1.1 Introduction

Lipids are essential constituent of all living systems. In plants, the vegetative cells contain 5 to 10% lipids by dry weight, and almost all of this weight is found in the membranes. Other type is the storage lipids that are the major form of carbon storage in the seeds of many plant species, constituting up to 60% of the dry weight of such seeds (Ohlrogge and Browse, 1995). Unlike the other major constituents of plants (proteins, carbohydrates, and nucleic acids), lipids are defined on the basis of their physical rather than their common chemical structure. Thus, lipids are often loosely defined as those compounds that are insoluble in water and that can be extracted from cells by non-polar organic solvents (such

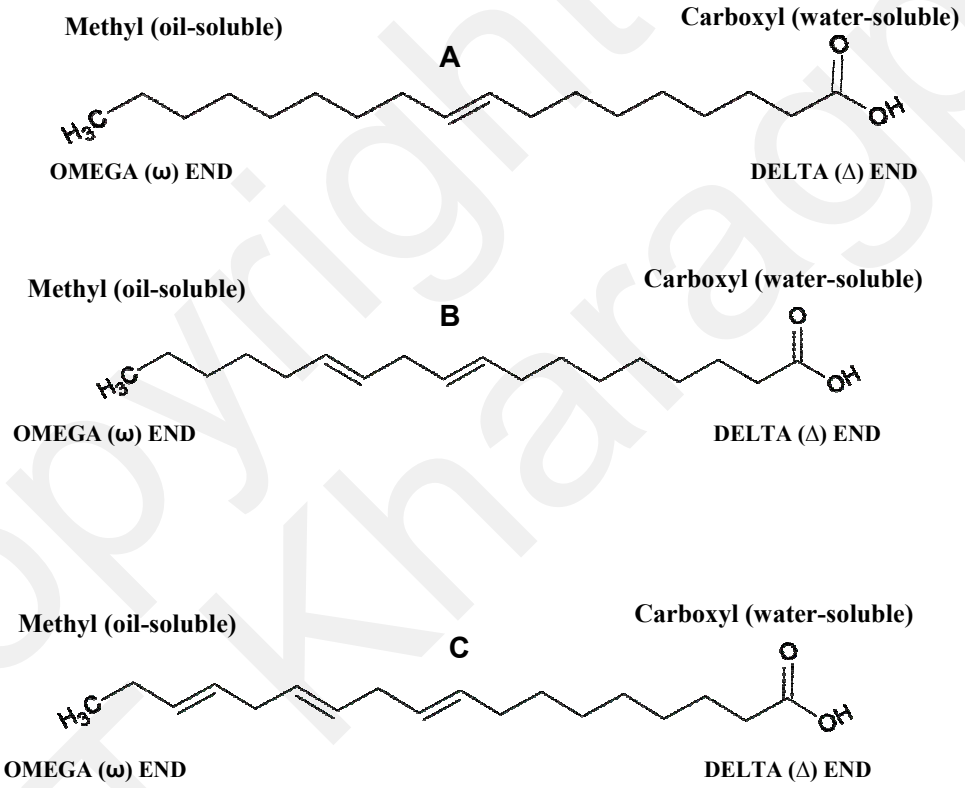

Figure 1.1: Examples of unsaturated fatty acids A. Oleic acid (C18:1) B. Linoleic acid (C18:2), C. α-linolenic acid (C18:3).

as chloroform). As such, this class of components are extremely diverse in structure and actually constitutes the products of severa1 distinct biosynthetic pathways. The most

abundant types of lipid in majority of cells, however, are those that are derived from the fatty acids and are the products of glycerolipid biosynthetic pathway. Defining the fatty acids, they are aliphatic monocarboxylic acids derived from, or contained as esterified molecular form in lipids (fat, oil or wax) of microbes, animals or plants. Natural fatty acids commonly have a chain of 4 to 28 carbons (usually unbranched and even numbered) and further they may be saturated or unsaturated depending on the presence of double bonds in the acyl chain. The unsaturated fatty acids may be monounsaturated or polyunsaturated fatty acids depending on the number of double bonds in their chemical structure (Figure 1.1). Unsaturated fatty acids are essential for normal cell function and have recently generated scientific interest as targets for genetic manipulation. Polyunsaturated fatty acids (PUFAs) are long chain (usually > 18 carbon) fatty acids containing two or more double bonds introduced by specific desaturase enzymes. PUFAs have entered into the biomedical and nutraceutical areas as a result of the elucidation of their biological role in certain clinical conditions as well as in normal growth and development of human beings.

1.2 Review of literature

1.2.1 Polyunsaturated fatty acids and their role in human health

There are two families of essential fatty acids (EFAs), the omega-6 and omega-3 polyunsaturated fatty acids (PUFA). They are essential because human beings cannot make them due to lack of desaturase enzymes required for their production and therefore they must be obtained from the diet. The essential fatty acids start with the short chain polyunsaturated fatty acids (SC-PUFA) i.e. linoleic acid or LA (C18:2) (ω-6 fatty acids) and α-linolenic acid or ALA (C18:3) (ω-3 fatty acids). They form the starting point for the generation of longer and more desaturated fatty acids, which are also referred to as long-chain polyunsaturated fatty acids (LC-PUFA, C18-24) and very long chain fatty acids (VLC-PUFA,C>24). The important LC-PUFA are ω-3 fatty acids e.g. eicosapentaenoic acid or EPA (C20:5), docosahexaenoic acid or DHA (C22:6) and ω-6 fatty acids e.g. gamma-linolenic acid or GLA (C18:3), dihomo-gamma-linolenic acid or DGLA (C20:3), arachidonic acid or AA (C20:4). These two families of EFA are not interconvertible, are

metabolically and functionally distinct and often have important opposing physiological functions.

The dietary balance of EFA is important for good and normal health. There are evidences that our ancestral dietary fat composition showed an omega-6: omega-3 ratio of 2:1, this same ratio is today in excess of 10:1 (Simopoulos, 1999; Simopoulos, 2006). Excessive amounts of omega-6 PUFA and a very high omega-6: omega-3 ratio, as is found in today's diets, promote the pathogenesis of many diseases, including cardiovascular disorders, cancer, inflammatory and autoimmune diseases; whereas increased levels of omega-3 PUFA (a lower omega-6:omega-3 ratio), exert suppressive effects (Simopoulos and Cleland, 2003; Harris *et al*., 2008; Horrobin, 1991). It has been observed that, in the secondary prevention of cardiovascular disorder, a ratio of 4:1 was associated with a 70% decrease in total mortality **(**Lorgeril, 1994). A ratio of 2.5:1 reduced rectal cell proliferation in patients with colorectal cancer, whereas a ratio of 4:1 with same amount of omega-3 PUFA had shown no effect on patients (Simopoulos and Cleland, 2003). Research has shown that docosahexaneoic acid (DHA), an omega-3 fatty acid found in fish oil, is essential for the development of the premature infant relative to visual acuity, visual function and maturation. In the full term infant, DHA may influence visual acuity and neural pathways associated with the developmental progression of language acquisition (Simopoulos, 2006). These findings have led to inclusion of DHA and arachidonic acid (AA), an omega-6 fatty acid, in infant formula by most countries around the world. As a consequence, dietary recommendations now include not only LA and ALA but also EPA, DHA and AA for optimal nutrition in human beings (Kris-Etherton *et al*., 2009; Calder and Yaqoob, 2009). The GLA is also nutritionally important fatty acid and low levels of GLA have been found to cause certain deficiency disorders, risks of which can be reduced by having a diet supplemented with GLA (Horrobin, 1990; Huang and Milles, 1996).

Although VLC-PUFA were discovered more than two decades ago, very little is known about their biosynthesis and functional role in human health. This is due to mainly intrinsic difficulties associated with working on these unusually long polyunsaturated hydrocarbon chains and their existence in small amounts. However a recent study

emphasized the importance of these fatty acids in maintaining the structural and functional integrity of retinal photoreceptors **(**Agbaga *et al*., 2010).

1.2.2 Sources of PUFA

Many higher plants are rich in PUFAs such as LA and ALA, but VLC-PUFA are completely absent in them. The commercial source of VLC-PUFAs are cold water marine fish (such as salmon, tuna, mackerel and sardines) but there is a growing global concern regarding the sustainability of global fish stocks because marine fish reserves are in severe decline as a result of decades of overfishing (Pauly *et al*., 2005). The primary sources for *de novo* synthesis of VLC- PUFAs are marine microalgae which form the base of an aquatic food web that culminates in the accumulation of these fatty acids in the lipids of the marine fish (Williams and Burdge, 2006). The other sources i.e. some fungi, mosses, bacteria and lower plants also have capacity to synthesize significant amounts of VLC-PUFAs (Domergue *et al*., 2005). The most obvious alternative to fish oils is via contained culture of PUFA synthesizing aquatic microbes in fermenters and their subsequent extraction for use as food supplements. However, such systems are expensive to maintain and have limited flexibility for significant scale-up and require the appropriate microbiological facilities (Lee, 2001). In view of all these factors, there is an obvious need for an alternative sustainable source of these important fatty acids and transgenic crops producing PUFAs, present a promising scenario in this direction. During the last few years, genes encoding the primary enzymes involved in the biosynthesis of these fatty acids have been successfully isolated from a range of PUFA synthesizing organisms with a number of these being heterologously expressed (singly or in combination) in oilseed crops for PUFA production (Napier and Graham, 2010).

1.2.3 Fatty acid biosynthesis in plants

To bring about genetic modifications utilizing key genes of fatty acid biosynthesis, a thorough knowledge of the fatty acid biosynthesis pathway in general and PUFA biosynthetic pathway in specific is needed. Fatty acids in cells are almost never found in the form of 'free' fatty acids. Most of the fatty acids in membranes are found esterified to glycerol; this class of lipids essentially is termed as glycerolipids. Membrane

glycerolipids have fatty acids attached to both the sn-1 and sn-2 positions of the glycerol backbone and a polar head group attached to the sn-3 position. The combination of non-polar fatty acyl chains and polar head groups leads to the amphipathic physical properties of glycerolipids, which is essential to formation of membrane bilayers. When all three positions on glycerol are esterified with fatty acids, a "triacylglycerol" structure (Figure 1.2) results that is not suitable for membranes but instead constitutes the major form of storage lipid in seeds (Ohlrogge and Browse, 1995) and interestingly, the triacylglycerol is the major component of edible seed oil of most oilseed crops.

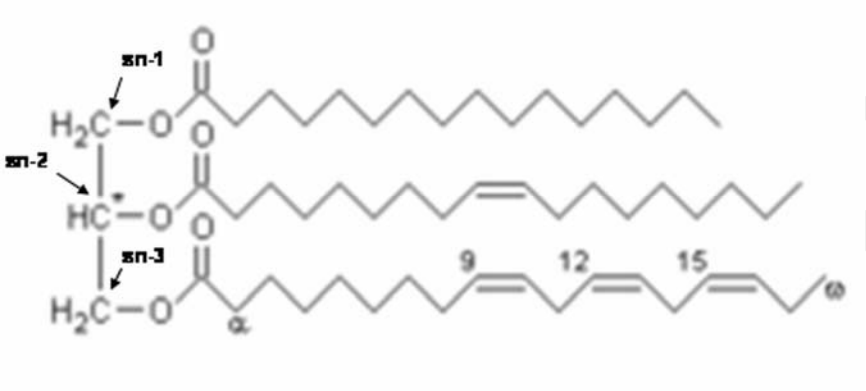

Figure 1.2: Example of an unsaturated fat triglyceride or triacylglycerol. Left part: glycerol backbone, right part from top to bottom: palmitic acid, oleic acid, α-linolenic acid; sn-1, sn-2 and sn-3 positions of the glycerol backbone are marked.

In living system, biosynthesis of fatty acids is carried out by fatty acid synthase complex (FAS) consisting of several enzymatic components. There are two types of FAS complex in living organisms with respect to organization, they are type I FAS and type II FAS Type I FAS is present within the cytoplasm of animal and yeast cells, and all the components of FAS reside in one or two multifunctional polypeptide chains. However, bacteria and plant plastids possess type II FAS where enzymes are soluble and individual units; yet both the types of FAS are functionally similar (Wakil, 1989). Plant fatty acid biosynthesis starts almost exclusively inside the plastid (Somerville and Browse, 1991), and is carried out by the FAS complex (Harwood, 1988). Newly synthesized fatty acids are then utilized directly in the plastid for the production of plastid glycerolipids (prokaryotic pathway) or exported to the cytoplasm. The cytoplasmic acyl lipid pool is

used to produce other glycerolipids via processing in endoplasmic reticulum (eukaryotic pathway) (Somerville and Browse, 1991).

Plastidial or prokaryotic pathway of fatty acid synthesis

The first committed step (step1 in Figure 1.3) in fatty acid biosynthesis is the conversion of acetyl coenzyme A (CoA) and HCO_3^- into malonyl-CoA in an ATP dependent reaction catalyzed by acetyl CoA carboxylase (ACCase). Acyl carrier protein (ACP), a small (~9 kDa) protein is a crucial cofactor in fatty acid biosynthesis.

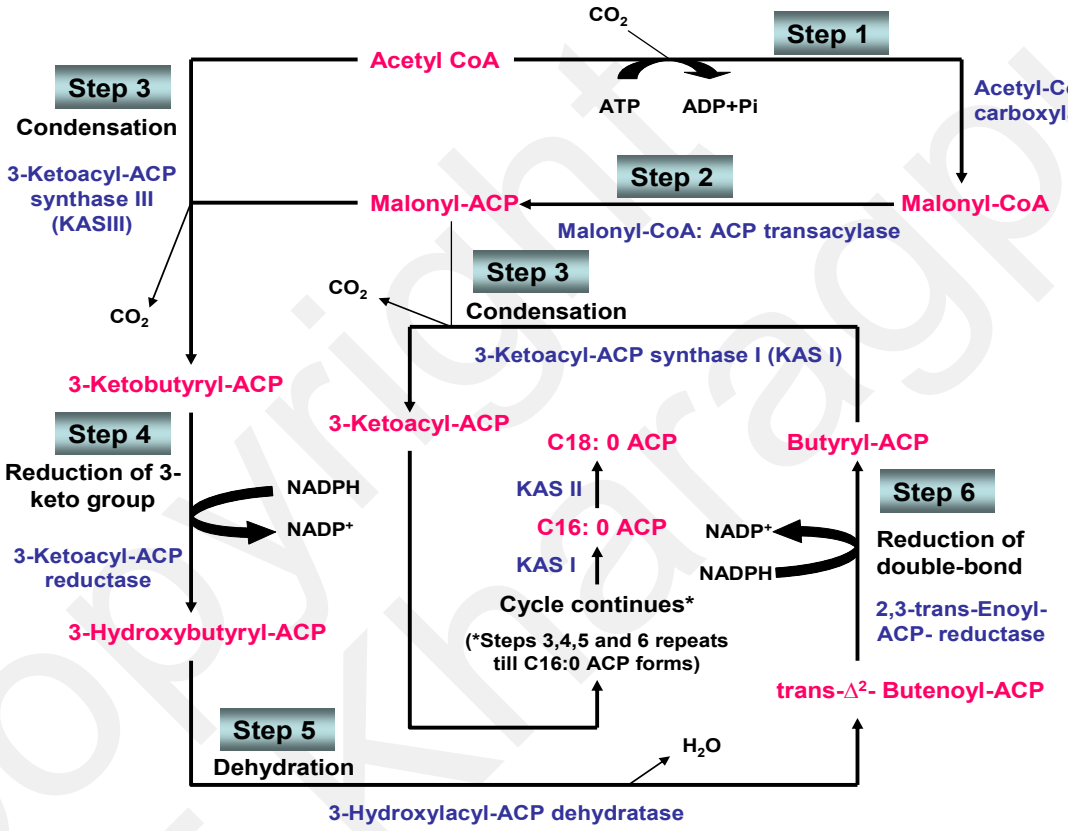

Figure 1.3: Diagram showing biochemical pathways of fatty acid synthesis in plant. [Based on Biochemistry & Molecular Biology of Plants by Buchanan et al., 2000]

For initiation of cyclic chain elongation processes, malonyl-ACP is essential and this is produced by transfer of malonyl moiety from malonyl-CoA into the 4'-phosphopantetheine-SH group of ACP through the activity of malonyl-CoA: ACP transacylase (step 2 in Figure 1.3). Each cycle of acyl chain elongation proceed by adding two carbon units (donated by malonyl-ACP) to the growing acyl chain that is bound to

ACP by fatty acid synthase (FAS) complex consisting of atleast seven enzymes (Figure 1.3). Each cycle consisted of four reactions- condensation (step 3), reduction of 3-keto group (step 4), dehydration (step 5) and reduction of double bond (step 6).

As shown in Figure 1.3, the plastidial *de novo* fatty acid synthesis usually ends at C16:0 or C18:0 and forms the total acyl-ACP pool of the plastids. It is interesting to note that the fatty acid biosynthetic pathway produces saturated fatty acids, but in most plant tissues, over 75% of the fatty acids are unsaturated (Ohlrogge and Browse, 1995). The first double bond is introduced in the saturated acyl-ACP by the plastidial soluble enzyme, steroyl-ACP desaturase. The above process i.e. the formation of the first unsaturated fatty acid (i.e. C18:1-ACP) is very crucial for formation of membrane lipids in any living system and ensures the availability of other unsaturated fatty acids that are derived from C18:1-ACP during the growth of any cell.

A portion of total acyl-ACP pool formed in the plastid is used for the biogenesis of plastidial membrane lipids through ACP-acyl transferases but the larger portion of the fatty acids from acyl-ACP pools are released from the plastid by the action of fatty acyl-ACP thioesterase enzymes. Then the acyl groups are exported to the cytoplasm through the formation of acyl-CoA esters by the activity of an enzyme, acyl-CoA synthase, located at the outer membrane of the plastids.

Cytoplasmic or eukaryotic pathway of fatty acid synthesis

Acyl-CoA pools exported to the cytoplasm undergo various types of acyl chain modifications, chain elongation, desaturation and exchange or transfer catalyzed by different enzymes bound to endoplasmic reticulum (ER) membrane which constitute the 'eukaryotic pathway' of lipid biosynthesis. Both the prokaryotic and eukaryotic pathways serve the cellular needs for membrane lipids but the eukaryotic pathway is the major route for the storage lipids (Harwood, 1996). In different plants, the different kinds of acyl chain modifications occur in cytoplasmic organelle mainly in ER while the acyl groups are normally maintained as acyl-CoA or acylglycerolipid pools. The eukaryotic pathway is of special interest in this research work since polyunsaturated fatty acids are produced by this pathway. Thus, PUFA biosynthetic pathway in plants is discussed in little detail in the following section.

1.2.4 Polyunsaturated fatty acid biosynthetic pathway in plants and microbes

The two human EFAs i.e. linoleic (C18:2) and α-linolenic acid (C18:3), from which production of other long chain PUFA (LC-PUFA) and very long chain PUFA (VLC-PUFA) takes place, are naturally synthesized in some higher plants and microbes. In general, the linoleic acid is the primary precursor molecule for the (*n*-6) family of fatty acids and the α-linolenic acid is the primary precursor molecule for the (*n*-3) family of fatty acids. These EFAs are generated from oleic acid by the introduction of double bonds between the existing double bond and the terminal methyl group by the sequential action of Δ12 and Δ15 desaturases. Infrequently in plants, a double bond is inserted between an existing double bond and the carboxyl group as in the biosynthesis of γ-linolenic acid in evening primrose and borage seed oils. Rest of the LC-PUFAs and VLC-PUFAs are naturally rarely synthesized in higher plants. They are mostly synthesized in microalgae, some fungi and other microbes where the biosynthesis of PUFAs require a sequence of chain elongation and desaturation steps, as illustrated below (Figure 1.4), and the various enzymes require the acyl-CoA esters as substrates.

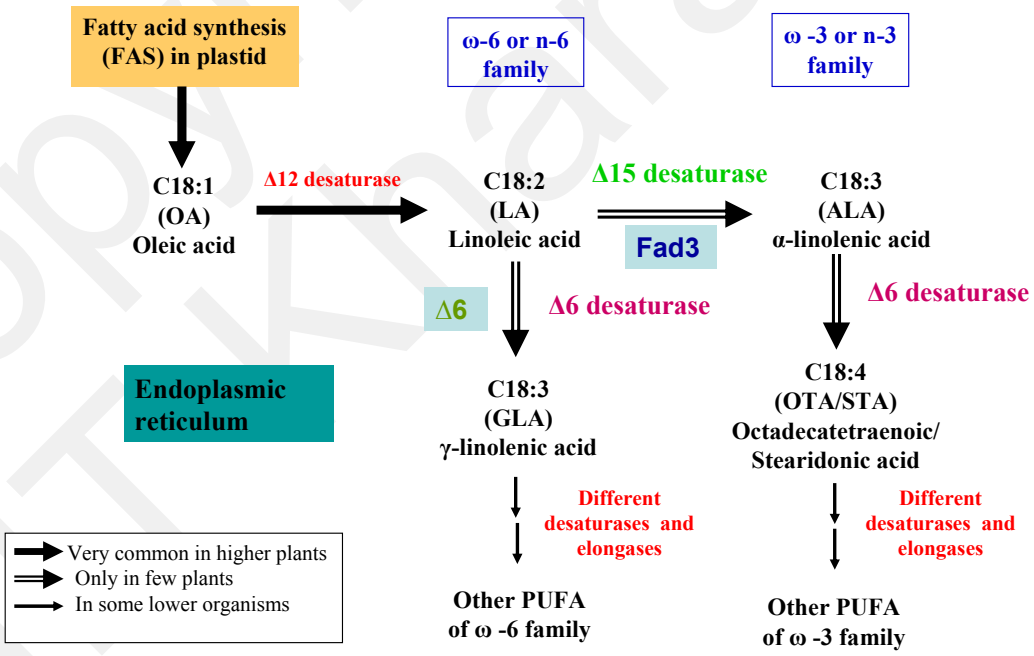

Figure 1.4: Metabolic pathway of mono- and polyunsaturated fatty acid synthesis in plants and microbes.

Recently, a novel alternative pathway for the biosynthesis of >C20 PUFAs has been described in prokaryotes and eukaryotes and this system does not require the multiple desaturase and elongase enzymes mentioned above, but instead uses a polyketide synthase-like gene cluster to synthesize PUFAs (Metz *et al.* 2001). Certain species of marine bacteria e.g. *Shewanella* sp. synthesize C_{20+} PUFAs in this way and thus represent the primary input of these fatty acids into the marine food web, which culminates in the accumulation of these PUFAs in fish oils (Napier, 2002).

1.2.5 Desaturase enzymes and their role in PUFA production

Generally in plants the fatty acid acyl carrier protein (ACP) desaturases (EC 1.14.99.6) convert saturated fatty acyl-ACPs into their *cis*-monounsaturated equivalents in an oxygen-dependent reaction (Bloomfield and Bloch, 1960; Schroepfer and Bloch, 1965; McKeon and Stumpf, 1982; Shanklin and Somerville, 1991; Thompson *et al*., 1991). The Δ9 desaturation of C18:0-ACP resulting in the formation of C18:1-ACP occurs ubiquitously in the plastids of plants and thus plays an important role in determining the fluidity of cell membranes (Buist, 2004). Plants can express distinct acyl-ACP desaturases, *e.g. Arabidopsis thaliana* has a family of seven acyl-ACP desaturases that are differentially expressed in different tissues (Shanklin and Cahoon, 1998).

Desaturase enzymes have evolved independently twice, first as the acyl-ACP desaturases which are soluble enzymes found in the plastids of higher plants, whereas the second is a more widespread class of integral membrane desaturases found in endomembrane systems in prokaryotes and eukaryotes. The soluble desaturases use acyl-ACP thioesters as substrates and NADPH: ferredoxin reductase and ferredoxin as electron donors. The membrane bound desaturases use the acyl moeity esterified to complex lipids as substrate and NADPH: cytochrome b_5 oxidoreductase and cytochrome b_5 as electron donor (Shanklin and Cahoon,1998) The membrane bound desaturases can be further divided into two groups: carboxyl–directed desaturases and methyl-directed desaturases. The carboxyl-directed desaturases are also known as 'front end desaturases' which introduce a new double bond between the existing double bond and carboxyl-terminus of the fatty acyl chain e.g. Δ6 desaturases. Methyl-end directed desaturases introduce a new double bond between the existing double bond and methyl-terminus of the fatty acyl chain e.g

Δ9, Δ12, Δ15 desaturase (Sayanova *et al*., 1997, Qui *et al*., 2001). These methyl-directed and front-end desaturases are the key enzymes in production of polyunsaturated fatty acids (PUFA), which have wide commercial importance.

1.2.6 Genetic engineering approach for seed oil modification

Alteration of fatty acid composition of seed oil in a defined and accurate manner was possible with the advent of rDNA technology coupled with the knowledge of the biochemical pathways and the genes involved in lipid metabolism. Through expression of suitable transgene(s) or silencing of endogenous gene(s), many successful attempts have been made to alter the fatty acid profile in plants for edible oil quality improvement or for industrial applications (Thelen and Ohlrogge, 2002). For example, California bay and *Cuphea* seeds accumulate up to 90% short chain saturated fatty acids as triacylglycerol. On expression of a California bay thioesterase in the seeds of non-laurate (12:0)-accumulating plants, *Arabidopsis* and *Brassica napus* (rapeseed), resulted in the ‘‘short-circuiting’’ of acyl chain elongation to produce laurate up to 24 and 58% of total seed fatty acids, respectively (Voelker *et al.*, 1992; Voelker *et al.*, 1996).

Similarly for industrial applications, Jojoba was found to be interesting in being the only plant species known to accumulate waxes (up to 60% dry weight) rather than triacylglcerol (TAG) as a seed storage reserve. Coordinated expression of three genes—a *Lunaria annua* long-chain acyl-CoA elongase, a jojoba reductase and an acyltransferase in *Arabidopsis* resulted in wax production up to 70% of the lipid present in mature seeds (Lardizabal *et al.*, 2000). If this trait can be successfully transferred to commercial crops this would represent a large potential source of waxes for a variety of applications, including cosmetics and industrial lubricants.

Recent efforts to produce unusual fatty acids in engineered seeds for novel industrial oils have focused largely on divergent forms of the Δ12-oleic acid desaturase (Fad2) (Cahoon and Kinney, 2005). The typical Fad2 enzyme catalyzes the introduction of the cis-Δ12 double bond in oleic acid to produce linoleic acid. However, divergent forms of Fad2 have been identified in seeds from several non-agronomic species that catalyze a remarkably wide range of fatty acid modifications, including hydroxylation, epoxygenation, and double bond conjugation (Cahoon and Kinney, 2005). For example,

divergent Fad2 enzymes termed 'conjugases' can catalyze the formation of conjugated double bonds, to generate seed oils with improved drying properties for paint, ink and other coating applications. The transfer of a Fad2 conjugase from pot marigold (*Calendula officinalis*) to soybean, for example, produced seed oils that contain 20% of the unusual conjugated fatty acid, calendic acid (18:3 $^{\Delta 8trans, \Delta 10trans, \Delta 12cis}$) (Cahoon *et al*., 2006). On the other hand, the seed oil of flax (*Linum usitatissimum*) is notable for its high level of linolenic acid, generally around 45% to 65%, which gives it a high drying quality, making it useful for industrial purposes. The development of low-linolenic acid flax lines (Green, 1986; Rowland, 1991), known as 'solin' or 'linola' types, expanded the potential markets for flaxseed oil but the molecular mechanism underlying this trait was not known for long. Two independently inherited genes, *LuFAD3A* and *LuFAD3B* that encode desaturases capable of desaturating linoleic acid were identified as a step towards understanding the microsomal omega-3 desaturases (Vrinten *et al*., 2005).

The production of LC-PUFAs in oilseed crops has been an even greater challenge because of the apparent complexity of the biosynthetic pathways for these fatty acids. As the oils from most crop species are enriched in linoleic acid, the production of EPA requires the introduction of at least four genes that encode Δ15-, Δ6-, and Δ5- desaturases and a fatty acid elongating enzyme (ELO). DHA production requires an additional Δ4 desaturase and a second ELO for elongation of the C20 fatty acid precursor (Wu *et al*., 2005). To date, the genes for these enzymes have been isolated from several marine algal, thraustochytrid, mammalian, and fungal sources (Robert *et al*., 2005; Wu *et al*., 2005; Abbadi *et al*., 2004), and engineering of these pathways into *Brassica juncea* seeds, for instance, has yielded EPA and DHA levels of 8% and 0.2% of the total fatty acids, respectively (Wu *et al*., 2005). Similar experiments conducted in Arabidopsis generated seed oils that contained 2.4% EPA and 0.5% DHA (Robert *et al*., 2005).

The above studies imply that the pathways of plant lipid metabolism can be genetically modified to produce designer fatty acids in the seed oils of many crops. However, the desired goals in all cases can be affected or be successfully achieved depending upon the candidate genes (in few cases single gene is enough and other cases many genes are needed to produce the desired trait in the plants) and target crops. The choice of target crop plant is important since the endogenous biochemical machinery and the metabolites

of the selected plant may not be amenable to the fatty acid profile modifications expected by introgression of these foreign genes into their genome. In the present research work, investigations were made in isolating suitable genes encoding desaturases for PUFA production and identifying a target crop which support the PUFA generation in its seed oil.

1.2.7 Sesame (*Sesamum indicum)* crop as the target oilseed crop for PUFA production with suitable heterologous desaturases

There is a wide range of oilseed crops grown throughout the world in different agro-climatic zones yielding different qualities of vegetable oils e.g. mustard, groundnut, soybean, sunflower, sesame, olive, rice bran, cotton seed and corn oil. Inhabitants of any region decide on the edible oil of their choice depending on the availability, cost and culinary preferences. For example, in India, coconut, groundnut and sesame oil is preferred in South and West whereas mustard (rapeseed oil) is chosen as edible oil in Eastern and Northern parts of India.

Sesame (*Sesamum indicum* L.) belongs to family Pedaliaceae and is an important oilseed crop and predominantly cultivated in India, China, Myanmar, Sudan, Egypt and other African countries. It is grown primarily for its nutritious seed that is rich in linoleic acid, protein and calcium as well as vitamin E and small quantities of vitamin A, B_1 and B_2 (Morris, 2002). Nearly 70% of the world's sesame seed is processed into edible oil and meal for animal feed while the remainder is chanelled for food and confectionary industries (Morris, 2002).

Sesame seed has a relatively superior oil quantity as well as quality in comparison to many oilseed crops. The oil content ranges from 34.4% to 59.8% in different cultivars, the average is generally about 50% of the seed weight (Ashri, 1989). However, values upto 63.2% have been reported in some varieties (Baydar *et al*., 1999). Both genetic and environmental factors influence the oil content in sesame seeds. The oil contains four major fatty acids namely palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic acid (C18:2) with oleic and linoleic acid occurring in nearly equal amounts, constituting about 85% of the total fatty acids (Ashri, 1998; Were *et al*., 2006). Despite having a high content of linoleic acid, sesame oil is unusually stable to oxidation during storage

compared to other vegetable oils with a similar fatty acid composition. This feature is attributed to antioxidant activities of sesamol and sesaminol together with tocopherols present in the oil (KamalEldin an Appelqvist, 1994). A combination of the high stability and a nutritionally acceptable fatty acid profile contributes significantly to the excellent oil quality making it high value edible oil. Having a relatively high proportion of linoleic acid (C18:2) in its seed oil, sesame could be a desirable target crop for introduction of a suitable desaturase gene for endogenous production of other PUFAs that are derived from linoleic acid for human health benefit. Thus, in the present study, attempts have been made to isolate, clone and characterize two desaturase genes, the products of which are suitable for PUFA generation.

However, there are some problems limiting sesame production e.g. plant diseases that reduces its yield potential, indeterminate flowering, dehiscent capsules and insufficient diversity in oil composition. Cultivation of sesame crop suffers from considerable yield loss because of pathogenic diseases like Phytophthora blight and root/stem rot (Gangopadhyay *et al*., 1998). In addition, it is difficult to determine the time of harvest of sesame crop to maximize the yield because plant growth and flowering is indeterminate in nature and capsules or pods dehisce spontaneously when mature (Day, 2000). Thus, an interdisciplinary concerted effort with the participation of both conventional breeding and genetic transformation is urgently required for germplasm improvement of sesame in order to harness potential benefits of this crop.

For genetic modification of sesame, a highly reproducible plant regeneration protocol is necessary. However, very little information is available on sesame regarding this aspect and is found to be highly recalcitrant in nature. The early reported studies on tissue culture in sesame were that of Lee *et al*., (1985) and George *et al*., (1987) who have established *in vitro* cultures from different parts of sesame plant. Kim *et al*., (1987) studied on the effect of explants and hormone combinations on callus induction. Chae *et al*., (1987) have established herbicide resistant cell lines of sesame without achieving the plant regeneration. Lee *et al*., (1988) investigated the effect of growth regulators on callus induction and organogenesis from different explants of sesame. Somatic embryogenesis from hypocotyl segments (Mary and Jayabalan, 1997) and cotyledon, root and subapical hypocotyl segments from young seedlings (Zeevaart and Creelman, 1988, Seo *et al*.,

2007) of *S. indicum* has been reported. However, Xu *et al*., (1997) reported that plant conversion rate from somatic embryos was very low (less than 12-13%). Multiple shoot induction from seedling shoot tips (George *et al*., 1987; Rao and Vaidhyanath 1997) and nodal segments with axillary buds (Gangopadhyay *et al*., 1998) also have been reported; however, these protocols are hardly applicable for production of transgenic plants because of low transformation frequency. Studies have been also carried out on micropropagation of sesame from leaf disc culture (Sharma and Pareek 1998). It has also been reported that somatic embryos could be obtained from zygotic embryos (Ram *et al*., 1990). Recently, a few attempts have been made for *in vitro* regeneration of sesame from hypocotyls and cotyledonary explants (Rao and Vaidhyanath, 1997b; Younghee, 2001; Were *et al*., 2006), the success of regeneration was low in most cases**.** There are no established reports for either stable or transient gene expression protocol in sesame. Therefore, the present research work also includes optimization of parameters for *in vitro* plantlet formation as well as developing methods for *Agrobacterium*-mediated transformation of sesame leading to transient gene expression.

1.3 Objectives of the present study

The overall goal of this research program is to isolate and clone suitable desaturase gene(s) from plants, that could be transgenically introduced into sesame crop in future for production of other health beneficial PUFAs that are derived from the linoleic acid which is naturally high in sesame oil. Based on above literature review and the target goal of the overall program, the present research work had the following objectives:

1. Isolation, cloning and characterization of a putative microsomal *Fad3* desaturase gene by heterologous expression in model plant tobacco.
2. Isolation, cloning and characterization of a putative Δ6 desaturase gene by heterologous expression in the tobacco plant.
3. Optimization of methods for *in vitro* plantlet regeneration and transient gene expression in sesame plant.