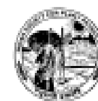

ISOLATION AND CHARACTERIZATION OF *fad2-1* cDNA SEQUENCE FROM *GLYCINE MAX* L.

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SUMMARY

Microsomal ω-6 desaturase encoded by *fad2-1* gene catalyzes the production of polyunsaturated fatty acids in seed storage lipids. A cDNA library was constructed in λTriplEx2 vector using poly (A) RNA isolated from developing seeds (19-26 DAF) of *Glycine max* L. The putative clones from library screening were converted into plasmid clones following *in vivo* excision and analyzed for insert size by restriction digestion. A positive clone PR2 with largest insert size spanning a total of 1139 bp revealed a 3' UTR region of 184 bp specific to *fad2-1*. Conceptual translation indicated an open reading frame of 955bp encoding a 317 amino acid protein with a predicted molecular weight of 37kD. A high degree of sequence identity was found with *Glycine max* cDNA for ω-6-desaturase and with *fad2-1* of *Arachis hypogea*. PR2 was also used as a labelled probe to analyse the expression of *fad2-1* transcripts by northern blotting. High levels of *fad2-1* mRNA transcripts were detected at the mid maturation stages of seed development (19-26 DAF).

Key words: **Developing seeds, *fad2-1*cDNA, omega-6 desaturase, soybean**

INTRODUCTION

Oils rich in oleic acid are oxidatively stable, tend to have good flavour characteristics and also impart positive health benefits, whereas, oils rich in polyunsaturated fatty acid have poor flavour as well as low stability. Polyunsaturated fatty acids (linoleic 55%, and linolenic acid 7%) are major precursors of the off-flavour compounds in soybean as they are susceptible to oxidation and their oxidized products are commonly associated with rancidity (Frankel 1980) and atherogenic effects (Katan *et al*. 1995) . The major polyunsaturated fatty acid in soybean, linoleic acid, is synthesized from the main product of the plastidial fatty acid biosynthesis, oleic acid, by the membrane bound microsomal ω-6 desaturase encoded by gene, *fad2-1* (Heinz 1993). Reduction in the expression of *fad2-1* results in an increased accumulation of oleic acid (18:1) and a corresponding decrease in polyunsaturated fatty acid (PUFA) content in the soyoil, thereby, making it more healthier and oxidatively stable. A chemical solution to enhance soyoil stability is industrial hydrogenation which decreases the content of polyunsaturated fatty acids and increases the relative abundance of monounsaturated oleic acid while also producing substantial quantities of the trans isomers of oleic acid and other trans isomers which are linked with unfavorable plasma lipoprotein profiles and coronary heart disease (Katan *et al*. 1995). Soybean germplasm being extremely narrow, derived from very few parental lines thereby leaving little scope for continued improvement by classical breeding approach. The cDNAs, encoding this gene have been isolated from *Brassica juncea* (Singh *et al*. 1995), *Glycine max* (Heppard *et al.* 1996), *B. napus* (Scheffler

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et al. 1997) and *Arabidopsis* (Okuley *et al.* 1994). Since the seed specific *fad 2-1* gene of *Glycine max* L. is strongly expressed only in developing embryos of soybean (Heppard *et al.* 1996, Tiwari *et al.* 2004), the high levels of polyunsaturated fatty acid content in the seed lipids can be effectively reduced by suppressing the expression of *fad 2-1* by gene silencing technologies. In the present study, we report isolation, characterization and expression of a *fad2-1* cDNA clone from the cDNA library of developing seeds of soybean for further manipulation.

MATERIALS AND METHODS

Developing seeds of *Glycine max* L. cv. Pusa 9702 were collected from the farm of Indian Agricultural Research Institute, New Delhi. Etiolated seedlings of *Glycine max* L. were harvested for DNA isolation. Tissues of fully developed soybean stem, leaves and roots were frozen and stored at –80°C until used for expression studies. Gene specific primers were designed using sequence information of *fad-2-1* gene available in the GenBank database. The sequence of the primers was as follows:

Forward Primer: 5' GTATTGATGGAGCAACCAAT 3'

Reverse Primer: 5' GGCAGAAAGCTATAAGCAGA 3'

DNA from 6-7 days old etiolated seedlings of soybean was isolated by CTAB method (Murray and Thomson 1980). For PCR amplification reagents were added in a 0.2 ml PCR tube: 2.5 µl 10 x PCR buffer, 2.5 µl 1 mM dNTP mixture, (1 µl) 100 pmol forward primer, (1 µl) 100 pmol reverse primer, (0.5 µl) 1.25 units Taq DNA polymerase, (5 µl) 50 ng template DNA. Final volume was made to 25 µl with sterile water. The polymerase chain reaction was carried out in a thermal cycler (Minicycler™, MJ Research) following a programme of initial denaturation at 94°C for 4 min. followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 1.5 min and a final extension at 72°C for 10 min after the completion of the cycles. PCR amplified product was gel eluted , purified and cloned into a T-A cloning vector, $pGEMT_{Easy}$. Ligated fragment was transformed into DH5α cells and the recombinant clone named as PR1 was sequenced at Department of Biochemistry (DBT facility) University of Delhi , South Campus, New Delhi. Total RNA from developing seeds (19-26DAF), leaf, stem and root tissues was isolated by a modified procedure of Sambrook *et al.* 1989.

The cDNA library was constructed according to the protocol described for SMART™ cDNA library construction kit (Clonetech Laboratories, Inc) . Poly $(A)^+$ RNA was obtained from total RNA of seeds 19-26 DAF, using OligoTex resin (oligo dT primer bound to polystyrene resin, Qaigen,Germany). First strand synthesis was performed in a total volume of 10µl using ~3 µl poly $(A)^+$ RNA (0.5 µg); 1 µl SMART IV Oligonucleotide; 1 µl CDS III/3' PCR primer. Following reagents were also added to the reaction tube , 2 µl 5x First Strand Buffer, 1 µl DTT (20 mM), 1 µl dNTP mixture (10 mM), 1 µl Power Script Reverse Transcriptase. The contents of the tube were mixed by pipetting and tube was incubated at 42°C for 1 hr. The reaction was terminated by placing the tube on ice. The second strand cDNA synthesis and cDNA amplification was done by PCR in presence of Advantage 2 Polymerase Mix. Following the addition of *Eco R* I linkers the cDNA was ligated into the λTriplEx2 vector and packed *in vitro* with Giga PackIII Gold Cloning Kit as recommended by the manufacturer. The primary library was amplified in SM buffer [0.05M Tris-HCl (pH7.5), 0.58% NaCl, 0.2% MgSO4 and 0.01% gelatin] according to manufacturer's instructions and the aliquots of the amplified library were stored in 7% DMSO(v/v) at –80°C.

For preparation of the radiolabelled probe the plasmid DNA of clone PR1 was restricted with *Eco* RI (10 units each/µg of DNA) at 37°C for 5 hrs and electrophoresed on a 1% LMP agarose gel. The insert fragment excised and purified using the GENECLEAN kit was denatured by heating at 95ºC for 10 min and radiolabelled with $\alpha^{32}P$ dCTP following the Prime-a-Gene Labeling System (Promega). The unincorporated nucleotides were removed using Spin Column 30 (Sigma) and this radiolabelled fragment was used as a probe to screen the cDNA library.

The cDNA library was screened in duplicate by plaque hybridization using 200 bp radiolabelled fragment from clone PR1 as probe (Ausubel *et al.* 1999). The putative clones from library screening were converted

into plasmid clones following *in vivo* excision and analyzed for insert size by restriction digestion with *Sfi* I. One of the clones PR2 showing a strong signal on Southern hybridization was sequenced at Department of Biochemistry (DBT facility) University of Delhi , South Campus, New Delhi.

RNA concentrations were determined spectrophotometrically. Ten microgram of total RNA from mid maturation stages of seed development (19-26DAF), leaf, stem and root tissues were electrophoresed on a denaturing agarose gel and blotted onto a nitrocellulose membrane following the method as described by Sambrook *et al* (1989). The membrane was UV cross-linked using the GS Gene Linker (Bio Rad Laboratories). The membrane was pre-hybridized at 42ºC in 5 x SSC, 50% formamide, 5 X Denhardt's solution [50 x stock solution consisting of 1% Ficoll 400, 1% polyvinylpyrrolidone, 1% BSA (Bovine Serum Albumin, Fraction IV)] in distilled water, 0.5% SDS and 100 μg/ml denatured calf thymus DNA for 4 hrs. The 1.2kb *Eco* RI fragment of soybean PR2 was excised, radiolabelled and used as probe. Hybridization was performed at 42ºC for overnight. Blots were washed twice at 65ºC in a solution containing 1x SSC and 0.1% SDS for 30 min. Hybridized membrane was exposed to Kodak-X ray film and the autoradiogram developed.

The DNA sequence similarity search was done by computing at BLAST server developed by NCBI, USA for searching the DNA and protein databases over the internet. The software EMBOSS at the URL:http://www.ebi.ac.uk was used for primary structure analysis i.e. DNA translation and WEBCUTTER V2.0 was used for restriction site mapping. The protein sequence similarity search was done by computing at FASTA server and the multiple sequence alignment at CLUSTAL-W server.

RESULTS AND DISCUSSION

The coding sequences of *fad2-1* and *fad2-2* show 73% homology at the deduced amino acid level (Heppard *et al.* 1996), thus the primer sequences were retrieved from 3' UTR region of the cDNA sequences available in the data base to amplify a the 200 bp fragment unique to 3' untranslated region (UTR) of *Gmfad 2-1* using genomic DNA as template for PCR (Fig. 1). The amplified fragment was gel eluted , purified and ligated into pDrive vector and transformed into *E. coli* DH5α competent cells. The recombinants were identified by blue white screening and the plasmid DNA isolated from putative recombinants was restricted with *Eco*R1 . Using SP6 primer, a nucleotide sequence of 191 bp was read. It contained 55 adenine, 26 cytosine, 46 guanine, 64 thymine residues. Analysis on BLASTN search showed 100 per cent homology of amplicon with *Glycine max* L. *fad 2-1* cDNA sequence available in the GeneBank

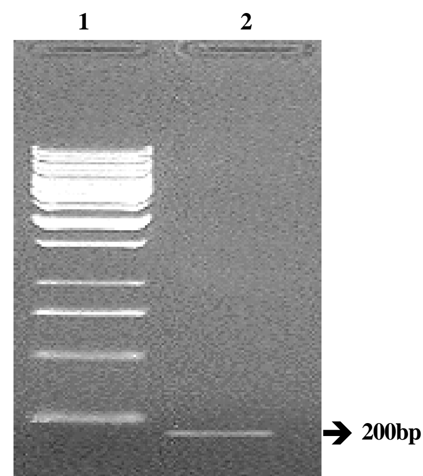

Fig.1. Agarose gel (1.2%) showing PCR amplified *fad* 2-1 gene fragment from genomic DNA of soybean (Lane 2); M-1 kb DNA ladder (Lane1)

A cDNA library was constructed using the RNA isolated from developing seeds (19-26 DAF) of *Glycine max* L. as *fad 2-1* gene expression predominates at this stage. The titer of the primary library was found to be $3x10^6$ *pfu* in 550 μl volume and blue white selection indicated that the library had >90% recombinants. However, to make a large, stable quantity of higher-titer library, the library was amplified and the titer of the amplified library was calculated to be $3.0x10^9$ pfu/ml.

Partial DNA sequence (PR1) of *Glycine max* L. *fad 2-1* was used as a probe to screen cDNA library. Different approaches have been followed by researchers to isolate desaturase genes in various crops. Heppard *et al* (1996) isolated *Gmfad 2-1* cDNA clone by screening a developing soybean embryo library (Yadav *et al.* 1993) using *Arabidopsis fad 2* cDNA fragment (Okuley *et*

*al.*1994). Scheffler *et al.*1997 isolated cDNA clones of genes encoding the four different desaturase enzymes (e2, e3, p2 and p3) from cDNA of *Brassica napus* using PCR and RACE techniques. Okuley *et al.* (1994) identified a *fad 2* allele in a population of *Arabidopsis* in which the mutation had been created by T-DNA insertion. Genomic DNA flanking the T-DNA was cloned by plasmid rescue and used to isolate cDNA and genomic clones of *fad2*. Hongtrakul *et al.* (1980) isolated a full length cDNA clone (OLD-7) from a developing seed cDNA library of sunflower using *Arabidopsis thaliana* L. (*At OLD*) cDNA as a probe.

The cDNA library was screened by plaque hybridization using *fad2-1* specific probe and eight, single, well isolated, positive plaques were picked and processed further. These clones were excised in *E. coli* BM 25.8 to liberate ampicillin resistant pTRIplEx2 plasmid to allow insert characterization in a plasmid system. Restriction digestion of the plasmids of all the eight putative clones was done with *Sfi* I and only one clone (PR2) gave a large sized insert of 1.2 kb (Fig. 2). The cDNA clone (PR2) was restricted and electrophoresed on a 0.8% agarose gel along with DNA marker, transferred on to a nylon membrane and Southern hybridized to the *fad2-1* specific probe PR1 The insert fragment in clone PR2 was prominently highlighted in the autoradiogram (Fig. 3 a,b) indicating the presence of *fad2-1* sequences in it. To further confirm the presence of the gene, the plasmid DNA from PR2 was PCR amplified using the *fad2-1* specific primers. An amplicon of size 0.2 kb resolved on a 1% agarose gel and its BLAST search revealed a high degree of nucleotide homology with *fad2-1* cDNA sequence in GenBank.

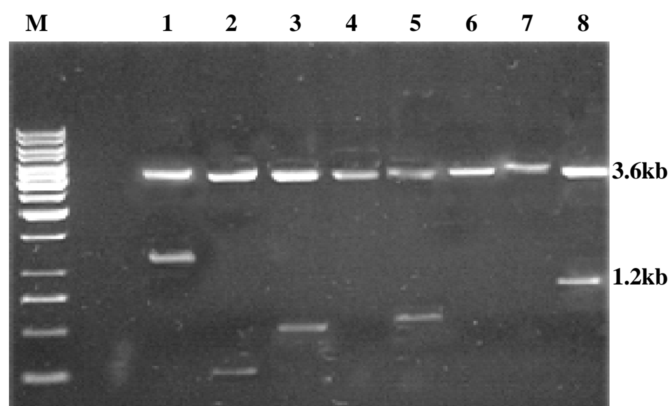

Fig. 2. EtBr stained 1% agarose gel showing the *Sfi*I digestion pattern of eight randomly selected putative clones from cDNA library of *Glycine max*.L after excision . The gel shows 3.6 kb vector bands and cDNA inserts.(M- 1kb DNA ladder)

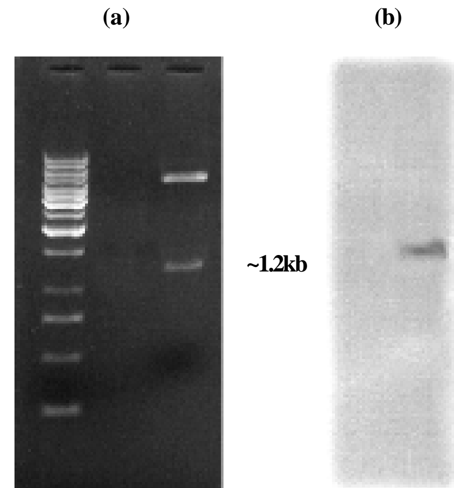

Fig. 3. (a) Restriction digestion of the positive cDNA clone with *Sfi* I showing ~1.2 kb insert (Lane1); 1kb DNA ladder (LaneM). (b) Autoradiogram showing positive signal at ~1.2 kb using PR1 as probe.

The PR2 plasmid in BM 25.8 strain of *E. coli* was transformed into DH5α competent cells before sequencing at Department of Biochemistry (DBT facility), Delhi University, South Campus. The complete sequencing of the insert PR2 revealed a total length of 1139 base pairs. The nucleotide composition of cDNA was 281 adenine, 261 cytosine, 253 guanine and 344 thymine residues (Fig. 4). The homology search for the cDNA done by BLASTN revealed maximum homology with *Glycine max* L. cDNA for omega-6-desaturase (*fad 2-1*). BlastN search also revealed homology (Table 1) with other *fad 2-1* cDNAs of other crops viz. *Arachis hypogea* (85%), *Cucurbita pepo* (81%), *Arabidopsis thaliana* (82%), *Nicotiana tabacum* (81%) and *Gossipum hirsutum* (79%). This partial fragment of *fad 2-1* gene had a single large exon of 955 bp, a 184 bp long 3' UTR regions but no introns as expected. This finding is in line with single uninterrupted exons for desaturase genes reported in other crops like *Arachis hypogea*, *Cucurbita pepo*, *Arabidopsis thaliana*, *Nicotiana tabacum* and *Gossipum hirsutum.* Hongtrakul *et al.* 1980 reported a full length cDNA clone of 1492 bp in sunflower Δ^{12} oleate desaturase gene (OLD-7). It

NUCLEOTIDE COMPOSITION OF PR2

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T T T C T A C A T T G C C A C C A C C T A C T T C C A C C
T C C T C C C T C A C C C C T T T T C C C T C A T T G C A
T G G C C A A T C T A T T G G G T T C T C C A A G G T T G
C A T T C T T A C T G G C G T G T G G G T G A T T G C T C
A C G A G T G T G G T C A C C A T G C C T T C A G C A A G
T A C C C A T G G G T T G A T G A T G T T A T G G G T T T
G A C C G T T C A C T C A G C A C T T T T A G T C C C T T
A T T T C T C A T G G A A A A T A A G C C A T C G C C G C
C A C C A C T C C A A C A C G G G T T C C C T T G A C C G
T G A T G A A G T G T T T G T C C C A A A A C C A A A A T
C C A A A G T T G C A T G G T A C A C C A A G T A C C T G
A A C A A C C C T C T A G G A A G G G C T G C T T C T C T
T C C C A T C A C A C T C A C A A T A G G G T G G C C T T
T G T A T T T A G C C T T C A A T G T C T C T G G C A G A
C C C T A T G A T G G G T T T T G C A G C C A C T A C C A
C C C T T A T G C T C C C A T A T A T T C A A A T C G T G
A G A G G C T T T T G A T C T A T G T C T C T G A T G T T
G C T T T G T T T T C T G T G A C T T A C T T G C T C T A
C C G T G T T G C A A C T A T G A A A G G G T T G G T T T
G G C T G C T A T G T G T T T A T G G G G T G C C A T T G
C T C A T T G T G A A C G G T T T T C T T G T G A C C A T
C A C A T A T C T G C A G C A C A C A C A C T A T G C C T
T G C C T C A C T A T G A T T C A T C A G A A T G G G A T
T G G C T G A G G G G T G C T T T G G C A A C T A T G G A
C A G A G A T T A T G G A A T T C T G A A C A A G G T G T
T T C A C C A C A T A A C T G A T A C T C A T G T G G C T
C A C C A T C T T T T C T C T A C A A T G C C A C A T T A
C C A T G T A A C G G A G G C A A C C A A T G C A A T G A
A G C C A A T A T T G G G T G A G T A C T A C C G A T T T
G A T G A C A C A C C A T T T T A C A G G C A C T G T G G
A G A G A A G C A A G A G A G T G C C T C T A T G T G G A
G C C A G A T G A A G G A G C A T C C G A G A A G G G C G
T G T A T T G G T A C A G G A A C A A G T A T T G A T G A
A C C A A G C A A T G G G C C A T A G T G G G A G T T A T
G G A A G T T T T G T C A C T T A T C A C T T A A T T A
G T A G A A T G T T A T A A A T A A G T G G A T T T G C
C G C G T A A T G A C T T G T G T G C A T T G T G A A A C
A G C T T G T A G C G A T C C A T G G C T A T A A T G T A
A A A A T A T G T G G A A A G T G T T C T G C T T A T A
G C T T T C T G C C
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A=281 C=261 G=253 T=344 Total 1139 bp

Fig. 4. Nucleotide sequence of PCR amplified *fad 2-1* fragment in PR2 from genomic DNA of soybean.

had an ORF of 1137 bp from 122 to 1258 bp encoding 378 amino acids, a 121 bp 5' UTR and 234 bp 3' UTR region. Heppard *et al.* (1996) using *Arabidopsis fad 2* cDNA as a probe screened soybean cDNA library and reported a 1453 bp cDNA clone pSF2-169K containing an ORF encoding a predicted protein of 387 amino acids residues in soybean.

In order to examine the expression of *fad2-1* transcripts in plants using labeled probe derived from *Sfi* I fragment of PR2 as probe, the total RNA from leaf, stem, root and developing seeds (19-26 DAF) was

Table 1. Similarity data of microsomal omega-6-desaturase gene (*fad 2-1*) in cDNA clone PR2 with microsomal omega-6-desaturase genes of other crop species as obtained in BLASTN homology search.

Plant	Identity (%)	Score(BITS)
Glycine max	100	2258
Arachis hypogaea	85	367
Cucurbita pepo	81	168
Arabidopsis thaliana	82	92
Nicotiana tabacum	81	139
Gossipium hirsutum	79	94
Helianthus annuus	81	66
Sesamum indicum	80	151
Ricinus communis	82	105

analyzed by northern hybidization. As expected northern blotting detected high levels of *fad2-1* gene expression in the developing embryos of soybean at mid maturation stages i.e.19-26 DAF**.** The *fad2-1* expression was not detected in northern blots of mature leaf, stem and root tissues (Fig. 5) clearly indicating its seed specificity (Heppard *et al.*1996, Tiwari *et al.* 2004).

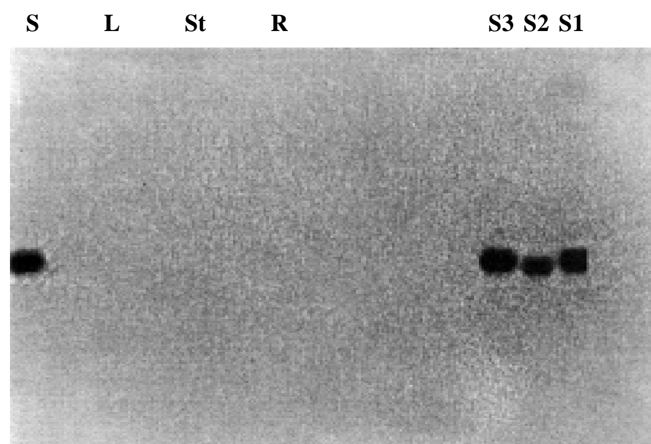

Fig. 5. Northern hybridization of *fad2-1* transcripts with PR2 (probe) in seed (S), leaf (L), stem (St), root (R) tissues and developing seeds,19,21 and 26 DAF(S1, S2 , S3).

The predicted protein encoded by *Glycine max* L. *fad 2-1* cDNA consisted of 317 amino acid residues (Fig. 6). The BLASTP homology search program at the EMBL site: http://www.ebi.ac.uk revealed the molecular

AMINO ACID SEQUENCE OF PR2

F Y I A T T Y F H L L P H P F S L I A W P I Y W V L Q G C I
L T G V W V I A H E C G H H A F S K Y P W V D D V M G L T
V H S A L L V P Y F S W K I S H R R H H S N T G S L D R D E
V F V P K P K S K V A W Y T K Y L N N P L G R A A S L P I
T L T I G W P L Y L A F N V S G R P Y D G F C S H Y H P Y A
P I Y S N R E R L L I Y V S D V A L F S V T Y L L Y R V A T
M K G L V W L L C V Y G V P L L I V N G F L V T I T Y L Q H
T H Y A L P H Y D S S E W D W L R G A L A T M D R D Y G
I L N K V F H H I T D T H V A H H L F S T M P H Y H V T E
A T N A M K P I L G E Y Y R F D D T P F Y K A L W R E A R
E C L Y V E P D E G A S E K G V Y W Y R N K Y

Fig. 6. Deduced amino acid sequence of *fad 2-1* gene in cDNA clone PR2.

weight of the protein to be 37 kDa and an isoelectric point (pI) of ~ 7.84. The gross amino acid composition of the protein showed abundance of valine (7.94%), histidine (6.6%), alanine (6.6%) and leucine (11%). The least abundant amino acids were glutamine (0.6%) and cysteine (1.6%). The BLASTP search result for PR2 also revealed maximum homology with *Glycine max* L. and with other omega-6-desaturase enzymes with score 687 and E value. A strong homology which extended from 100% in *Glycine max* L. to 85% in *Arachis hypogea,* 76% in *Gossypium hirsutum*, 73% in *Cucurbeta pepo*, 72% in *Brassica juncea* and 74% *Sesamum indicum* . A comparison of the clone PR2 *fad 2-1* sequence showing phylogenetic relationship at the deduced amino acid level with those of other plant species is shown in dendrogram (Fig. 7). The microsomal omega-6-desaturase of *Glycine max* L. and *Arachis hypogea*, *Arachis duranensis* are grouped in the same cluster revealing close evolutionary relationship as also evident

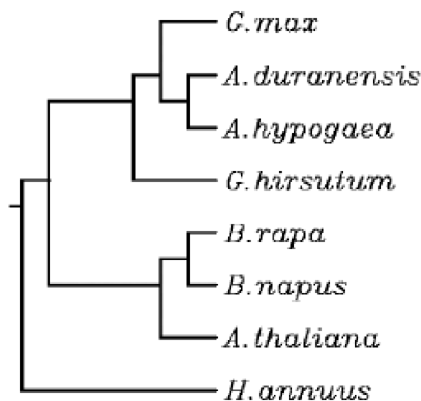

Fig. 7. Dendrogram constructed with microsomal ω-6-desaturase sequence of *Glycine max* (PR2 *fad2-1*), *Arachis duranensis*, *Arachis hypogaea*, *Gossypium hirsutum*, *Brassica rapa*, *Brassica napus*, *Arabidopsis thaliana* and *Helianthus annus*

from the BLASTP and BLASTN analysis, reflecting approximately 80% sequence similarity at both amino acid and nucleotide levels. Also, *G. hirsutum* and *Brassica rapa* are much closely related to soybean evolutionarily as compared to *Arabidopsis thaliana* and *Helianthus annuus.* 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 (Schmidt *et al.* 1994). In the present study, analysis of the deduced amino acid sequence also shows the presence of metal ion binding conserved histidine boxes in soybean microsomal omega-6-desaturase, as present in all membrane bound desaturases. The presence of these conserved histidine boxes in desaturase enzyme is important for enzyme activity and specificity. Many studies have shown that any alteration or mutation in these conserved regions may lead to reduction or loss of enzyme activity (Sayanova *et al.* 2001). The restriction sites present in the *fad 2-1* gene sequence were studied using WEBCUTTER. Restriction pattern analysis revealed the absence of restriction sites for *Hin*d III*, Bam*H I*, Eco*R V enzymes and presence of restriction site for *Eco*R I*, Pst* I and *Sca* I enzymes.

Furthermore, based on these comparisons, it is expected that all higher plant microsomal omega-6-desaturases show an overall identity of 60% or more and hence may provide vital information for the isolation of homologous fatty acid desaturase sequences using sequence dependent protocols.

The *fad 2-1* cDNA sequence generated in the present study can ultimately be used to improve the quality of soyoil containing high levels of polyunsaturated fatty acids. It will help in designing various gene silencing constructs, like cosuppression, antisense and intron spliced hairpin constructs, using the entire or partial sequences (Smith *et al.,* 1990, Napoli *et al.* 1990, Cartea *et al*. 1998, Stoutjesdik *et al*. 2002) to inhibit plant target genes in a tissue specific manner. Furthermore, *fad2-1* cDNA can be used to assess its potential in enhancing and/or altering oil quality by making transgenics in model plants. Also, the cDNA library constructed in the present study can be utilized to isolate other important genes from soybean *Glycine max* L.

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REFERENCES

Ausubel, H.F., Brent, R., Kingston, E.R., Moore, D.D., Seidman, J.G., Smith, A.J., Struchl, K. (1999). Single step RNA isolation from cultured cells or tissues. Short Protocols in Molecular Biology (IV ed.), unit 4.2, pp. 4-4. John Wiley and Sons, New York.

Cartea, M.E., Migdal, M., Galle, A.M., Pelletier, G., Guerche, P. (1998). Comparison of sense and antisense technologies for modifying fatty acid composition of *Arabidopsis thaliana* oil seed. *Plant Sci.* **136**: 181-194.

Frankel, E.N. (1980). Soybean oil flavour stability. In: D.R. Erickson, E.H. Pryde, O.L. Brekke, T.L. Mounts and R.A. Falb (eds.), Handbook of Soyoil Processing and Utilization, pp 229-244 . Am. Soybean Assoc. St. Louise, Missouri, USA.

Heinz, E. (1993). Biosynthesis of polyunsaturated fatty acids. In: T.S Morre Jr. (ed.), Lipid Metabolism in Plants, pp. 33-89. CRC Press, Boca, Raton.

Heppard, E.P, Kinney, A.J., Stecca, K.L. and Miao, G.H. (1996). Development and growth temperature regulation of two different microsomal ω-6 desaturase genes in soybean. *Plant Physiol.* **110** : 311-319.

Hongtrakul, V., Slabaugh, M.B. and Knapp, S.J. (1980). A seed specific delta-12 oleate desaturase gene is duplicated, rearranged, and weakly expressed in high oleic acid sunflower line. *Crop Sci.* **38**: 1245- 1248.

Katan, M,B., Zock, P.L. and Mensink, R.P. (1995). Trans fatty acids and their effects on lipoproteins in humans. *Ann Rev. Nutr.* **15:** 437- 439.

Murray, M.G., and Thomson, W.F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* **8**: 4321- 4325.

Napoli, C., Lemieux, C. and Jorgensen, R.(1990). Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes *in trans*. *Plant Cell* **2**: 279-289.

Okuley, J, Lightner,J., Feldmann,K., Yadav, N. and Lark, E.(1994). Arabidopsis *fad2* gene encodes the enzyme that is essential for polyunsaturated lipid synthesis. *The Plant Cell* **6** : 147-158.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning : A Laboratory Manual, 2nd edition, Cold spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sayanova, O., Beaudoin, F., Libisch, B., Castel, A., Shewry, P.R., and Napier, J.A., (2001). Mutagenesis and heterologous expression in yeast of a plant Δ^6-fatty acid desaturase. *J. Exp. Bot.* **52** :1581-1585.

Scheffler, J.A., Sharpe, A.G., Schmidt, H., Sperling, P., Parkin, I.A.P., Luhs, W., Lydiate, D.J. and Heinz, E. (1997). Desaturase multigene families of *Brassica napus* arose through genome duplication. *Theor. Appl. Genet.* **94** : 583-591.

Schmidt, H., Dresselhaus, T., Buck, F. and Heinz, E. (1994). Purification and PCR-based cDNA cloning of a plastidial w-6 desaturase. *Plant Mol. Biol.* **26**: 631-642.

Singh, S., Heide, T.V., Mc Kinney, S. and Greenn, A. (1995). Nucleotide sequence of a cDNA (Accession no. 91139) from *Brassica Juncea* encoding a microsomal omega-6 desaturase (PGR 95-107). *Plant Physiol*. **109** : 1498.

Smith,C.J.S., Watson,C.F, Bird CR, Ray, J., Schuch, W. and Grierson, D. (1990) Expression of a truncated tomato polygalacturonase gene inhibits expresssion of the endogenous gene in transgenic plants. *Mol Gen Genet.* **224**: 477- 481.

Stoutjesdijk, P.A, Hurlstone, C., Singh, S.P. and Green, A. (2002). hpRNA–mediated targeting of the *Arabidopsis FAD2* gene gives highly efficient and stable silencing. *Plant Physiol*.**129**:1723-1731.

Tiwari, Nirupama, Sachdev, A., Kumar, J., Swathi K. and Johari, R.P. (2004). Developmental expression of microsomal omega-6 desaturase gene. (*fad 2-1*) in soybean seeds. *J. Plant Biochem. Biotech.* . **13** : 93-99.

Yadav, N.S., Wierzbicki, A., Aegerter, M. Caster, C.S., Perez, G.L., Kinney, A.J., Hitz, W.D., Booth, J.R., Schweiger, B., Stecca, K.L., Allen, S.N., Blackwell, M., Rciter, R.S., Carlson, T.J., Russel, S.H., Feldmann, K.A., Pierce, J. and Browse, J. (1993). Cloning of a higher plant omega-3-fatty acid desaturase. *Plant Physiol.* **103** : 467-476.