50594736	366	42488.2	9.16	0.076	+
13	MtFAD2.3	Medtr7g093200.1	chr7:37015652..37021227	434	50190.8	9.13	−0.066	+
14	MtFAD3.1	Medtr3g464330.1	chr3:25836811..25841263	385	44917.6	8.47	−0.219	−
15	MtFAD3.2	Medtr5g071170.1	chr5:30184947..30187279	391	45684.1	7	−0.21	−
16	MtFAD5	Medtr4g010150.1	chr4:2249018..2251257	394	45616.3	8.87	−0.13	+
17	MtFAD6.1	Medtr2g460790.1	chr2:25077127..25082950	447	51903.4	9.35	−0.187	−
18	MtFAD6.2	Medtr4g130980.1	chr4:54616212..54622427	446	51503.9	9.3	−0.145	−
19	MtFAD7.1	Medtr4g015630.1	chr4:4703749..4708197	440	50084.6	9.08	−0.244	+
20	MtFAD7.2	Medtr7g068780.1	chr7:25266118..25269838	446	50909	6.65	−0.258	−