Genome-wide analysis of fatty acid desaturase genes in rice (*Oryza sativa* L.)

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Fatty acid desaturases can catalyze saturated or unsaturated fatty acids to form a double bond at various locations in the hydrocarbon chain. In the present study, a total of 20 full-length desaturase genes were identified from rice genome. An exhaustive analysis was performed to describe their chromosomal locations, gene structures, phylogeny, cis-regulatory elements, sub-cellular localizations and expression patterns. The rice desaturase genes were distributed on ten of 12 chromosomes and phylogenetically classified into six subfamilies with the *Arabidopsis* counterparts, FAB2, FAD2, FAD3/7/8, FAD6, DES1 and SLD1. Among of them, 9 members were expanded via chromosomal tandem or segmental duplications. The gene structures and motif constituents were evolutionarily conserved in the same subfamilies. The majority of desaturase genes showed tissue-specific expression patterns and response to abiotic stresses and hormones based on microarray data and qRT-PCR analyses. This study will provide useful clues for functional validation of desaturase genes and contribute to produce nutritionally important fatty acids by genetic modification in rice.

Rice is one of the most important staple crops, provides almost half of the world’s population a dietary source of energy1. Apart from starches and proteins, rice grains contain a low proportion of lipids consisted mostly in the bran. Rice bran oil (RBO) extracted from rice bran is a commercial valuable by-product of milling, consisting of various fatty acids such as 36% oleic acid, 37% linoleic acid, 18% palmitic acid, 2.4% stearic acid, 1.5% linolenic acid and so on. RBO is as effective as other commonly used vegetable oils in lowering plasma cholesterol level2. The oxidative stability and nutritional value of RBO, which are already being considerable of commercial importance, are also affected by the levels of fatty acids3.

Fatty acid desaturases catalyze saturated or unsaturated fatty acids to form a double bond at various locations in the hydrocarbon chain4. They exist very widely in eukaryotic cells and contain three or two conserved histidine regions. Histidine-rich motifs are thought to form a part of the diiron center where oxygen activation and substrate oxidation happen5–7. The desaturation processes occur in both the endoplasmic reticulum (ER) membrane and the plastid membrane through two distinct pathways8. The genes encoding ER- and plastid-localized desaturases have been cloned and characterized from many plant species up to now. In rice, OsSSI2 encoding a stearoyl acyl carrier protein fatty-acid desaturase, and is involved in producing oleic acid from stearic acid. Suppression of OsSSI2 enhances resistance to blast and leaf blight diseases in rice5. A microsomal Δ12 fatty acid desaturase gene designated as OsFAD2-1, is responsible for the conversion of oleic acid into linoleic acid, and can improve the tolerance of rice to low temperature stress10–12. OsFAD3 code a ω-3 (Δ-15) fatty acid desaturase localized to the ER membrane, which catalyzed linoleic acid conversion to α-linolenic acid in rice seeds13,14. Chloroplast-localized OsFAD7 and OsFAD8 have also ω-3 desaturase activity for forming trienoic fatty acids and they negatively regulate the disease resistance against *Magnaporthe grisea*, in addition, OsFAD8 plays a significant role in stress tolerance at low temperature13–16.

Currently, the rice genome has been deeply sequenced and assembled, approximately 373 Mb captured in 12 chromosomes, and its 39102 predicted gene loci annotated by MSU-RGAP are publicly available17. So, rice has become the primary model for cereal species in plant science research, and its genome and annotated genes facilitate comparative genomic studies which will help to discover novel genes and explain some biological problems in silico18. In the present study, although several orthologous genes encoding fatty acid desaturases from rice were identified, our understanding of the biological function of the majority of these enzymes is limited. As such, there

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seven introns and eight exons, and are all nearly 1155 bp in length (Fig. 1B). OsFAD3-1 to share similar exon-intron structure and transcript length. For example, OsFAD3-2 and OsFAD3-8 each have three, chromosome 1 has two and chromosomes 4, 6, 9, 11 and 12 each only contain one member (Fig. 2). Eight each have three, chromosome 1 has two and chromosomes 4, 6, 9, 11 and 12 each only contain one member (Fig. 2).

Table 1. The general information and sequence characterization of 20 fatty acid desaturase genes in rice.

| Gene Name/Alias | Accession Number | RGAP Locus | FL-cDNA | Protein* | subcellular localization* | Arabidopsis ortholog locus |
|-----------------|------------------|------------|---------|----------|--------------------------|----------------------------|
| OsFAD2-1        | LOC_Os02g48560   | AK061506   | 1       | 389      | 44350.1      | 8.23 | ER | FAD2(AT3G12120) |
| OsFAD2-2        | LOC_Os07g23430   | AK105371   | 1       | 470      | 52845.2      | 9.35 | plasma membrane | FAD2(AT3G12120) |
| OsFAD2-3        | LOC_Os07g23410   | AK070559   | 1       | 391      | 44981.0      | 9.51 | ER | FAD2(AT3G12120) |
| OsFAD2-4        | LOC_Os07g23390   | NA         | 1       | 224      | 24677.1      | 12.61 | plasma membrane | FAD2(AT3G12120) |
| OsFAD3-1        | LOC_Os11g01340   | AK242740   | 7       | 385      | 43902.4      | 7.48 | ER | FAD3(AT2G29980) |
| OsFAD3-2        | LOC_Os12g01370   | AK071185   | 7       | 386      | 43899.5      | 8.01 | ER | FAD3(AT2G29980) |
| OsFAD6          | LOC_Os08g34220   | AK060449   | 9       | 455      | 52243.7      | 9.51 | chloroplast | FAD6(AT3G09590) |
| OsFAD7          | LOC_Os03g18070   | AK058242   | 7       | 459      | 50772.1      | 7.66 | chloroplast | FAD7(AT3G11170) |
| OsFAD8          | LOC_Os07g49310   | AK061531   | 6       | 414      | 47012.0      | 8.73 | chloroplast | FAD8(AT3G05580) |
| OsFAB2-1        | LOC_Os01g65830   | AK059526   | 1       | 382      | 42942.9      | 7.10 | chloroplast | FAB2.1(AT1G43800) |
| OsFAB2-2/OsSS12 | LOC_Os01g69080   | AK058979   | 2       | 401      | 45320.7      | 7.07 | chloroplast | FAB2.2(AT2G43710) |
| OsFAB2-3        | LOC_Os02g30200   | AK069683   | 3       | 388      | 42513.3      | 7.02 | chloroplast | FAB2.3(AT2G43710) |
| OsFAB2-4        | LOC_Os03g30950   | AK070282   | 1       | 419      | 45365.5      | 7.94 | chloroplast | FAB2.4(AT3G02620) |
| OsFAB2-5        | LOC_Os04g31070   | AK065340   | 2       | 391      | 44341.4      | 7.01 | chloroplast | FAB2.5(AT2G43710) |
| OsFAB2-6        | LOC_Os03g53010   | NA         | 0       | 264      | 30087.6      | 10.47 | mitochondrial | FAB2.6(AT2G43710) |
| OsFAB2-7        | LOC_Os06g30780   | NA         | 0       | 190      | 22534.2      | 12.46 | mitochondrial | FAB2.7(AT3G02620) |
| OsFAB2-8        | LOC_Os08g09950   | AK105852   | 1       | 423      | 46491.2      | 7.98 | chloroplast | FAB2.8(AT3G02610) |
| OsFAB2-9        | LOC_Os08g10010   | AK241294   | 1       | 405      | 44829.0      | 7.41 | mitochondrial | FAB2.9(AT3G02610) |
| OsDES1          | LOC_Os02g42660   | AK101968   | 1       | 329      | 37868.6      | 8.93 | plasma membrane | DES1(AT4G04930) |
| OsXLD1          | LOC_Os09g16920   | AK058543   | 0       | 467      | 51723.8      | 8.55 | plasma membrane | XLD1.1(AT2G46210) |

is an urgent need for an entire feature about the fatty acid desaturase family. This study provides a global overview of the desaturases which contains the gene structures, chromosome locations, phylogeny, and the expression profiling resulting from various organs/tissues of rice. The identification of novel desaturases will give us new insights into the pathways involved in unsaturated fatty acid metabolism and signaling transduction in rice. Moreover, the characterization of desaturases from rice will offer scientists abundant candidate genes for the production of nutritionally beneficial fatty acids in transgenic crops.

Results and Discussion

Identification of fatty acid desaturase genes in rice genome. We used several bioinformatics resources in our efforts to thoroughly explore the entire FAD gene family in rice. 29 gene models were obtained through FAD domain searching (FA_desaturase, PF00487; FA_desaturase_2, PF03405) with the MSU Rice Genome Annotation Project Database (MSU-RGAP, http://rice.plantbiology.msu.edu/analyses_search_domain.shtml). In the Rice Annotation Project Database (RAP-DB, http://rapdb.dna.affrc.go.jp/), using a keyword search for “fatty acid desaturase”, 10 genes were identified. The orthologous protein sequences of Arabidopsis desaturases were used as queries in BLASTP searches against the rice genome entries in the RAP-DB databases. Following removal of the redundant sequences and eliminating alternate splice variants of the same gene, a total of 20 fatty acid desaturase genes were thus identified in rice. Among of them, nine members homologous with Arabidopsis FAB2.1 were designated as OsFAB2-1–9. Analogously, four FAD2 subfamily members, four FAD3/FAD7/FAD8 subfamily members, and one each in DES1, SLD1 and FAD6 subfamily were named. The detailed information about each gene locus, FL-cDNA, ORF length, and characteristics of corresponding proteins are detailed in Table 1.

The numbers and positions of exons and introns of each desaturase gene were determined by the comparison of the CDS sequences and the corresponding genomic sequences via using the Gene Structure Display Server website (GSDS, http://gsds.cbi.pku.edu.cn/). 19 Three gene (OsFAB2-6, OsFAB2-7 and OsXLD1) lacked introns; the number of introns in the coding sequences of the other 17 genes ranged from one to nine (Fig. 1B). Four genes (OsFAB2-3, OsFAB2-6, OsFAB2-7 and OsFAD2-4) had no untranslated regions. Most orthologous genes tended to share similar exon-intron structure and transcript length. For example, OsFAD3-1 and OsFAD3-2 each contain seven introns and eight exons, and are all nearly 1155 bp in length (Fig. 1B).

Chromosomal localization and gene duplication. Based on the MSU-RGAP loci coordinates (http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/), the accurate locations and orientation of the fatty acid desaturase genes on the rice chromosomes were determined. All of the 20 genes were distributed on ten of 12 chromosomes, excluding chromosomes 5 and 10. Rice chromosome 7 has four desaturase genes, chromosomes 2, 3 and 8 each have three, chromosome 1 has two and chromosomes 4, 6, 9, 11 and 12 each only contain one member (Fig. 2).
To explore the impacts of duplication events during evolution on the expansion of the fatty acid desaturase family, we investigated tandem and segmental duplications. According to the criterion of separation by less than 5 intervening genes and >50% homology at protein level, a total of 5 genes fell into two groups were tandemly

Figure 1. Phylogenetic relationship and intron-exon structures of desaturase genes. (A), Phylogenetic tree of 20 desaturase genes using maximum-likelihood methods. The MEGA software (version 7.0.25) was used to generate the phylogenetic tree. Scale bar represents 0.2 amino acid substitution per site. The proteins on the tree can be divided into six distinct subfamilies. The number in the line represents the goodness of fit (%). (B), intron-exon structures of desaturase genes. The numbers 0, 1 and 2 indicate the phase of introns.

Figure 2. Chromosomal localization and gene duplication events of desaturase genes. The circlize package in R (version 3.6.1) was used to generate the circo map. Respective chromosome numbers are indicated at the center of each arc. The scale on the arc is in megabases (Mb). The segmental duplication genes are connected by straight line in color. The tandemly duplicated genes are shaded with ovals in light blue. The arrows next to gene names show the direction of transcription.
Table 2. Conserved histidine-rich boxes of fatty acid desaturases in rice.

| Subfamily | H-box 1 | H-box 2 | H-box 3 |
|-----------|---------|---------|---------|
| FAB2      | WT(S)AE(K)ENR(H)GHD | AAS(D)EKRE | / |
| FAD2      | WVF(I)A(G)HGGCHHAFS | FSWKTS(T)H(R)QRRHHSNT | TDTTVHXHLPF(S) |
| FAD3/7/8  | WLFVFLHDGCGRGFS | YKGWRSHHTRNHGQ | HDHGTIVIHHLFPQHYHL |
| FAD6      | FFVIGHDCAHRKKSFS | EPWRFHKEHRHAKTN | HDNIVHPFHERSPR |
| SLD1      | GHDSGHH | WWKCHNTHHHTCNSLDD | GGLPQIEHLHLFPLFRPR |
| DES1      | NLFALHELHSHNLAF | FQKYHLEHHHRFGVQGDID | HVGYSHEHHDFPFRPG |

Multiple sequence alignment and phylogenetic analysis. To clearly apprehend the sequence characteristics of fatty acid desaturases in rice, we performed a multiple sequence alignment using the deduced amino acid sequences of the 20 proteins. The 20 gene products contained typical histidine-rich boxes (Table 2), which accorded with the standard of different types of desaturase genes. For instance, majority of genes from FAD3/7/8 subfamily matched the standard for plastidial \( \Delta 12 \) desaturase, namely, GHDCXH, HXXHH and HVXXH; two histidine-boxes of nine FAB2 subfamily members were consistent with these of stearoyl-[acyl-carrier-protein] desaturases characterized by EERNGH and DEXRHE. To be sure, an exception was that LOC_Os07g23390 appeared to be truncated at the C-terminus missing the third histidine-rich box, so no FAD domain (either PF00487 or PF03405) could be detected in Pfam database. But in keeping with previous published article11, LOC_Os07g23390 was still considered as OsFAD2-4.

To examine in detail the phylogenetic relationship and functional divergence among the 20 desaturases in rice, a phylogenetic tree was constructed (Fig. 1A). The 20 desaturases were classified into 6 distinct subfamilies; the subfamilies were named according to their identity to Arabidopsis desaturases and included subfamilies FAB2, FAD2, FAD3/7/8, FAD6, DES1 and SLD1. The rice homologs of Arabidopsis AtFAD5 and AtAD5/1 were not present. Among the 6 subfamilies, subfamily FAB2 with nine members was the largest (Fig. 1A).

In order to identify orthologous genes between Arabidopsis and rice, an integrated phylogenetic tree composed of members of the two species was constructed (Fig. S1). In this analysis, similar subfamilies were formed as compared with the evolutionary tree of rice. Except for the subfamily FADS/AD5 absent in rice genome, each clade of a distinct subfamily consisted of desaturases from both rice and Arabidopsis. Furthermore, the orthologues between Arabidopsis and rice in subfamily FAB2, FAD2, FAD3/7/8, FAD6, DES1, and SLD1 showed very high sequence identities. These results indicated that the formation of desaturase family in Arabidopsis and rice occurred before the split of dicots and monocots.

Expression analysis of desaturase genes during development. To gain valuable insights into the possible function of desaturase genes during rice development, we analyzed their expression patterns in various organs/tissues at different developmental stages using microarray data (http://ricexpro.dna.affrc.go.jp/GGEP/). Data for the following tissues was examined: vegetative roots (VR), reproductive roots (RR), vegetative leaf blades (VL), reproductive leaf blades (RL), vegetative leaf sheaths (VS), reproductive stems (RS), inflorescences (I1-I3), male gametophytes between Arabidopsis and rice, an integrated phylogenetic tree composed of members of the two species was constructed (Fig. S1). In this analysis, similar subfamilies were-formed as compared with the evolutionary tree of rice. Except for the subfamily FADS/AD5 absent in rice genome, each clade of a distinct subfamily consisted of desaturases from both rice and Arabidopsis. Furthermore, the orthologues between Arabidopsis and rice in subfamily FAB2, FAD2, FAD3/7/8, FAD6, DES1, and SLD1 showed very high sequence identities. These results indicated that the formation of desaturase family in Arabidopsis and rice occurred before the split of dicots and monocots.
inconsistent expression patterns, for example, SLD showed high expression levels in all tissues except for reproductive roots in rice, but was barely expressed in Arabidopsis. The similarities and differences of expression profiles of these orthologous genes may imply their functional conservatism and evolution.

Regulation of desaturase gene expression in response to abiotic stresses. To examine the response of desaturase genes to various abiotic stresses, the microarray data (GSE6901) for 7-day-old seedlings treated with drought, salt and cold stress was analyzed. A total of 8 genes were significantly \( P < 0.05 \) down-regulated and only one was up-regulated in at least one of the stress conditions (Fig. 5A–D). The transcriptional level of OsSLD1 was down-regulated under all three stresses (Fig. 5A). Four genes (OsFAD3-2, 6, 8 and OsFAB2-9) were significantly down-regulated by both drought and salt stresses (Fig. 5B). Three genes (OsFAD2-2, OsFAD2-3 and OsDES1) were specifically down-regulated by drought stress (Fig. 5C). OsFAB2-1 was up-regulated under drought stress (Fig. 5D). All in all, it appeared that cold stress had only very limited influence, but drought stress had a much higher effect on the expression of these desaturase genes. These results suggest that desaturase genes may be involved in drought signaling pathways and play important roles in responses to drought stresses by changing the content ratio of the various fatty acid compositions in rice.

Differential expression of desaturase genes in response to hormones. To determine if desaturase genes in rice are involved in hormone signaling pathways, we investigated their expression profiles under different phytohormone treatments. Total RNA was isolated from seedlings of Nipponbare rice at three-leaf stage treated with ABA (Abscisic Acid), 6-BA (6-Benzylaminopurine), IAA (Indole-3-Acetic Acid) and GA (Gibberellin Acid), and the expression levels of the desaturase genes were evaluated using quantitative RT-PCR (Fig. 6). The results showed that the expressions of 8 genes (OsSLD1, OsDES1, OsFAB2-1, OsFAB2-4, OsFAB2-8, OsFAB2-9, OsFAD2-2, OsFAD2-4) were markedly down- or up-regulated (<50% or >2-fold) under at least one of the hormone treatments at 3 h time point, as compared with the untreated control. It implied that these genes may be involved in plant’s early response to the hormones.

Under IAA treatment, the transcript levels of four genes (OsFAD2-1, OsFAD3-1, OsFAD6 and OsFAD7) and two genes (OsFAB2-4, OsFAD2-4) were decreased and increased at 6 sampling time points, respectively. Two genes (OsSLD1, OsFAB2-5) were almost unaffected by IAA. These data indicated that the various desaturase genes showed different induction kinetics in response to auxin.
Under GA treatment, four genes (OsFAD3-1, OsFAD6, OsDES1, OsSLD1 and OsFAB2-6) were induced slightly at the early time points and reached peak value at 9 h, but were reduced at the later time points. The expressions of two genes (OsFAB2-3, OsFAB2-4) were repressed during the whole early-middle period, but were induced strongly at 48 h time point. Two genes (OsFAB2-1, OsFAB2-9) showed high express level at the whole time points. The results revealed that most of the desaturase genes were responsive to gibberellin.

Under ABA treatment, the expression levels of 10 genes (OsFAB2-2, OsFAB2-6, OsFAB2-7, OsFAB2-8, OsFAD2-1, OsFAD2-4, OsFAD3-1, OsFAD6, OsFAD8 and OsDES1) showed different changes at the different time points of the treatment; that is to say, these genes were repressed (<50%) at some time points, but induced (>2-fold) at other time points. It illustrated that desaturase genes could play a complex role in abscisic acid signaling pathways in rice. Meanwhile, the expression patterns of quite a part of genes under 6-BA treatment were similar with the result of ABA treatment; for example, the transcript levels of eight genes (OsSLD1, OsDES1, OsFAB2-1, OsFAB2-3, OsFAB2-6, OsFAB2-7, OsFAB2-9 and OsFAD2-1) were noticeable increased at particular time points, but decrease at other times. Moreover, the three genes (OsFAB2-2, OsFAB2-1 and OsFAD8) were reduced throughout the treatment time courses; this is also consistent with ABA treatment.

Comparison of the induction kinetics of desaturase genes under different hormones stimulus revealed that the expression levels of the majority of genes increased under at least two hormones treatments. Three genes (OsFAB2-6, OsFAB2-7 and OsFAB2-8) and one gene (OsFAD2-1) were up-regulated and down-regulated by all the four hormone treatments, respectively. The hormone-responsive expression profiles of this family suggested that almost all of the genes were responsive to the four hormones tested in these experiments expect OsFAB2-5, which was not responsive to ABA.

Figure 4. Real-time PCR analysis of tissue-specific expression of the desaturase genes. The ggplot2 package in R (version 3.6.1) was used to generate the cluster bar chart. Relative mRNA levels of individual genes normalized to Os03g0234200 are shown. The genes with preferential expression levels in spikelets (A), flag leaves and sheaths (B), seedlings (C) and radicles (D) were showed. Error bars indicate standard deviations of independent biological replicates (n = 3 or more).
Cis-regulatory elements in the promoter of desaturase genes. Conserved regulatory elements in promoter sequences were involved in response to various growth factors and environmental stresses. Here, the promoter sequences (~2.0 kb) of nine abiotic stress and hormone-induced desaturase genes (OsFAB2-1/9, OsFAD2-2/3, OsFAD3-2, OsFAD6, OsFAD8, OsSLD1 and OsDES1) were selected to compare with each other (Fig. 7). It was observed that dozens of different cis-acting elements predicted by PlantCARE were discovered. Among them, several cis-acting elements, like G-boxes, CAAT-boxes and TATA-boxes and so on, were mutual in nine examined genes. To more intuitively explore the promoter regions, a total of nine developmental or stress-related cis-regulatory elements were used for promoter analysis, including abscisic acid responsive element (ABRE), anaerobic response element (ARE), auxin-responsive element (AuxRE), gibberellin-responsive element (GARE-motif), low temperature responsive element (LTR), myb-binding site involved in drought-inducibility (MBS), Methyl jasmonate-responsive element (MeJA-RE), defense and stress responsive element (TC-rich repeat) and wounding and pathogen responsive element (WUN-motif). The results showed that eight out of nine examined genes except OsFAD2-2 possessed multiple ABRE elements and at least four different other regulatory elements in their promoter regions; it was in accordance with their expression profiles in response to abiotic stress and ABA treatment. In addition, each gene contained a number of various light responsive elements, such as Box 4, G-Box, GT1-motif, TCT-motif, Sp1, and so forth. It was estimated that desaturases might be also involved in light response.

Further, the PlantPAN 3.0 database was used to screen the predicted transcription factors (TF) and their binding sites of the nine desaturase genes. Subsequently, the TFs from PlantCARE and cis-acting elements from PlantPAN were integrated into a table, depending on the promoter motifs and their positions (Table S1). A total of 189 members of thirteen TF families were identified. Among them, numerous bZIP, bHLH, Homeodomain and SBP transcription factors were predicted to bind the ABRE and MeJA-RE elements. Similarly, the WUN-motif elements could be bound by NF-YB, NAC and AT-Hook transcription factors. Besides, AP2, MYB and WRKY etc. transcription factors were able to interact with other regulatory elements. The results indicated that these cis-acting elements and corresponding trans-acting factors may be activated in response to hormones and environmental stresses.

Materials and Methods
Identification of fatty acid desaturase genes in rice. To explore all of the putative desaturase members in rice, three approaches were employed, as described in detail previously29. First, the five protein sequences of Arabidopsis desaturases (FAD2/AT3G12120, FAB2/AT2G43710, DES1/AT4G04930, SLD1/AT2G46210 and ADS1/AT1G06080) were used as queries to execute BLASTP program against rice protein database (https://rapdb.dna.affrc.go.jp/tools/blast) with default parameters except for E-value set to e^-10 (default value is 0.1). Secondly, two Pfam domains (PF00487 and PF03405) searching was performed using the MSU-RGAP website (http://
Thirdly, in the Rice Annotation Project Database (RAP-DB, http://rapdb.dna.affrc.go.jp/), using a keyword search for “fatty acid desaturase”. After removing the redundant sequences, the remaining protein sequences were defined as putative fatty acid desaturases. The genomic, transcript, coding sequence (CDS) and peptide sequences of identified genes were retrieved from MSU-RGAP (http://rice.plantbiology.msu.edu/), and full-length cDNA accessions were obtained from NCBI (https://www.ncbi.nlm.nih.gov/). The gene structures of the desaturase members were analyzed using the GSDS2.0 website (Gene Structure Display Server, http://gsds.cbi.pku.edu.cn/)19. 

**Sequence analysis of desaturase genes/proteins.** The physical positions of desaturase genes given by the MSU-RGAP database were used to chromosomal mapping, and their distribution on chromosomes was drawn by R package circlize21. Multiple sequence alignment and conservation analysis were performed using...
CLC Genomics Workbench 12.0 with default parameters. The un-rooted phylogenetic tree of all desaturases was constructed by MEGA 7.0 using the neighbor-joining (NJ) method with 1000 bootstrap replicates, poisson model and complete deletion\textsuperscript{22}. Main criteria used for analyzing potential segmental gene duplication events included: (a) length of alignable region covers > 75\% of longer gene, and (b) similarity of aligned regions > 75\%.\textsuperscript{23}

Subcellular localization was predicted by CELLO2GO (http://cello.life.nctu.edu.tw/cello2go/\textsuperscript{24}).

**Promoter analysis.** The 2000 base pairs upstream from the ATG translational start codon of the desaturase genes were extracted from the RAP-DB database. The upstream sequences were subsequently scanned in the PlantCARE website (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) and PlantPAN 3.0 database (http://plantpan.itps.ncku.edu.tw/promoter.php) to analyze the presence of various cis-acting regulatory elements and corresponding trans-acting factors\textsuperscript{25,26}.

**Plant material and treatments with hormones.** As described in detail previously\textsuperscript{20}, in order to evaluate the spatio-temporal expression profiles of desaturase genes, the rice seedlings of the cultivar Nipponbare were grown in the field during the normal growing season at 30–34: 22–26 °C (day: night) and 80–95\% humidity with a photoperiod of 14 h. The eight materials tested in the expression analysis were: (1) 7-day-old radicle (R); (2) 7-day-old seedlings (Se); (3) 90-day-old node (N); (4) 90-day-old internode (In); (5) flag leaf (Fl); (6) sheath (Sh); (7) 1 cm spikelet (Sp); and (8) flowering spikelet (Fp). For hormones treatments, rice seeds were immersed in water at 37 °C for 30 h, and then were sown on a plastic net that was floating on a nutrient solution in a growth chamber at 28 °C [light: dark = 14 h: 10 h]. Then, seedlings at three-leaf stage were transferred into containers and treated with 10 μM IAA, 10 μM 6’-BA, 10 μM GA, 25 μM ABA or non-treatment for control, and placed in a 28 °C illumination incubator\textsuperscript{20}. At 3, 6, 9, 12, 24, 48 h after these treatments, seedlings were harvested.

All materials harvested were immediately frozen in liquid nitrogen and stored at −80 °C prior to RNA extraction.

**Digital expression and qRT-PCR analysis of desaturase genes.** The microarrays expression data of desaturase genes were extracted from the Rice Expression Profile Database (http://ricexpro.dna.affrc.go.jp/), and were used to analyze expression profiles in various organs at different developmental stages (RXP_0001). Additionally, the total RNA isolation from various tissues, cDNA synthesis and quantitative RT-PCR were performed to analyze the expression profiles under hormones treatments. Real-time PCR was performed using a SYBR Green Realtime PCR Master Mix (TOYOBO) on an ABI StepOnePlus Real-Time PCR System. The 2$^{-\Delta \Delta CT}$ method was used to analyze relative changes in gene expression\textsuperscript{27}. The rice ubiquitin gene (Os03g0234200) was used as a reference in the experiment. The gene-specific primers were listed in Table S2.
Conclusions
Making an intensive study of fatty acid desaturase genes will be conducive to deep understanding of the gene family. The present study investigated thoroughly the fatty acid desaturase gene family in rice. We identified several tissue-specific, abiotic stress and hormone-responsive desaturase genes and analyzed their chromosomal locations, gene structures and phylogeny. Tandem and segmental duplications of desaturase genes and the presence of various cis-regulatory elements in the promoter were also analyzed. The evidence of altered expression of desaturase genes in response to hormone and stress may explain their role for developmental processes and drought, salt or cold stress tolerance. However, their true functions in growth, development and stress tolerance require more experimental confirmations, such as inspecting the phenotype of knock-out and over-expressing mutants.

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Author contributions
Z.E. and H.C. conceived the original screening and research plans; J.Y. and T.L. performed the experiments; C.C. and L.W. collected and analyzed the data; E.Z. drafted the manuscript with contributions of all the authors; H.C. and H.T. revised the manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare no competing interests.

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