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Biosynthesis of very long polyunsaturated Omega-3 and Omega-6 fatty acids in transgenic Japonica rice (Oryza sativa L)

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Abstract

In this research, the fatty acid pathway was modified to produce the C20 polyunsaturated fatty acid arachidonic acid (ARA) and eicosapentaenoic acid (EPA) by co-expression the genes encoding omega-3 fatty acid desaturase (*SK-FAD3*) from *Saccharomyces kluyveri*, Δ^9–elongase (*IgASE1*) from *Isochrysis galbana*, Δ^8-desaturase (*efd1*) from *Euglena gracilis* and Δ^5-desaturase from *Mortierella alpina* (*Mort*Δ^5) in *japonica* rice plant through *Agrobacterium tumafaciens* transformation method. The Southern hybridization analysis has confirmed the integration of these four transgenes and showed the presence of one copy number for each transgene in all five lines of T_0 transgenic rice (T_0-1, T_0-2, T_0-3, T_0-4 and T_0-5). The GC analysis showed the presence of new fatty acids eicosadienoic acid (EDA), eicosatrienoic acid (ETrA) and arachidonic (ARA) in transformed leaves of T_0 and T_1 compare to wild type. Further analysis of total fatty acid content in seeds showed that linolenic acids (LA) was increased at 1-2% in T_0-2, T_0-4 and T_0-5 while α-linolenic acid (ALA) content accumulated up to 0.2% in T_0-4 of transformed plant.

Keywords: *Agrobacterium tumefaciens*, omega-3, omega-6, *Oryza sativa* L., Transgenic plant, VLCPUFA.
Abbreviations: ALA_α-linolenic acid; ARA_ Arachidonic acid; BAP_Benzylaminopurine; 2,4-D_2,4-dichlorophenoxy acetic acid; DGLA_Dihomo-γ-linolenic acid; DHA_Docosahexaenoic acid; EDA_ ω6-eicosadienoic acid; EPA_Eicosapentaenoic acid; ETA_ Eicosatetraenoic acid; ETrA_ω3-eicosatrienoic acid; GUS_β-glucuronidase; Hyg_Hygromycin; LA_Linoleic acid; PTGS_ posttranscriptional gene silencing; VLCPUFA_Long-chain polyunsaturated fatty acid; NAA_α-Napthalene acetic acid.

Introduction

The very long chain polyunsaturated fatty acids (VLCPUFAs) such as arachidonic acid (ARA; C20:4n-6), eicosapentaenoic acid (EPA; C20:5n-3), and docosahexaenoic acid (DHA; C22: 6n-3) are important components of neuronal cells in brain and retina tissues and are considered to have profound effects on cell function and development and nutritionally important components for human health (Qi et al., 2004). Production of nutritionally enhanced foods via metabolic engineering has been showed to be feasible in oilseeds plant (Wu et al., 2005; Abbadi et al., 2004). Plant metabolic engineering would provide a novel and cost effective source of VLCPUFA fatty acids by altering desaturation/elangotaion pathways of n-6 and n-3 fatty acids. Alteration of desaturation/elongation pathways of n-6 and n-3 fatty acids in oilseeds resulted of increased fatty acids as reported by Wu et al., (2005). The beneficial of VLCPUFA are not only required for fetal neuronal system, components of membrane phospholipids in specific tissues or as precursors for the synthesis of the different groups of eicosanoid effectors (e.g., prostaglandins) but also reducing the incidence of cardiovascular diseases and Alzheimer's (Demaison and Moreau, 2002; Dolecek, 1992; Okuyama et al., 2007). At present, the sources of ALA are mainly deep-sea fish and specific oilseed plants (e.g., falx, soybean, rape, walnut and perilla). However, the deep-sea fish supply has been limited by heavy metal contamination and overfishing, while the yield of oilseed plants has been low (Yokoo et al. 2003). There are several pathways the biosynthesis of VLCPUFAs exists in nature (Qiu, 2003). The biosynthesis of VLCPUFA using desaturation/elongation pathways of n-6 and also n-3 fatty acids has been proven to enhance yields of fatty acids in *B. juncea* seeds (Wu et al., 2005). The major end product of ω6 pathways is arachidone (20:4n-6, ARA) while eicosapentaenoic acids (20:5n-3, EPA) and docosahexanoic acid (22:6n-3) in ω3 pathways. The ω6 pathway started with linoleic acid (18:2n-6), LA) and convert to α-linolenic by Δ6-desaturase enzyme followed by coversion to dihomo-γ-linolenic acid (20:3n-6, DGLA) by Δ6 elongase. Arachidone (ARA) is produced by DGLA by a Δ5desaturase. In ω3-pathways, EPA is produced from eicosatetraenoic acid (20:4n-3) that catalyze byΔ5-desaturase. The eicosatetraenoic acid is synthesized from stearidonic acid (STA) (18:4n-3) by the action of an elongase complex. The stearidonic acid is produced from α-linolenic acids (ALA) by Δ6 desaturase. Both pathways were interconnected by a ω3 desaturase that converts ARA to EPA or conversion of linolenic acids to α-linolenic. Further Δ5 elongation and Δ4 desaturations reactions lead to the

Table 1. GUS percentage of callus rice mediated by *Agrobacterium tumefaciens.*

Exp	Total no. of callus infected (A)	Hyg^R callus	GUS^+ callus	Hyg^R regenerated plant	GUS^+ plants (B)	Transformation efficiency (B/A, %)
1	126	11	3	3	3	2.38
2	131	12	2	2	2	1.52
3	125	7	5	4	4	3.20
4	119	13	3	3	3	2.52
5	92	13	4	4	4	4.35
6	102	8	3	3	3	2.94
Total	695	64	20	19	19	2.82

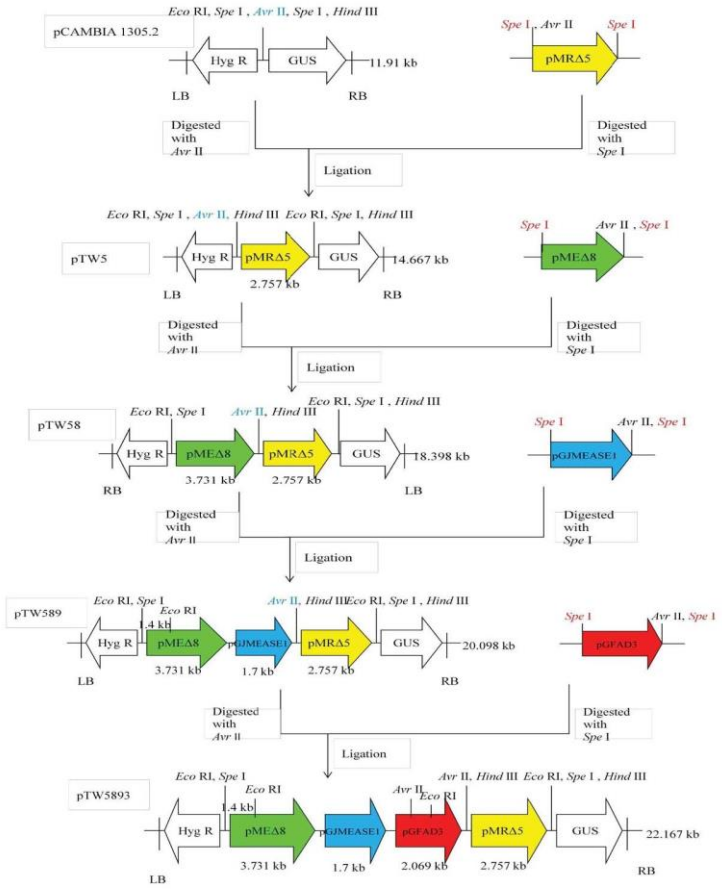

Fig 1. Schematic diagram of construction expression vector. pMEΔ8: expression cassette for *efd*1; pGJMEASE1: expression cassette for *IgASE*11; pGFAD3: expression cassette for *SK-FAD*3; pMΔ5: expression cassette for *Mort*Δ^5.

synthesis of docosatetraenoic acid (DPA) and finally DHA in these pathways. To date, the metabolic engineering of an artificial pathway has been reported in *Physcomitrella patens*, where a gene from the marine algae *Pavlova* sp., encoding D5-elongase, was used to activate the C_{22}-PUFA pathway to increase docosatetraenoic acid (DPA) or adrenic acid (ADA) and n-3 docosapentaenoic acid (DPA) (Chodok et al., 2012). Therefore, there is a considerable interest in obtaining a rice cultivar with high yield of VLCPUFA by fatty acid pathway modification. The present study demonstrated production of C20 polyunsaturated fatty acids, ARA and EPA by co-expression of the genes encoding omega-3 fatty acid (*SK-FAD3* desaturase), Δ^9–elongase, Δ^8-desaturase and Δ^5-desaturase. The present study demonstrates a single construct containing four genes driven by constitutive promoters resulted in the accumulation of fatty acids in transgenic putative rice plant.

Results and Discussions

Agrobacterium-mediated transformation of rice

The expression vector pCAMBIA1305.2 containing four genes was successfully constructed (Fig 1) and later transformed in *A. tumefaciens* EHA105. Embryogenic callus was proliferated at the skutellar region on LSD medium following 2 months of culture (Fig 2 A,B,C) and is used as starting materials for transformation. The present study shows that only 9.2% from 695 pieces of embryogenic callus used in 6 experiments showed hygromycin resistant callus (Table 1). Analysis of GUS activity of hygromycin resistant callus shows low percentage of GUS positive callus (2-5%). A total of 19 regenerate putative transgenic plants were obtained from hygromycin callus showed 100% of GUS activity. An average of transformation efficiency of all

Table 2. Total Fatty acid composition (% w/w) of leaves from wild type and 5 putative transgenic plants T_0 and T_1. Each value represents the mean ± standard error from three separate measurements of five putative transgenic plants.

Fatty Acids	Plants Source										
	Wild Type	T_0					T_1				
		0-1	0-2	0-3	0-4	0-5	1-1	1-2	1-3	1-4	1-5
C12:0	6.2±2.00	6.3±1.60	7.0±2.20	6.9±0.50	6.3±1.10	6.21±2.16	0.53±0.86	7.05±2.20	6.44±1.95	4.27±0.22	5.62±0.77
C13:0	0.83±0.10	0.8±0.05	1.1±0.20	0.8±0.05	0.69±0.07	0.75±0.10	0.8±0.02	1.07±0.22	0.75±0.10	0.78±0.08	0.9±0.10
C14:0	0.4±0.04	0.5±0.20	0.25±0.05	0.31±0.05	0.67±0.07	0.49±0.17	0.46±0.21	0.28±0.08	0.46±0.20	0.44±0.12	0.27±0.02
C16:0	16.2±0.90	17.7±1.80	18.1±1.80	15.9±0.40	17.5±1.30	18.9±1.50	17.4±1.90	18.1±1.77	18.3±2.30	18.4±2.17	15.9±0.39
C16:1Δ^{7}	2.1±0.10	2.5±0.10	1.9±0.30	2.6±0.30	2.57±0.30	2.29±0.24	2.65±0.45	1.86±0.25	2.29±0.24	2.39±0.15	2.6±0.50
C18:0	5.3±2.10	6.0±3.00	4.5±0.20	3.6±1.00	6.9±3.90	9.56±1.68	5.88±3.30	4.4±0.18	7.54±3.70	5.95±2.90	3.68±1.07
C18:1Δ^{9}	.9±0.40	2.3±0.30	3.6±0.10	2.0±0.10	2.3±0.80	2.84±1.07	2.69±0.82	2.75±0.85	2.85±1.08	3.49±0.43	2.73±0.86
C18:2$\Delta^{9,12}$(LA)	12.1±0.90	10.8±0.50	10.5±1.2	10.3±1.50	10.7±1.70	8.76±0.60	10.5±1.30	10.3±1.41	9.04±0.27	9.47±1.27	10.5±1.30
C18:3$\Delta^{9,12,15}$ (ALA)	43.8±3.10	43.5±2.90	41.7±5.90	48.9±0.80	43.5±3.00	37.1±2.20	45.7±3.40	47.3±0.83	40.7±1.41	37.7±2.85	47.7±0.54
C20:2$\Delta^{11,14}$(EDA)		0.2±0.10	0.15±0.05	0.13±0.03	0.12±0.02	-	0.21±0.01	0.22±0.12	-	-	0.16±0.02
C20:3$\Delta^{11,14,17}$(ETrA)		0.1±0.05	0.35±0.05	0.16±0.09	0.14±0.03	0.11±0.02	0.2±0.12	0.37±0.03	0.22±0.05	0.25±0.05	0.34±0.02
C20:4$\Delta^{5,8,11,14}$(ARA)		0.8±0.10	0.32±0.01	0.63±0.20	0.65±0.25	0.29±0.05	0.71±0.27	0.84±0.07	0.36±0.07	0.56±0.20	0.6±0.26

Table 3. Total Fatty acid composition (% w/w) of transgenic seeds from five selected putative transgenic plants of T_0. Each value represents the mean ± standard error from three separate measurements of five putative transgenic plants.

Fatty acids	Plant Source, T_0					
	Wild type	1	2	3	4	5
C12:0	2.12±0.1	2.4±0.2	2.0±0.2	2.2±0.1	2.1±0.1	2.0±0.1
C13:0	2.08±0.1	2.3±0.1	1.8±0.2	2.8±0.2	2.0±0.1	1.9±0.2
C14:0	1.2±0.1	1.2±0.1	1.0±0.1	1.3±0.1	1.0±0.1	1.1±0.1
C16:0	18.2±0.93	18.5±0.5	18.2±0.2	18.4±0.3	19.2±0.2	18.1±0.1
C16:1Δ^{7}	0.17±0.2	0.16±0.2	-	0.16±0.1	0.13±0.2	0.17±0.1
C18:0	3.2±0.2	2.1±0.2	1.7±0.1	2.1±0.1	2.0±0.2	1.9±0.1
C18:1Δ^{9}	29.2±0.1	30.7±0.2	32.5±0.4	29.7±0.3	29.1±0.4	31.3±0.3
C18:2$\Delta^{9,12}$(LA)	37.1±0.9	36.6±0.0.3	38.1±0.7	36.9±0.2	38.3±0.4	39.5±0.2
C18:3$\Delta^{9,12,15}$ (ALA)	1.3±0.1	1.4±0.1	1.4±0.2	1.45±0.1	1.5±0.2	1.4±0.1

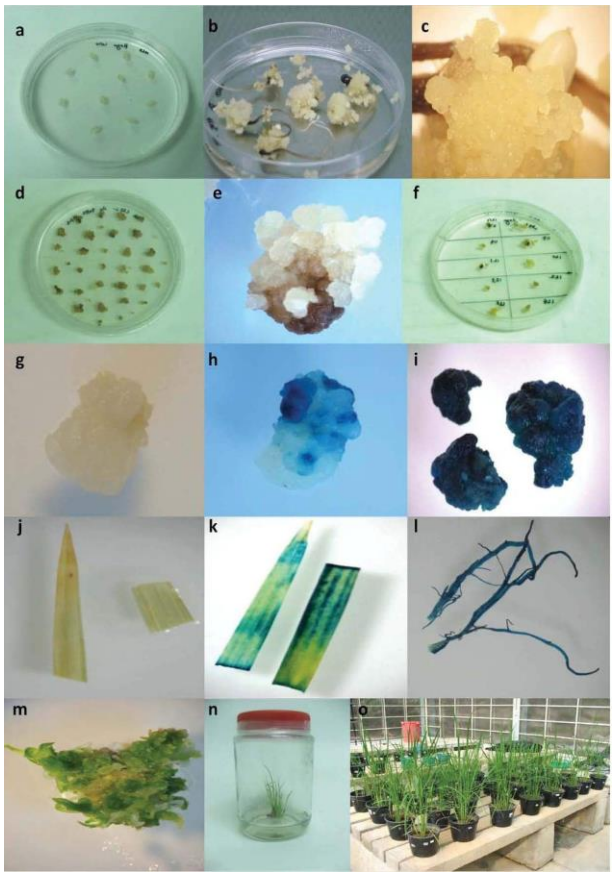

Fig 2. Histochemical GUS assay of callus and regeneration of transgenic rice. (a,b,c) Callus proliferated on LSD media. (d) *Agrobacterium* infected callus during co cultivation. (e,f) Hygromycin-resistant callus that proliferated after 4 weeks in selection media. (g) Negative control of GUS assay. (h) GUS positive callus after 5 days of infection. (i) Blue spot on hygromycin-resistant callus. (j) GUS assay of negative control (non-transformed) leaf. (k, l): GUS positive in transgenic leaf and root respectively. (m) Shooting of hygromycin-resistant callus on MSRg media. (n) *in vitro* plantet regenerated from hygromycin-resistant callus. (l) Putative transgenic rice plant in the greenhouse.

experiment conducted is 2.7%. Blue color were not observed on non-transformed callus (Fig 2G) while evenly distributed blue color was observed on transformed callus (Fig 2H) after five days of co-cultivation. The hygromycin-resistant callus proliferation was white to yellowish in color (Fig 2D,E,F) and even distribution of GUS expression was observed on hygromycin resistant callus (Fig 2I). After four cycles of selection procedure, the callus was transferred to the regeneration media. Further analysis of GUS assay on leaves and roots section showed GUS expression in comparison to non-transformed plant (Fig 2J,K,L). Regenerated resistant callus was maintained and allowed to grow *in vitro* (Fig 2M & 2N) before acclimatization. All the surviving putative transformed rice plants shows normal characteristic (Fig 2O) and maintained in greenhouse for further analysis. Low percentage of transformation efficiency in this present study may be because of multiple gene that may contributed to gene silencing phenomenon which not directly associated to copy number but with some epigenetic which lead to block the transcription or inhibition of mRNA accumulation (Halpin et al., 2001). Genes silenced by posttranscriptional gene silencing (PTGS) tend to be reactivated upon meiosis

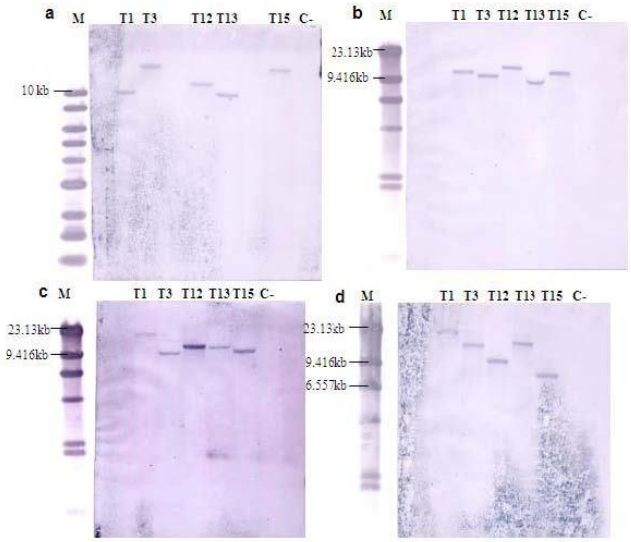

Fig 3. Southern-blotting hybridization pattern of DNA prepared from putative transformed plants, line T1, T3, T12, T12 and T15. (a) Genomic DNA digested with *Hind* III and hybridized with *IgASE*1 probe. (b) Genomic DNA digested with *Xho* I and hybridized with *SK-FAD*3 probe. (c) Genomic DNA digested with *Avr* II and hybridized with *efd*1 probe. (d) Genomic DNA digested with *Avr* II and hybridized with *Mort*Δ^5 probe. Lane C- : Control line (non-transformed rice plant).

(Dehio and Schell, 1994; Balandin and Castresana, 1997), and then re-silenced later in development. Furthermore several reasons have been highlight that lead to low transformation efficiency in rice transformation system via *Agrobacterium tumefaciens* (Tie et al., 2012).

Southern blot, RT-PCR and fatty acid composition

Five selected putative transgenic plants designated as T_0-1, T_0-2, T_0-3, T_0-4 and T_0-5 were subjected to Southern blot hybridization. Southern hybridization showed that the genome was successfully integrated with a single copy of the expression cassette (Fig 3). Further analysis of successful transformation and expression of these four genes was conducted in the developing seeds by RT-PCR. The results of RT-PCR showed that the expression of ω3-desaturase gene (*SK-FAD3*), Δ^9-elongase gene (*IgASE1*), Δ^8-desaturase gene (*efd1*) and Δ^5-desaturase gene (*Mort*Δ^5) in five selected putative transgenic plants line of T_0-1, T_0-2, T_0-3, T_0-4 and T_0-5 (Fig 4). The expected fragment size of the four genes by RT-PCR analysis were 1260 bp for *SK-FAD*3, 792 bp for *IgASE*1, 1260 bp for *efd*1 and 1341 bp for *Mort*Δ^5. The RT-PCR was also carried out in T_1 putative transgenic plants and showed the same results as generation T_0 (data not shown) that exhibited the transcript expression. Five selective putative transgenic plants designated as T_0-1, T_0-2, T_0-3, T_0-4 and T_0-5 was used for analysis of fatty acid composition in leaves and seed. The GC analysis results showed several new peaks were detected such as eicosadienoic acid (EDA; C20:2$\Delta^{11,14}$), dihomo-γ-linolenic acid (DGLA; C20:3$\Delta^{11,14,17}$) and arachidonic acid (ARA; C20:4$\Delta^{5,8,11,14}$) in the transgenic leaves rice (Table 2; Fig 2b). The fatty acids anaylisis of the recombinant putative selective seed are shown in Table 3. The results showed palmitic acids (PA; C16:0) was increase to 1% in putative transformed seeds samples of T_0-4 while

remain the same with other samples. The total fatty acids of linolenic acids (LA; C18:2) was increased at 2% in T_0-2 and T_0-4 while 2% in T_0-5 compare to wild type. The α-linolenic acid (ALA) content accumulated up to 0.1%. of total fatty acids in transformed seeds of T_0-1, T_0-2, T_0-4 and T_0-5 while 0.2% in T_0-3. The results showed increasing copy number of expression cassette, the contents of LA and ALA increased; however the pattern was not similar in the sample tested. Other reports show that ALA content is increased 2.5 times and 13-fold when transformed with heterologous microsomal genes driven by CaMV35S and Ubi-1 promoter, respectively (Shimada et al., 2000; Anai et al., 2003) but not in the present study. Therefore, it is suggested that CaMV35S constitutive expression promoters are not sufficiently strong in rice seed, as has also been reported by others (Qu and Takaiwa 2004). Based on this finding and other previous analysis, it is suggested that the use of a strong endosperm-specific promoter might help to increase the accumulation of ALA in rice endosperm. To date, rice transformed with *FAD* genes driven by the endosperm-specific promoter GluC have shown ALA content increase by up to 27.9-fold compared with non-transformants (Liu et al., 2012). Puttick et al. (2009) also reported that the ALA content of *Arabidopsis* seed was increased from 19% to nearly 40% of total fatty acids by seed-specific promoters. Preliminary finding by Wu et al., (2005) shows that constitutive expression of the 6 elongase pathways in *B. juncea* resulted in higher EPA levels in leaves compared to seed. In line with this, the present study found that the use of constitutive promoter do not change the content of fatty acids in putative transgenic seed. These results indicate the following; (1) the promoters used unable to give sufficient expression in rice seed; (2) to obtain high proportions C20 PUFA, the host plants should have high endogenous levels of linolenic and/or α-linolenic acids in the wild-type seed, (3) the plant enzymes (algal elongase and algal desaturase) seem to increase the ARA contents. Other researchers have also found that an increase in the copy number of desaturase and elongase genes in *Pichia pastoris* results in an increase of the total fatty acid levels compared to a single copy number (Li et al. 2009). The present study demonstrated that DHA synthesize in seeds, albeit at low levels, is a basis for further optimization to attain commercially viable levels, as has been demonstrated here for ARA.

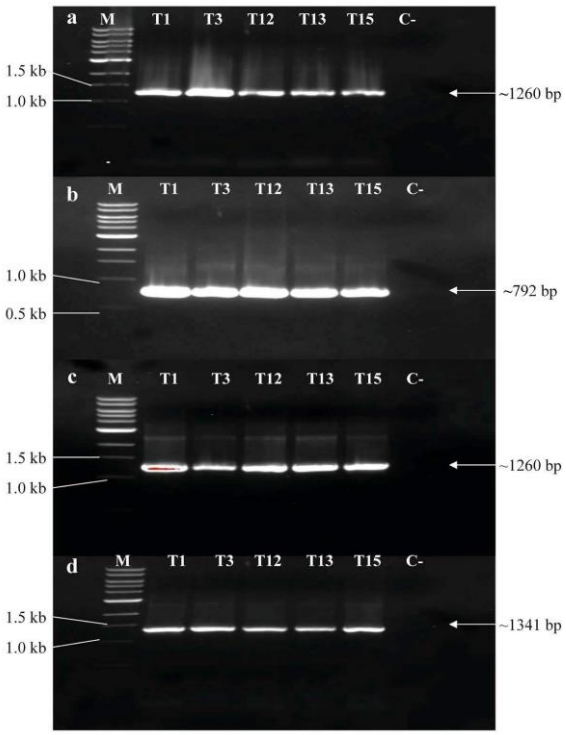

Fig 4. Result of RT-PCR from 5 selected putative transgenic plants line T1, T3, T12, T13 and T15. (a) *SK-FAD3* (b) *IgASE*1. (c) *efd1*. (d)*Mort*Δ^5. Lane C- : negative control (non-transformed)

Materials and Methods

Vector construction and Agrobacterium-mediated transformation

The binary vectors pCAMBIA1305.2 (CAMBIA, Canberra, Australia) was used as final expression vector with *nptII* gene as selectable marker and ligate with four cassette containing the coding sequence of ω3-desaturase (*SK-FAD3*), Δ^9-elongase (*IgASE1*), Δ^8-desaturase (*efd1*) and Δ^5-desaturase (*Mort*Δ^5). The cassette were names as pGFAD3 containing ω3-desaturase gene driven by modified CaMV35S promoter; pGJMEASE1 containing Δ^9-elongase gene driven by modified CaMV35S promoter; pMEΔ8 containing Δ^8-desaturase (*efd1*) driven by ubiquitin promoter and pMΔ5 cassette containing Δ^5-desaturase controlled by CaMV35S promoter. The resulting four cassettes were digested with *Avr*II and *Spe*I and ligated into binary vector of pCAMBIA 1305.2 (Fig 1; Table 1: Supplementary Data (The origin and accession number of gene). The orientation of the plasmid was confirmed by digestion. The expression vector containing pCAMBIA1305.2 and all four cassettes designated as pTW5893 were transferred into *A. tumefaciens* strain EHA 105 via heat shock method. For plant transformation procedure, two months old embryogenic calli from mature seeds of japonica rice var. Taipei 309 were used as explants. The embryogenic rice calli were immersed in suspension bacteria for three min before being allowed to stand for 40 min. After infection, calli were blotted dry on sterile tissues paper and placed on LSC medium. The culture was later incubated at 25°C for five days in the dark before being transferred into LSW media. The infected calli on LSW media were then placed on selection LSS1 media for two weeks before being transferred on LSS2 media containing 50 mgL^{-1} of hygromycin. The proliferated hygromycin-resistant calli were excised and cultured on the LSS2 media for 2 cycles before being transferred onto MSRg and incubated at 25 °C for two weeks in the dark. When the plant reached a height of five cm, the *in vitro* plant were subculture to MSRt media for rooting induction. The surviving plants were transferred to soil in pots and grown in the greenhouse before histochemical GUS assay was conducted. The GUS assay was conducted according to Jefferson (1997). Each petri dish contained 10 explants and all experiments for transformation procedures were performed six times with three replicates. All the composition media used in the present study was shown in Table 2 (Supplementary Data).

Identification of transgenic plants

Total DNA were extracted from rice leaves according to the SDS methods (Shah et al., 1994) with minor modifications for Southern Blot analysis. Aliquots (25 µg) of genomic DNA were digested with *Hind*III, *Xho*I or *Avr*I and

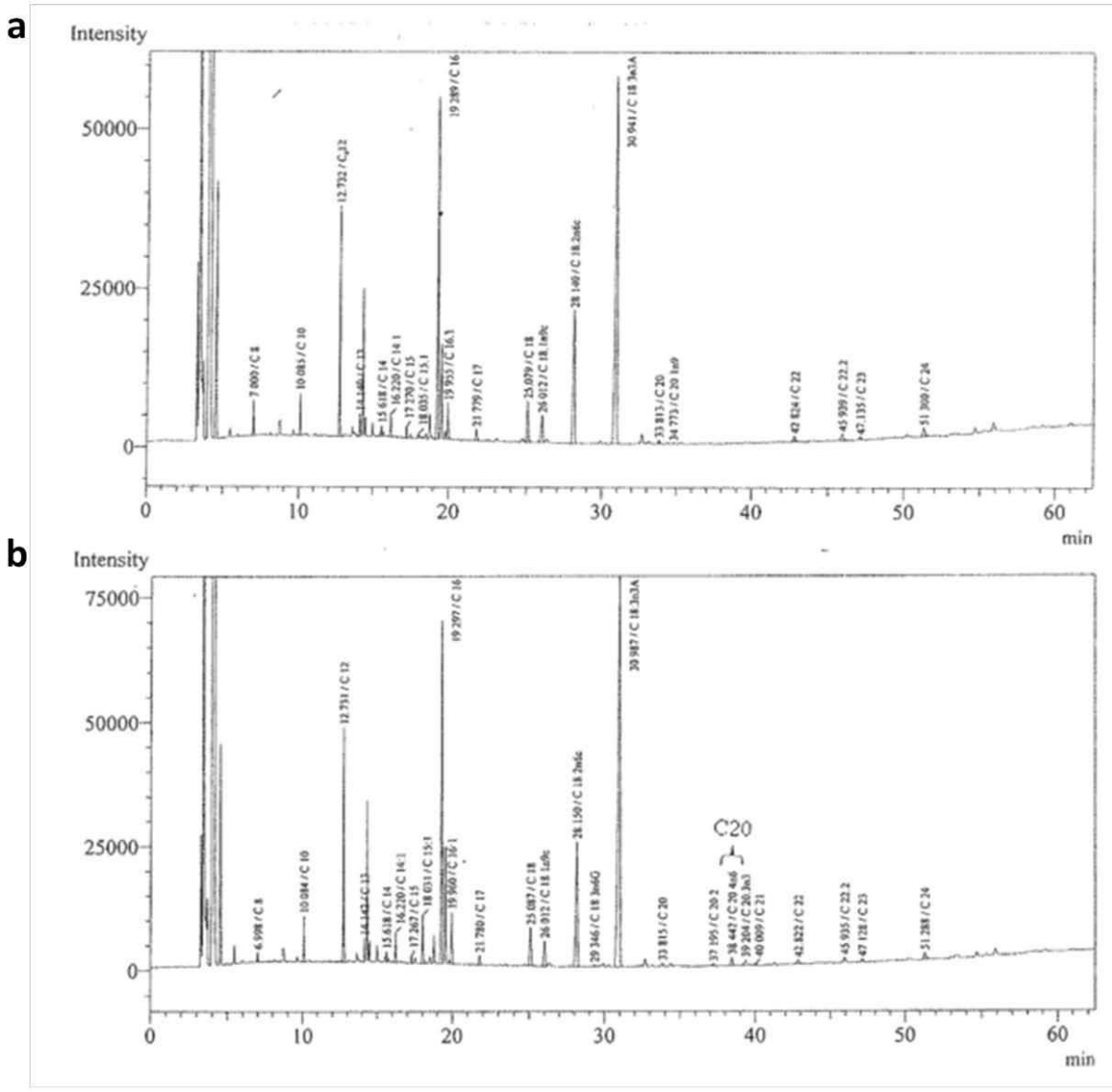

Fig 5. GC analysis of fatty acid from putative transgenic leaf rice (a) while non-transformed leaves (b).

fractionated on a 0.8% (w/v) agarose gel. Following the electrophoresis, the fractionated DNA was transferred to a nylon membrane (Hybond N^+, Amersham, UK) according to the manufacturer's instruction. The probes were prepared from PCR containing full-length coding region of the *SK-FAD*3, *IgASE*1, *efd*1 and *Mort*Δ^5. The probes were subsequently labeled with digoxigenin-dUTP from Random Primed DNA Labeling Kits (Roche Applied Science, USA) and hybridized, washed and detected according to the manufacturer's protocol. For RT-PCR analysis, total RNA from leaves was extracted using TRI Reagent (Sigma). The first cDNA strand was synthesized from 5 µg of total RNA using SuperScript III Reverse Transcriptase (Invitrogen, USA) with oligo$(dT)_{12-18}$ (Invitrogen, USA). The reaction mixture (25 µL) for RT-PCR consisted of 1 µL of template of cDNA, 1.0 µM forward and reverse primers, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 1X PCR buffer, 0.2 U Taq DNA Polymerase (1U/µL) (Promega, USA). The program for PCR was Thermal cycling for 45 sec at 94 °C, 30 sec at 58 °C and 2 min at 72 °C, performed for 30 cycles. Forward and reverse primers, respectively, in RT-PCR were: 5' Forward ATG TCT ATT GAA ACA GTC GGA TCA TCG 3'; 5' Reverse TCA TTG ACT GGA ACC ATC TTC CGG C-3' (*SK-FAD*3); 5' Forward ATG GCC CTC GCA AAC GAC GCG GGA G3' and 5' Reverse CTA GAG CTG CTT GCC CGC CTT GGC C3' (*IgASE*1); 5' Forward ATG AAG TCA AAG CGC CAA GCG CT3' and 5' Reverse TTA TAG AGC CTT CCC CGC GGG TTG CT3' (*efd*1); 5' Forward ATG GGT ACG GAC CAA GGA AAA ACC T-3' and 5' Reverse CTA CTC TTC CTT GGG ACG GAG TCC A-3' (*Mort*Δ^5).

Fatty acid extraction and analysis

Fatty acid extraction was conducted according to Kanchanamayoon and Kanenil (2007). 0.5 gram of dried transgenic leaves or seed were mixed with five mL of toluene and five mL of fresh solution of methanolic hydrochloric (methanol: hydrochloric acid; 5:95 v/v) and incubated in a water bath at 70 °C for two hours. Next, five mL of 6 % potassium carbonate solution and 1 mL of toluene were added and vortexed for 1 min. The organic phase was separated by centrifugation at 2500 rpm for 5 min. The organic phase was dried with anhydrous sodium sulfate and filtered by a 0.45 µm millipore. A volume of 1-µL aliquot was subjected to gas chromatography. The standard FAME (Fatty Acid Methyl Ester) (gred GC) was the SUPELCO 37 Component FAME Mix, and acid heptadecanoic (C17:0) was used as an internal standard. The fatty acids were separated by a gas chromatography system (Shimadzu) equipped with DB23-cis/trans fused with silica capillary column (30 m x 0.32 mm I.D., 0.25-µm thickness) and detected with a flame ionization detector. The mass spectrum of new peak was compared with that of standard for identification of fatty acids.

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Conclusion

The present study shows putative transgenic rice plants expressed the target gene under one cassette with the control of constitutive promoter. The new fatty acid acids (eicosadienoic acid (EDA), eicosatrienoic acid (ETrA) and arachidonic, ARA) were detected in transformed leaves rice but none in transformed rice seed. Therefore, future study needs to explore the mechanism and regulation of the target gene controlled by specific promoters.

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