Indian Journal of Experimental Biology
Vol. 45, April 2007, pp. 390-397

Isolation and characterization of microsomal ω-6-desaturase gene (*fad2-1*) from soybean

K Kishore, S K Sinha, R Kumar, N C Gupta, N Dubey & A Sachdev*
Division of Biochemistry, Indian Agricultural Research Institute, New Delhi 110 012, India

Received 19 June 2006; revised 8 December 2006

A genomic DNA sequence (*fad2-1*) encoding seed specific microsomal ω-6 desaturase was isolated from soybean (*Glycine max. L* cv. Pusa – 9702). A positive genomic clone of 1852 nucleotides containing a single uninterrupted 3′ end exonic region with an ORF of 1140 bp encoding a peptide of 379 amino acids, a complete 3′ UTR of 206 bp and 86 bp of 5′ UTR interrupted by a single intron of 420 bp was obtained on screening the sub-genomic library of soybean. Southern blots revealed at least two copies of the gene per haploid genome. Analysis of the translated product showed the presence of three histidine boxes, with the general sequence HXXXH and five probable transmembrane segments reported to be involved in substrate specificity.

Keywords: Exon, *fad 2-1*, Genomic clone, Histidine box, Intron, Microsomal ω-6 desaturase gene, Soybean,

An important determinant of usefulness and quality of edible plant oils is their fatty acid composition. High levels of both cholesterol and saturated fatty acids in the diet increase the risk of cardiovascular diseases therefore there has been a reduction in the consumption of animal fats. The ready availability of predominantly unsaturated, cholesterol-free vegetable oils from a wide range of oilseed crops (soybean, sunflower, rapeseed, cottonseed, peanuts etc) has facilitated the move away from animal fats. Most of these oils have levels of polyunsaturated fatty acids that significantly exceed that of palmitic acid and are highly recommended in human diet[1]. Unfortunately, these highly unsaturated oils are too unstable when exposed to high temperatures and oxidative conditions for long periods of time.

Soyoil, the major component of soybean seed, is a complex mixture of five fatty acids[2] ; palmitic (11%), stearic (4%), oleic (23%), linoleic (55%), and linolenic (7%). It is used in a wide variety of food applications due to its nutritional qualities, economic values and abundancy. One of the major constraints associated with its versatility is its oxidative instability, which refers to the susceptibility of oil to oxidative reactions during storage and processing[3]. Much of the interest in altering the fatty acid composition of soyoil is centered on reducing the levels of polyunsaturated fatty acids (PUFA) contributing to the instability and increasing the precursor monounsaturated fatty acid (MUFA), thereby producing oils high in MUFA which are particularily more stable in high temperature food frying and have improved nutritional value[4]. The chemical solution to enhance stability i.e selective hydrogenation, greatly decreases the content of PUFA and increases the relative abundance of MUFA but at the same time produces substantial amounts of transisomers and these trans isomers can constitute upto 40% of the fatty acid content of commercially prepared foods[5]. Their consumption has been linked to unfavourable plasma lipoprotein profile and coronary heart diseases[6]. Traditional plant breeding/mutational breeding approaches too have resulted in limited success rates in developing the required fatty acid composition mainly because of environmental instability of the resulting phenotypes and the lack of natural genetic variation[4,1].

In soybean seeds, most of the triacylglycerol PUFAs (linoleic and linolenic) are derived from the activity of ω-6 desaturase that is encoded by the seed specific *fad2-1* gene, only a small amount (~5%) is derived from a separate pathway in the plastid and a negligible amount from the activity of a second ω-6 desaturase that is encoded by the constitutive *fad2-2* gene[7]. Since the seed specific expression of *fad2-1* plays a major role in the conversion of oleic to linoleic acid within storage lipids during seed

*Correspondent author
Email: arcs_bio@yahoo.com

development, the most effective way, therefore, of reducing PUFA content in soybean oil is by suppressing the expression of *fad2-1* by gene silencing technologies[8]. To achieve this ultimate goal it is necessary to isolate and characterize *fad2-1* gene for further genetic manipulation. In the present study we have reported the isolation of *fad2-1* gene encoding microsomal ω-6-desaturase in soybean (*cv. Pusa-9702*) and its characterization.

Materials and Methods

Mature seeds of soybean *cv. Pusa-9702* were collected from the farm at I.A.R.I, New Delhi. Plant DNA was isolated by CTAB method[9] from 6-7 days old etiolated seedlings of soybean raised under controlled conditions in the Phytotron facility, I.A.R.I., New Delhi and the purified DNA was restricted with *Eco*RI, *Bam*HI, and *Hind*III. Electrophoresis was carried on 0.8% agarose gel and for Southern blotting the gel was soaked in NaOH (0.5*M*), NaCl (1.5*M*) for 30 min before blotting onto a nylon membrane (Amersham). The filters were then probed with [α-^{32}P]-dCTP labelled *fad2-1* specific probe[10] *pGM* (~1.2kb) generated by PCR amplification of soybean DNA using gene specific oligo primers (forward primer : 5′ TTTCTACATTGCCACCACC 3’ and reverse primer : 5′ GCAGAAAGCTATAAGCAGAT 5′). The forward and reverse primers were designed from the conserved region of cDNA sequence data of soybean *fad2-1* available in NCBI Genbank. Hybridization was carried out at 65°C over night in 6XSSC(Saline Sodium Citrate) 0.5% SDS (Sodium Dodecyl Sulphate), 5X Denhardt’s solution and 100 µg/ml denatured Salmon sperm DNA. The membrane was washed thrice with 2XSSC and 0.1%SDS for 5 min each at room temperature with constant vigorous shaking followed by another set of two washings with 0.2XSSC and 0.1% SDS for 30 min each at 50°C. The DNA from the region corresponding to hybridized fragment in *Hin*dIII lane was recovered by phenol-chloroform extraction method and cloned into phagemid vector SK^{+} at *Hind*III site. Plasmids were transformed into *E. coli* (DH5α) in the presence of IPTG and X-Gal. Transformed colonies (113) were picked up and grown over night in LB medium containing ampicillin. Colony hybridization of the recombinant clones was carried out with the probe *pGM*. Ten putative clones showing strong hybridization signals were taken up for restriction analysis followed by Southern hybridization with [α-^{32}P]-dCTP labelled *pGM* probe to identify the positive clones.

Nucleotide sequences of the positive clones were determined using automated sequencing facility at the University of Delhi, South Campus with T_7 and T_3 promoters as forward and reverse primers. Sequence analysis was done using various software programs.

Results and Discussion

Genomic Southern analysis with *fad2-1* specific probe *pGM* (Fig.1) showed two distinct bands each in case of *Eco*RI and *Hind*III restricted genomic DNA. The approximate size of bands was 4 and 2 kb in case of *Eco*RI and 4 and 3 kb in case of *Hind*III digested DNA. The results suggested at least two copies of gene (*fad*2-1) in the soybean genome. Sub-genomic library of genomic DNA region corresponding to the hybridizing band positions in the *Hind* III digested lane, was prepared in SK+ phagemid vector. On blue white screening of the recombinants, 113 white colonies were picked for analysis. Colony hybridization of the white colonies with probe *pGM* revealed strong hybridization signals in only ten. These colonies were identified and grown overnight in LB containing ampicilin (100µg/ml) and their plasmids excised. Restriction pattern is shown in Fig. 2. Southern hybridization of the *Hind*III restricted clones with *pGM* revealed only two positives (Fig. 3) with insert size of 1.9 kb each reflecting non-specific nature of the other clones. For further confirmation,

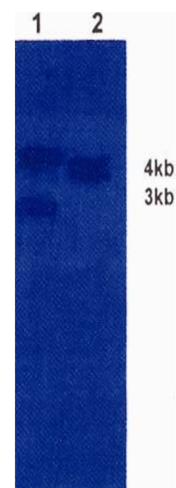

Fig. 1—Southern blot analysis of soybean genomic DNA. (Lane 1-DNA sample digested with *Eco*RI and probed with *fad2-1* specific fragment *pGM*, Lane-2 DNA sample digested with *Hind*III and probed with *pGM.*

PCR of these two clones was carried out with the same set of primers as used for probe generation. A single band of ~1.2 kb was amplified in both the cases (Fig. 4) confirming the presence of probe sequence. One of these, clones named as *pGMFAD2-1* was taken up for further analysis.

The sequence data of *pGMFAD2-1* revealed a total length of 1852 bp (Fig. 5). The *pGMFAD2-1* sequence was submitted to the NCBI nucleotide sequence database using Bankit (Accession number AY954300)[11]. The overall G+C content of the sequence was 42% and the per cent G+C content of the coding region was 46%. This is in accordance with the characteristics of eukaryotic nature of genes having G+C content always >40 per cent. The open reading frame of *pGMFAD2-1* encoded a single uninterrupted exon of 1140 bp towards 3′ end and an intron of 420 bp, between 65 and 66 nucleotides of the 5′ UTR. The sequence also contained the complete 3′ UTR of 206 bp and 86 bp of 5′ UTR. This finding is in line with single uninterrupted exons for desaturase genes reported in other crops. In sunflower a full length cDNA clone of 1492 bp was reported[12] for Δ-12 oleate desaturase gene (OLD-7). It had an ORF of 1137 bp from 122 to 1258 bp encoding 378 amino acids, a 121 bp 5′ UTR and 234 bp 3′ untranslated region. Analysis of genomic sequence amplified by PCR revealed no introns in the sunflower OLD-7 coding region. A 1372 bp cDNA clone pFb2 containing an ORF encoding a predicted protein of 383 amino acids has been reported[13] in *Arabidopsis*. Using this probe, they have screened the *Arabidopsis* genomic library and sequencing of a 3 kb genomic clone revealed the presence of a single intron of 1134 bp between nucleotides 88 and 89 of the cDNA. In peanut (*Arachis hypogaea*) Δ^{12} -*FAD* gene, a single intron of 1564 bp within the entire coding region at the 3' end has been revealed[14]. A single large intron (1133 bp) has also been reported in the 5′ UTR, located 9 bp upstream from the putative translation start site in *Gossypium* omega-6-desaturase gene *(ghFAD2-1)*[15]. Preliminary examination of the *ghFAD2-1* gene from five species (*G. arboreum, G. barbadense, G. hirsutum, G. raimondii* and *G. robinsonii*) reveals that the size and position of the introns are conserved.

BLASTN, similarity search of *pGMFAD2-1* sequence shows 99% similarity of its exonic region with *Glycine max FAD2-1* microsomal ω-6-desaturase mRNA sequence[7]. The intron too shows 99% similarity with *Glycine max FAD2* gene intron (Acc. No. AJ271842.1) sequence available in GenBank. Significant nucleotide sequence homology with omega-6-desaturase genes from other plant species namely *Arachis hypogaea*, *Cucurbita pepo*, *Sesamum indicum*, *Nicotiana tabacum Gossypium hirsutum* etc was also revealed (Table 1).

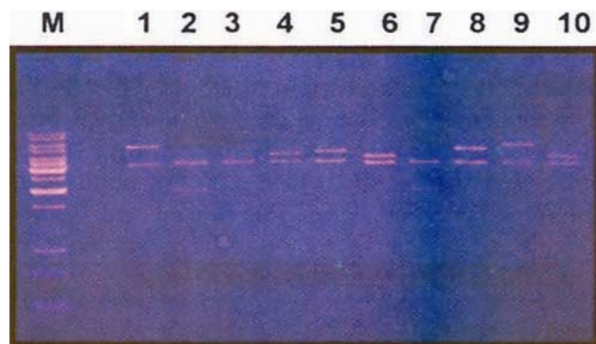

Fig. 2—Agarose gel (0.8%) showing restriction analysis of the subgenomic clones with *Hind*III (Lane 1-10)

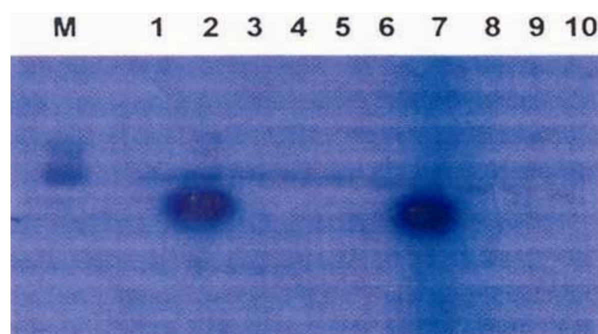

Fig. 3—Autoradiogram showing Southern blot hybridization pattern of the putative clones with probe *pGM*

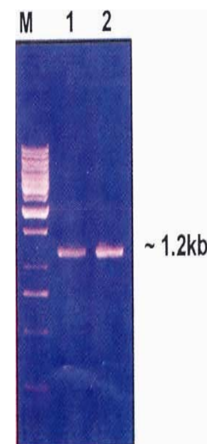

Fig. 4—1.2% agarose gel showing PCR amplified putative sub-genomic clones no. 2 (Lane 1) and 7 (Lane 2); 1kb DNA ladder (Lane M)

Table 1— Similarity data of microsomal omega-6-desaturas gene (*fad2*-1 in genomic clone *pGMFAD2*-1 with microsomal omega-6-desaturase genes of other species, as obtained in BLASTN homology search

Accession No.	Plant Species	Gene	% Similarity
gb L43920.1	*Glycine max*	Microsomal omega-6 desaturase	99
emb AJ271842.1	*Glycine max*	Microsomal omega-6 desaturase, intron 1	99
gb AF272950.1	*Arachis hypogaea*	Microsomal oleate desaturase	83
gb AY525163.1	*Cucurbita pepo*	Omega-6 fatty acid desaturase	86
gb AF192486.1	*Sesamum indicum*	Omega-6 fatty acid desaturase	81
gb AY660024.2.	*Nicotiana tabacum*	Microsomal omega-6 fatty acid desaturase	82
emb Y10112.2	*Gossipium hirsutum*	Omega- 6 desaturase	83
dbj AB094415.1	*Spinacia oleracea*	Delta-12 desaturase	82

5′
TATTGATAGCCCCTCCGTTCCCAAGAGTATAAAACTGCATCGAATAATACAAGCCACTAGGC
ATG**GTAAATTAAATTGTGCCTGCACCTCGGGATATTTCATGTGGGGTTCATCATATTTGTT**
GAGGAAAAGAAACTCCCGAAATTGAATTATGCATTTATATATCCTTTTTCATTTCTAGATTT
CCTGAAGGCTTAGGTGTAGGCACCTAGCTAGTAGCTACAATATCAGCACTTCTCTCTATTG
ATAAACAATTGGCTGTAATGCCGCAGTAGAGGACGATCACAACATTTCGTGCTGGTTACT
TTTTGTTTTATGGTCATGATTTCACTCTCTCTAATCTCTCCATTCATTTTGTAGTTGTCATTAT
CTTTAGATTTTTCACTACCTGGTTTAAAATTGAGGGATTGTAGTTCGGTTGGTACATATTAC
ACATTCAGCAAAACAACTGAAACTCAACTGAACTTGTTTATACTTTGACACAGGGTCTAGCA
AAGGAAACAACA**atg***GGAGGTAGAGGTCGTGTGGCCAAAGTGGAAGTTCAACGGAAGAAGC*
CTCTCTCAAGGGTTCCAAACACAAAGCCACCATTCACTGTTGGCCAACTCAAGAAAGCAATT
CCACCACACTGCTTTCAGCGCTCCCTCCTCACTTCATTCTCCTATGTTGTTTATGACCTTTCA
TTTGCCTTCATTTTCTACATTGCCACCACCTACTTCCACCTCCTTCCTCAACCCTTTTCCCTC
ATTGCATGGCCAATCTATTGGGTTCTCCAAGGTTGCCTTCTCACTGGTGTGTGGGTGATTGC
TCACGAGTGTGGTCACCATGCCTTCAGCAAGTACCAATGGGTTGATGATGTTGTGGGTTTG
ACCCTTCACTCAACACTTTTAGTCCCTTATTTCTCATGGAAAATAAGCCATCGCCGCCATCA
CTCCAACACAGGTTCCCTTGACCGTGATGAAGTGTTTGTCCCAAAACCAAAATCCAAAGTTG
CATGGTTTTCCAAGTACTTAAACAACCCTCTAGGAAGGGCTGTTTCTCTTCTCGTCACACTC
ACAATAGGGTGGCCTATGTATTTAGCCTTCAATGTCTCTGGTAGACCCTATGATAGTTTTGC
AAGCCACTACCACCCTTATGCTCCCATATATTCTAACCGTGAGAGGCTTCTGATCTATGTCT
CTGATGTTGCTTTGTTTTCTGTGACTTACTCTCTCTACCGTGTTGCAACCCTGAAAGGGTTG
GTTTGGCTGCTATGTGTTTATGGGGTGCCTTTGCTCATTGTGAACGGTTTTCTTGTGACTAT
CACATATTTGCAGCACACACACTTTGCCTTGCCTCATTACGATTCATCAGAATGGGACTGGC
TGAAGGGAGCTTTGGCAACTATGGACAGAGATTATGGGATTCTGAACAAGGTGTTTCATCA
CATAACTGATACTCATGTGGCTCACCATCTCTTCTCTACAATGCCACACTACCATGCAATGG
AGGCAACCAATGCAATCAAGCCAATATTGGGTGAGTACTACCAATTTGATGACACACCATTT
TACAAGGCACTGTGGAGAGAAGCGAGAGAGTGCCTCTATGTGGAGCCAGATGAAGGAACA
*TCCGAGAAGGGCGTGTATTGGTACAGGAACAAGTAT***tga**TGGAGCAACCAATGGGCCATAGT
GGGAGTTATGGAAGTTTTGTCATGTATTAGTACATAATTAGTAGAATGTTATAAATAAGTGGA
TTTGCCGCGTAATGACTTTGTGTGTATTGTGAAACAGCTTGTTGCGATCATGGTTATAATGTA
AAAATAATTCTGGTATTAATTACATGTGGAAA **3′**

TOTAL LENGTH: 1852 bp BASE COUNT: 481 a 407 c 377 g 587 t

Fig. 5—Nucleotide sequence of microsomal omega-6-desaturase gene *pGMFAD2-1* isolated from soybean (exon-italics, 3′UTR, 5′UTR-underlined, intron- normal)

The primary structure of the 379 amino acid long peptide encoded by the 1140 nucleotides of the ORF of *pGMFAD2-1* was analysed using Protparam tool available at http://au.expasy.org. The analysis revealed a molecular weight of 43.9 kDa and a theoretical pI of 9.10. The total number of negatively charged residues (Asp+Glu) was 27 and positively charged residues (Arg+Lys) was 36, reflecting the "basic" nature of the protein at physiological *p*H. The gross amino acid composition of the protein showed high leucine (11.1%), valine (8.7%), tyrosine (7.4%) and serine (6.9%) content, whereas cysteine (1.3%), methionine (1.3%) and glutamine (2.1%) were least abundant. A number of putative serine (26), tyrosine (28) and threonine (23) phosphorylation sites were present in its structure.

The hydropathy plot of the protein analysed by TMHMM package available at http://cbs.dtu.ak/cgi revealed multiple hydrophobic domains. It was predicted that the protein had 5 probable transmembrane segments (amino acids; 51-73, 78-100, 170-192, 219-236, 243-265). They may be involved in substrate specificity[16].

The deduced amino acid sequence of *pGMFAD2-1* was aligned with *B. juncea* (CAA62578.1), *A. thaliana* (AAM61113.1), *N. tabacum* (AAT72296.1), *G. hirsutum* (CAA65744.1), *A. hypogaea* (AAY67653), *S. indicum* (AAF80560.1) and *H. annus*

(AAX19895), using Clustal W alignment. A high degree of identity was observed throughout the sequence and not concentrated in any particular region, within all the members. Similarity data of *pGMFAD2-1* of soybean and microsomal ω-6 desaturases of other plant species, as obtained in BLASTP search results has been shown in Table 2 .

A comparison of the *pGMFAD2-1* sequences showing phylogenetic relationship at the deduced amino acid level with those of other plant species is shown in dendrogram (Fig. 6). The microsomal ω-6-desaturase of *Glycine max* and *Arachis hypogaea* (peanut) were grouped in the same cluster revealing close evolutionary relationship as is also evident from the BLASTP and BLASTN analysis, reflecting 83% sequence similarity at both amino acid and nucleotide levels. Also *Brassica* and *Arabidopsis* were found to be much closely related to soybean evolutionarily as compared to *Nicotiana tabacum* (tobacco) and *Helianthus annus* (sunflower).

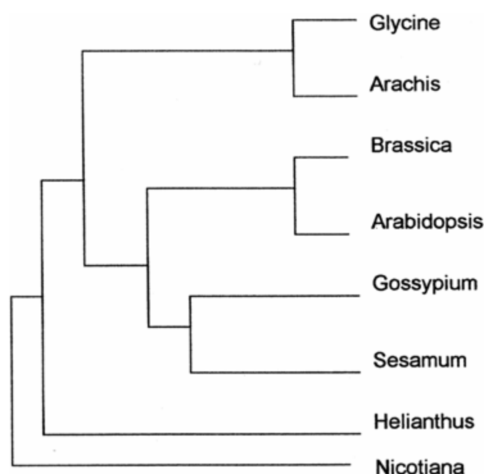

Fig. 6—Dendrogram showing relationship of the derived amino acid sequence of microsomal ω-6-desaturase of *Glycime max (pGMFAD2-1)* with various plant species.

One highly conserved feature of all membrane bound desaturases is the presence of three histidine boxes, with a general sequence HXXXH. These boxes may be involved in metal ion complexation required for oxygen reduction[17]. Fig. 7 shows the presence of the three histidine boxes in various plant desaturases aligned together. A tripartite histidine rich motif that is highly conserved in all membrane bound desaturases was identified between amino acids 101-106, 137-141 and 311-315 in *pGMFAD2-1* (*Glycine max*). The presence of three conserved histidine boxes in desaturases is important for the enzyme activity and specificity. Any alteration or mutation in these conserved regions may lead to reduction or loss of activity and specificity[18]. Examination of deduced amino acid sequences for the membrane desaturases from mammals, fungi, insects, higher plants, and cyanobacteria have also revealed three regions of conserved primary sequence containing HX(3 or 4)H, HX(2 or 3)HH, and HX(2 or 3)HH[19].

Restriction sites in the *fad2-1* sequence in *pGMFAD2-1* were analysed using NEB CUTTER available at http://tools.neb.com/NEBcutter2. Restriction pattern analysis revealed the absence of restriction sites for *Bam*HI, *Eco*RI and *Hind*III enzymes. Two of the enzymes were used to digest the genomic DNA for Southern analysis, which was carried out with *fad2-1* specific probe *(pGM)*. The autoradiogram showed the presence of two distinct hybridizing bands in case of *Eco*RI and *Hind*III indicating the presence of two copies of *fad2-1* which is consistent with the tetraploid nature of the soybean genome. This finding is well correlated with earlier findings[7,20]. Southern hybridization has been used to estimate the number of gene copies present for the cDNA clones in *Brassica napus*[21]. Based on the number of bands in the Southern blot, at least four homologous genomic DNA fragments for each of the cDNA clones in *B. napus*

Table2—Similarity data of microsomal omega-6-desaturase gene (*pGMFAD2-1*) of soybean with microsomal omega-6-desaturases of other species as obtained in BLASTP search result of coding sequence

Accession No	Plant Species	Gene	% Similarity
gb|AAB00859.1	*Glycine max*	Microsomal omega-6 desaturase	99
gb|AAY67653.1	*Arachis hypogae*	Oleate desaturase	83
gb|AAB84262.1	*Arachis hypogae*	Oleate desaturase	83
gb|AAW63040.1	*Olea europaea*	Microsomal delta-12 oleate desaturase	76
emb|CAA65744.1	*Gossypium* hirsutum	Omega-6 desaturase	75
gb|AAF80560.1	*Sesamum indicum*	Omega-6 fatty acid desaturase	73
gb|AAT72296.2	*Nicotiana tabacum*	Microsomal omega 6-desaturase	72
emb|CAD30827.1	*Brassica rapa*	Fatty acid desaturase2	71
gb|AAS92240.1	*Brassica napus*	Delta-12 oleate desaturase	70

```
Glycine        1   MGGRGRVAKVEVQR---KKPLSRVPNTKPPFTVGQLKKAIPPHCFQRSLLTSFSYVVYDL
Arachis        1   MGAGGRVTKIEAQ----KKPLSRVPHSNPPFSVGQLKKAIPPHCFERSLFISFSYVVYDL
Arabidopsis    1   MGAGGRMPVPTSSKKSETDTTKRVPCEKPPFSVGDLKKAIPPHCFKRSIPRSFSYLISDI
Brassica       1   MGAGGRMQVSPPSKKSETDNIKRVPCETPPFTVGELKKAIPPHCFKRSIPRSFSYLIWDI
Gossypium      1   MGAGGRMSVPTSPKKPEFNSLKRVPYSKPPFTLSEIKKAIPPHCFQRSVLRSFSYLLYDF
Nicotiana      1   MGAGGNMSVVTGKTGEKKNPLEKVPTSKPPFTVGDIKKAIPPHCFQRSLVRSFSYVVYDL
Helianthus     1   MGAGEYTSVTNEN-----NPLDRVPHAKPPFTIGDLKKAIPPHCFQRSLTRSFSYVLSDL

Glycine       58   SFA-FIFYIATTYFHLLPQPFSLIAWPIYWVLQGCLLTGVWVIAHECGHHAFSKYQWVDD
Arachis       57   LVAYLLFYIATTYFHKLPYPFSFLAWPIYWAIQGCILTGVWVIAHECGHHAFSKYQLVDD
Arabidopsis   61   IIASCFYYVATNYFSLLPQPLSYLAWPLYWACQGCVLTGIWVIAHECGHHAFSDYQWLDD
Brassica      61   IIASCFYYVATTYFPLLPHPLSYFAWPLYWACQGCVLTGVWVIAHECGHHAFSDYQWLDD
Gossypium     61   ILASLFYHVATNYFPNLPQALSNVAWPLYWAMQGCILTGVWVIAHECGHHAFSDYQWLDD
Nicotiana     61   ILVSVFYYIAITYFHLLPSPYCYLAWPIYWICQGCVCTGIWVIAHECGHHAFSDYQWVDD
Helianthus    56   TITAVLYHIATTYFHHLPTPLSSIAWASYWVVQGCVLTGVWVIAHECGHHAFSDYQWVDD

Glycine      117   VVGLTLHSTLLVPYFSWKISHRRHHSNTGSLDRDEVFVPKPKSKVAWFSKYLNNPLGRAV
Arachis      117   MVGLTLHSCLLVPYFSWKISHRRHHSNTGSLDRDEVFVPKPKSKVSWYNKYMNNPPGRAI
Arabidopsis  121   TVGLIFHSFLLVPYFSWKYSHRRHHSNTGSLERDEVFVPKQKSAIKWYGKYLNNPLGRIM
Brassica     121   TVGLIFHSFLLVPYFSWKYSHRRHHSNTGSLERDEVFVPKKKSDIKWYGKYLNNPLGRTV
Gossypium    121   TVGLILHSSLLVPYFSWKYSHRRHHSNTGSLERDEVFVPKKKSGLRWWAKHFNNPPGRFL
Nicotiana    121   TVGLILHSALMVPYFSWKYSHRRHHSNTGSLERDEVFVPKPKSQLGWYSKYLNNPPGRVM
Helianthus   116   TVGFVLHSSLLVPYFSWKYSHHRHHSNTGSLERDEVFVPKSRSKVPWYSKYFNNTVGRIV

Glycine      177   SLLVTLTIGWPMYLAFNVSGRPYDS-FASHYHPYAPIYSNRERLLIYVSDVALFSVTYSL
Arachis      177   SLFITLTLGWPLYLAFNVSGRPYDR-FASHYDPYAPIYSNRERLLIYVSDSSVFAVTYLL
Arabidopsis  181   MLTVQFVLGWPLYLAFNVSGRPYDG-FACHFFPNAPIYNDRERLQIYLSDAGILAVCFGL
Brassica     181   MLTVQFTLGWPLYLAFNVSGRPYDGGFACHFHPNAPIYNDRERLQIYISDAGILAVCYGL
Gossypium    181   SITIQLTLGWPLYLAFNVAGRPYDR-FACHYDPYGPIFSDRERLQIYISDAGVLAVAYAL
Nicotiana    181   SLTVTLTLGWPLYLAFNVSGRHYDR-FACHYDPYGPIYNDRERLQIFLSDAGVLGAGYLL
Helianthus   176   SMFVTLTLGWPLYLAFNVSGRPYDR-FACHYVPTSPMYNERKRYQIVMSDIGIVITSFIL

Glycine      236   YRVATLKGLVWLLCVYGVPLLIVNGFLVTITYLQHTHFALPHYDSSEWDWLKGALATMDR
Arachis      236   YHIATLKGLGWVVCVYGVPLLIVNGFLVTITYLQHTHASLPHYDSSEWDWLRGALATVDR
Arabidopsis  240   YRYAAAQGMASMICLYGVPLLIVNAFLVLITYLQHTHPSLPHYDSSEWDWLRGALATVDR
Brassica     241   YRYAAVQGVASMVCFYGVPLLIVNGFLVLITYLQHTHPSLPHYDSSEWDWLRGALATVDR
Gossypium    240   YRLVLAKGVGWVISVYGVPLLVVNAFLVMITYLQHTHPSLPHYDSSEWDWMRGALSTVDR
Nicotiana    240   YRIALVKGLAWLVCMYGVPLLIVNGFLVLITYLQHTH-----------------------
Helianthus   235   YRVAMAKGLVWVICVYGVPLMVVNAFLVLITYLQHTHPGLPHYDSSEWEWLKGALATVDR

Glycine      296   DYGILNKVFHHITDTHVAHHLFSTMPHYHAMEATNAIKPILGEYYQFDDTPFYKALWREA
Arachis      296   DYGILNKAFHHITDTHVAHHLFSTMPHYHAMEATNAIKPILGDYYQFDGTPFYKALWREA
Arabidopsis  300   DYGILNKVFHNITDTHVAHHLFSTMPHYNAMEATKAIKPILGDYYQFDGTPWYVAMYREA
Brassica     301   DYGILNKVFHNITDTHVAHHLFSTMPHYHAMEATKAIKPILGEYYQFDGTPVVKAMWREA
Gossypium    300   DYGILNKVFHNITDTHVAHHLFSTMPHYHAMVATKAIKPILGEYYQFDGMPVYKAIWREA
Nicotiana          ------------------------------------------------------------
Helianthus   295   DYGVLNKVFHHITDTHVVHHLFSTMPHYNAMEAQKALRPVLGEYYRFDKTPFYVAMWREM

Glycine      356   RECLYVEPDEGTSEKGVYWYRNKY
Arachis      356   KECLYVEPDDGASKKGVYWYKNKF
Arabidopsis  360   KECIYVEPDREGDKKGVYWYNNKL
Brassica     361   KECIYVEPDRQGEKKGVFWYNNKL
Gossypium    360   KECLYVEPDEGDKDKGVFWFRNKL
Nicotiana          ------------------------
Helianthus   355   KECLFVEQDDEG-KGGVFWYKNKM
```

Fig. 7—Alignment of deduced amino acids of various plant ω-6- desaturases showing the three conserved (HXXXH) histidine boxes (underlined)

have been detected. Genome organization of microsomal ω-6 desaturase genes in cotton revealed two copies of *Ghfad2-1* in two tetraploid cotton species and at least one copy in a diploid cotton species[22]. Three to four copies of Δ^{12} - FAD gene per genome were reported in *Arachis hypogaea,* based on the number of bands in Southern analysis[14].

A high degree of overall identity at the amino acid levels between *fad2-1* in *pGMFAD2-1* and microsomal delta-12 desaturase enzymes of *Arachis hypogaea, Gossypium hirsutum, Sesamum indicum Nicotioana tabacum, Brassica napus* and *Arabidopsis* thus, leads to the conclusion that *fad2-1* sequence of soybean in the genomic clone *pGMFAD2-1* encodes the microsomal delta-12 desaturase/omege-6-desaturase enzyme.

The sequence information of the untranslated regions (UTR) obtained in the present study can be used for designing of primers for inverse-PCR from the genome to obtain specific promoter sequences for genes which show constitutive or regulated expression.

Since all higher plant microsomal omega-6-desaturases show an overall identity of 60% or more, the conserved sequences may thus provide vital information for the isolation of homologous fatty acid desaturases especially from the oil producing species, for desirable modifications in their fatty acid profile.

The *fad2-1* sequence base generated in the present study can ultimately be used to improve the performance of soyoil by designing various gene-silencing constructs like cosuppression, antisense and intron spliced hairpin constructs using the entire or partial sequences to inhibit the endogenous *fad2-1* gene expression in a tissue specific manner.

The present study can hence play a significant role in promoting targeted research towards achieving the ultimate goal of improving soybean seed oil profile making it nutritionally more beneficial and stable for human consumption.

Acknowledgement

The financial assistance by National Agricultural Technology Project to carry out the research work is gratefully acknowledged [File.No:28(1) 2002-NATP/CGP-III/311.

References

1 Liu Q, Singh S & Green A, High oleic and high stearic cottonseed oils: Nutritionally improved cooking oils developed using gene silencing, *J Am Colle of Nutr,* 21 (2002) 206S.

2 Cahoon E B, Genetic enhancement of soybean oil for industrial uses; Prospects & Challenges, *AgBioForum,* 6(1&2) (2003) 11.

3 Frankel EN, in *Flavor chemistry of fats and oils*, vol.11 edited by D B. Min, & T H Smoure,(*American Oil Chemists' Society*, Champiagn) 1985, 111.

4 Kinney A J, Genetic modification of the storage lipids of plants. *Curr Opin Biotechnol*, 5 (1994) 144.

5 Popescu O, Koritala S & Dutton H J, High oleic oils by selective hydrogenation of soybean oil, *J Am Oil Chem Soc*, 46 (1969) 97

6 Katan M B, Zock P L & Mensink R P, Trans fatty acids and their effects on lipoproteins in humans, *Ann Rev Nutr*, 15 (1995) 437.

7 Heppard E P, Kinney A J, Stecca K L & Miao G H, Development and growth temperature regulation of two different microsomal ω-6 desaturase genes in soybean, *Plant Physiol*, 110 (1996) 311.

8 Kinney A J in *Lipid biotechnology* edited by T M Kuo and H W Gardener (Marcel Dekker, Inc. New York) 2002, 85

9 Murray M G & Thomson W F, Rapid isolation of high molecular weight plant DNA, *Nucleic Acids Res,* (1980) 8 4321.

10 Krishna Kishore, *Cloning and characterization of microsomal ω-6 desaturase gene fad2-1 from soybean (Glycine max L.*), Ph D thesis submitted to Indian Agricultural Research Institute, Delhi, 2005.

11 Kishore K, Sinha SK, Kumar R, Dubey N & Sachdev A, *Cloning and characterization of microsomal ω-6 desaturase gene (fad2-1) from Glycine max L*. NCBI Accession no AY954300, 2005.

12 Hongtrakul V, Slabaugh M B & Knapp S J, A seed specific Delta-12 oleate desaturase gene is duplicated, rearranged, and weakly expressed in high oleic acid sunflower line, *Crop Sci*, 38 (1980) 5 1245.

13 Okuley J, Lightner J, Feldmann K, Yadav N & Lark E, *Arabidopsis fad2* gene encodes the enzyme that is essential for polyunsaturated lipid synthesis, *The Plant Cell*, 6 (1994) 147.

14 Lopez Y, Nadaf H L, Smith O D, Connell J P, Reddy, A S & Fritz A K Isolation and characterization of the Δ^{12-} fatty acid desaturase in Peanut (*Arachis hypogaea* L.) and search for polymorphism for the high oleate trait in Spanish market type-lines, *Theor Appl Genet*, 101 (2000) 1131.

15 Liu L, Hammond E G, & Nikolau B J, *In vivo* studies of the biosynthesis of α-eleostearic acid in the seeds of *Momordica charantia* L. *Plant Physiol*, 113 (1997) 1343.

16 Minto R E, Gibbons W J Jr, Cardon, T B & Lorigan, G .A Synthesis and conformational studies of a transmembrane domain from a diverged microsomal D12-desaturase. *Analyt Biochem* , 308 (2002) 134.

17 Shanklin J & Cahoon E B Desaturation and related modifications of fatty acids, *Annu Rev Plant Physiol Plant Mol Biol*, 49 (1998) 611.

18 Sayanova O, Beaudoin F, Libisch B, Castel A, Shewry P R, & Napier J. A, Mutagenesis and heterologous expression in yeast of a plant Δ^6-fatty acid desaturase, *J Exp Bot*, 52 (360) (2001) 1581.

19 Shanklin J, Whittle E & Fox B G, Eight histidine residues are

catalytically essential in a membrane-associated iron enzyme, stearoyl-CoA desaturase, and are conserved in alkane hydroxylase and xylene monooxygenase. *Biochemistry*, 33 (43) (1994) 12787.

20 Tiwari N, Sachdev A, Kumar J, Swathi K & Johari R P Developmental expression of microsomal ω –6 desaturase gene (*fad2-1*) in soybean seeds, *J Plant Biochem Biotechnol*, 13 (2004) 93.

21 Scheffler J A, Sharpe A G, Schmidt H, Sperling P, Parkin I A P, Luhs W Lydiat D J & Heinz E, Desaturase multigene family of Brassica napus arose through genome duplication, *Theor Appl Genet*, 94 (1997) 583.

22 Liu Q, Singh S P, Brubaker C I, Sharp P J, Green A & Marshall D R, Molecular cloning and expression of a cDNA encoding microsomal ω-6- desaturase from cotton (*Gossypium hirsutum*). *Aust J Plant Physiol,* 26 (1999) 101.