
**Molecular Strategy for Development
of Value-Added Sesame Variety**

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Abstract

There are two groups of significant functional constituents in sesame seeds on the whole; one is the vegetable oils and another is the anti-oxidative compounds. However, although high amounts of major fatty acids are synthesized in sesame seeds, their composition is unfavorable because the contents of alpha- and gamma-linolenic acid, the essential fatty acids, are very low or do not produced in sesame seeds. So, to increase these fatty acids in sesame seeds, one strategy is to overexpress their genes, ω -3 fatty acid desaturase for alpha-linolenic acid and delta-6 fatty acid desaturase for gamma-linolenid acid, in them. Another molecular target is to enhance alpha-tocopherol, vitamin E, because its content is very low in sesame seeds. The enzyme, gamma-tocopherol methyltransferase, catalyzes the conversion of gamma-tocophero to alpha-tocopherol. Overexpression of this enzyme in sesame seeds could be also a good molecular breeding target. Reduction of phytic acid is also another molecular target in sesame seeds because phosphorus pollution may be caused by its high content in sesame seeds. Accordingly, to do so, one of target enzymes could be myo-inositol 1-phosphate synthase which is a key regulatory enzyme in the pathway of phytic acid biosynthesis. In this lecture, a molecular strategy for development of value-added sesame crop is described in association with some results of our experiments involved in the molecular characterizations of the genes mentioned above.

Introduction

Sesame (*Sesamum indicum* L.) has long been cultivated from ancient times and used as one of most important plant sources for production of high quality vegetable oils (Chung et al., 1995) or medicinal plant resources in the Oriental countries (Izawa, 1980). Some investigations on sesame crop have also shown that it can be used as functional food for human health, which enhances various human metabolic activities such as anti-oxidative activity and anti-aging effect (Namiki, 1995; Yamashita et al., 1995). In Korea, the sesame seed oils have been used as one of the most essential food spices. Recently, the molecular analysis of lipid biosynthesis provided us with a basic knowledge of molecular mechanisms involved in lipid biosynthesis of oilseeds, further

applying them to molecular breeding for genetic improvement of sesame crop (Alonso and Maroto, 2000; Opsahl-Ferstad, et al., 2003; Qi et al., 2004). As one of the future promising candidates for seed oil resources, the sesame oilseeds may be important in plant oil production because it has been known that the oils extracted from sesame oilseeds are relatively superior in oil quantity and quality to those from other species (Izawa, 1980). In sesame oilseeds, about 45-60% by seed weight consists of lipid and 18-20% comprise proteins (Izawa, 1980), which are deposited in membrane-bound organelles, lipid bodies and protein bodies, respectively (Stymne and Stobart, 1987). In addition, anti-oxidative compounds have been identified in the forms of lignophenols and carboxyphenols and phytic acid as the minor ingredients in sesame seeds, which reveal the high resistance to lipid peroxidation. Their anti-oxidative activity not only protects the sesame seed-oils from oxidation reaction caused by the air but also can be beneficial to human health (Namiki, 1995). In particular, the content of phytic acid in sesame seeds is relatively higher than those of other crops (Lott et al., 2002). Accordingly, in point of functional food sources, there are two groups of significant functional constituents in sesame seeds on the whole; one is the vegetable oils and another is the anti-oxidative compounds. However, although high amounts of major fatty acids are synthesized in sesame seeds, their composition is unfavorable because the content of linolenic acid, an essential fatty acid, is very low (Chung et al., 1995). So, this problem may be solved by molecular breeding technology for genetic improvement of sesame crop. Another interesting target in molecular breeding of sesame seeds is to overexpress the gamma-tocopherol transferase to enhance the biosynthetic activity in sesame seeds because the content of alpha-tocopherol, which shows the highest vitamin E activity and is beneficial to human health (Shintany and DellaPenna, 1998), is very low in them. One another interesting target is to reduce the content of phytic acid because the phytic acid undigested in the stomach of the monogastric animals may cause phosphorus pollution when the pomace of sesame seeds is fed for them (Wodzinski and Ullah, 1996).

An increase of the functional value of sesame seeds is important to the sesame growers and vegetable oil industries as well. Accordingly, some molecular technologies of sesame breeding to develop value-added sesame variety are described in association with the results from our experiments in this lecture.

◆ Two target genes for producing gamma-and alpha-linolenic acid in sesame oilseeds

In mammals, gamma-linolenic acid is formed from linoleic acid (18:2) by catalysis of $\Delta 6$ desaturase. Dietary gamma-linolenic acid may alleviate a variety of human diseases such as hypercholesterolemia, certain cancer and other clinical disorders (Huang and Mills, 1996). In

sesame seeds, this fatty acid is not synthesized. In contrast, alpha-linolenic acid is an essential fatty acid, which is synthesized by the action of omega-3 fatty acid desaturase. This fatty acid is not produced by the body and must be present in the diet to maintain health. In sesame seeds, very low amount of alpha-linolenic acid is produced (Chung et al., 1995). Accordingly, the genes encoding these two enzymes mentioned above would be good candidates for molecular breeding of sesame crop to increase the contents of the two fatty acids in sesame seeds.

1. Delta-6 fatty acid desaturase gene

Gamma-linolenic acid is synthesized in vivo from linoleic acid (18:2, $\Delta^{9, 12}$) by enzymatic desaturation of delta-6 fatty acid desaturase (Huang and Mills, 1996). The several isoforms of gene encoding this enzyme were isolated from the cyanobacterium *Synechocystis* sp. and some plant species such as borage, evening primrose and black current (Huang and Mills, 1996). Reddy and Thomas (1996) created transgenic tobacco plants producing gamma-linolenic acid by transforming them with a cyanobacteria-derived delta-6 desaturase gene. Although significant quantities of linoleic acid, which is the substrate for delta-6 desaturase, are produced in sesame oilseeds (Table 1), gamma-linolenic acid is not produced in them because probably of lack of

Table 1. This table indicates the amount of 5 species of fatty acids, which would be the major components of sesame seed oils

DAF ^a	C16:0	C18:0	C18:1	C18:2	C18:3
mg/g dry seed					
9	t ^b	T	t	t	t
12	0.7	0.2	2.0	1.6	0.3
15	3.0	1.0	9.1	8.4	0.8
18	4.9	2.1	20.3	18.9	0.8
21	6.4	2.9	24.7	23.8	0.9
24	8.0	4.1	37.9	35.7	1.0
27	12.0	6.3	59.3	63.0	1.4
30	13.7	8.0	79.3	79.6	1.3
33	16.0	8.4	81.5	94.2	1.9
36	16.9	10.3	97.3	111.7	1.4
39	17.1	10.4	99.1	110.6	1.5
42	17.2	10.6	100.6	108.5	1.6
D ^c	36.0	23.9	209.6	221.2	2.1

Note: The table also shows that as the sesame oilseeds develop, the rate of synthesis of oleic (18:1) and linoleic acid (18:2) was relatively much greater than that of other fatty acids. Each value is the mean of at least 3 independent preparations. ^adays after flowers, ^btrace amount (< 0.01 mg/g dry seed), ^cdry seed.

action of the delta-6 desaturase gene. So, this desaturase gene could be a good target gene for development of a sesame variety producing the nutritionally important gamma-linolenic acid in sesame oilseeds.

2. Omega-3 fatty acid desaturase gene (*FAD 3*)

Omega-3 fatty acid desaturases, which are plastid- or microsome-derived, are the enzymes to synthesize alpha-linolenic fatty acid (18:3) and located in the chloroplast membrane or endoplasmic reticulum (ER) membrane, respectively (Ohlrogge and Browse, 1995). In dry sesame, about 50~60% of sesame oil is composed of four major species of fatty acids, palmitic acid, (16:1), stearic acid (18:0), oleic acid (18:1) and linoleic acid (18:2). However, although it has been known that oils from sesame oilseed have good quality, the content of linolenic acid (18:3), which is an essential fatty acid and beneficial to human health (Holman, 1992), is very low in sesame oilseed (Table 1). Omega-3 fatty acid desaturase gene (*FAD 3*), therefore, for enhancement of this fatty acid in sesame oilseed could also be a good target gene for molecular breeding of sesame crop. We have isolated a seed-specific omega-3 fatty acid desaturase gene (*FAD3*) from developing perilla oilseeds and characterized (Chung et al., 1999).

2-1. Characterization and seed-specificity of *FAD3* gene

Perilla plant species has been used as plant resources for linolenate production in Oriental countries from ancient times because the seeds of this plant species synthesizes high amount of alpha-linolenic acid (65-70% of total fatty acid consists of alpha-linolenic acid). So, the perilla developing seeds were used to isolate *FAD3* gene. The cDNA encoding omega-3 fatty acid desaturase was assumed to be a new isoform of microsomal omega-3 fatty acid desaturase because the deduced amino acid sequence of this cDNA polypeptide showed high sequence identity and similarity with those of other microsomal omega-3 desaturase proteins (Chung et al, 1999). In addition, accumulation of mRNA for this cDNA revealed seed-specific expression pattern (Fig. 1).

◆ Gamma-tocopherol methyltransferase (gamma-TMT) gene: A target gene for high expression of gamma-TMT to increase the content of alpha-tocopherol in sesame oilseeds

Gamma-TMT is an enzyme catalyzing the reaction of S-adenosylmethionine dependent methylation in the conversion of gamma-tocopherol to alpha-tocopherol, so-called vitamin E. This enzyme leads to alpha-tocopherol as the final product in the biosynthetic pathway of tocopherol synthesis in higher plant chloroplasts (Hess, 1993). Of the tocopherols, alpha-tocopherol is the major constituent and contains the fully substituted benzoquinone ring, and is identified as

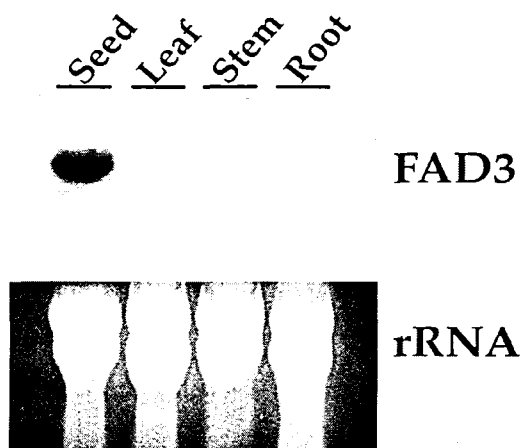


Fig. 1. Northern hybridization analysis of total RNAs prepared from developing perilla seeds, leaves, roots and stems. Approximately 20 ug of total RNAs per lane were separated on a 0.8% agarose gel containing 15% formaldehyde.

5,7,8-trimethyl tocol. In higher plants, alpha-tocopherol is synthesized in chloroplasts and proplastids. Generally, alpha-tocopherol is considered to be an effective quenching agent for both singlet O₂ and for alkyl peroxides and thus the most important to human health because of its highest vitamin E activity. Accordingly, to enhance function of food crops such sesame oils, an increase in the alpha-tocopherol content could be one of the major genetic targets to create functional food crops. In particular, the content of alpha-tocopherol in sesame oilseeds is very low (Table 2) and thus the gene for gamma-TMT would also be a good target for molecular breeding of sesame crop.

Table 2. The content of α -tocopherol in various crops

Crops	Tocopherol(mg/100g oils)	
	Total	α -TE
Corn	78~109	20~34
Sesame seeds	50~81	0
Rice	9~102	0.9~41
Peanut	37	16
Sunflower	46~67	35~63
Soybeans	90~110	20~31
Cotton seeds	78	43
Saffloweres	49~80	41~46

◆ **Myo-inosito 1-phosphate synthase (MIPS) gene: A target gene for lowering phytate in sesame seeds**

Myo-inositol-1-phosphate synthase (MIPS) catalyzes glucose-6-phosphate to *myo*-inositol-1-phosphate which is the first product in the biosynthetic pathways of *myo*-inositol, phytic acid and other essential cellular components (Loewus and Murphy 2000). MIPS plays important roles

in various essential metabolisms of both eukaryotic and prokaryotic organisms (Majumder et al. 1997). In plant, such roles involve the metabolic processes of inositols and inositol lipids (Loewus and Murphy 2000), and the biosynthesis of phytates in plant seeds (Hegeman et al. 2001). Phytic acid is a strong chelator of divalent minerals such as copper, calcium, magnesium, zinc and iron. The ability of phytic acid to chelate these minerals was recognized as a potential concern in animal and human mineral nutrition (Sathe and Reddy, 2002). The possible beneficial effects of food phytate include lowering of serum cholesterol and triglycerides, and protection against certain diseases such as cardiovascular diseases, renal stone formation and certain types of cancers (Sathe and Reddy, 2002). In the case of livestock production, however, the concerns over seed phytic acid are relatively problematic because monogastric livestock such as poultry, swine and fish, excrete all seed phytic acid contained in the feed, leading to phosphorus pollution and to the resulting eutrophication of surface waters (Raboy, et al., 2002). In general, the pomace of the sesame oilseeds after extraction of sesame oils has been used as a feed for the monogastric livestock. This may result in phytic acid problem because high amount of phytic acid is produced in sesame seeds (Table 3).

Consequently, to solve this problem, one genetic approach is to create low phytic acid sesame crop by such a molecular breeding technology as antisense technique using myo-inositol-1-phosphate synthase because this enzyme catalyzes the key committed step in the biosynthetic pathways of phytic acid (Loewus and Murphy, 2000). Recently, we isolated and characterized the gene coding for myo-inositol-1-phosphate synthase from developing sesame seeds (Chun et al., 2003) and introduce here some important results.

Table 3. Comparison of phytic acid content in various crops

Plant	Structure	% PA
Sesame	Dry seed	4.71
Pumpkin/squash	Embryo	4.08
Flax(linseed)	Dry seed	3.69
Rapeseed(canola)	Dry seed	2.50
Sunflower	Embryo	2.10
Brazil and other tree nuts	Embryo	1.80
Peanut	Seed in shell	1.70
Tomato	Seed only	1.66
Soybean	Dry seed	1.55
Peas	Dry seed	1.00

► Characterization of sesame *myo*-inositol-1-phosphate synthase cDNA (*SeMIPS*)

The *SeMIPS1* protein was highly homologous with those from other plant species (88~94%), while a much lower degree of sequence homology (53~62%) was found with those of other organisms such as human, mouse, algae, yeasts, *Drosophila*, bacteria and other prokaryotes Table 4).

Table 4. Sequence comparison of deduced amino acids and the conserved domains

Sources	Amino acid (%)	Conserved sequences in four functional domains			
	Identity (Similarity)	Domain 1	Domain 2	Domain 3	Domain 4
Sesame (AF284065) ^a	100 (100)	68 ^b GWGGNNG	228 ^b LNTANTERY	280 ^b NGSPQNTFVPGI	334 ^b SYNHLGNNDG
Tobacco (AB009881)	94 (97)	68 GWGGNNG	228 LNTANTERY	280 NGSPQNTFVPGI	334 SYNHLGNNDG
Wheat (AF120146)	92 (94)	68 GWGGNNG	228 LNTANTERY	280 NGSPQNTFVPGI	334 SYNHLGNNDG
Rape (U66307)	91 (95)	68 GWGGNNG	228 LNTANTERY	280 NGSPQNTFVPGI	334 SYNHLGNNDG
Iec plant (U32511)	91 (95)	70 GNGGNNG	230 LNTANTERY	282 NGSPQNTFVPGI	336 SYNHLGNNDG
Kidney bean (U38920)	90 (95)	69 GVDGNNG	229 LNTANTERY	281 NGSPQNTFVPGI	335 SYNHLGNNDG
Duckweed (Z11693)	89 (96)	68 GNGGNNG	228 LNTANTERY	280 NGSPQNTFVPGI	334 SYNHLGNNDG
Citrus (Z32632)	89 (94)	68 GNGGNNG	228 LNTANTERY	280 NGSPQNTFVPGI	334 SYNHLGNNDG
Rice (AB012107)	89 (94)	68 GNGGNNG	228 LNTANTERY	280 NGSPQNTFVPGI	334 SYNHLGNNDG
Barley (AF056325)	89 (94)	68 GNGGNNG	228 LNTANTERY	280 NGSPQNTFVPGI	334 SYNHLGNNDG
Maize (AF056326)	88 (94)	68 GNGGNNG	228 LNTANTERY	280 NGSPQNTFVPGI	334 SYNHLGNNDG
Fruit fly (AF071103)	62 (77)	68 GNGGNNG	229 LNTANTERY	281 NGSPQNTFVPGI	335 SYNHLGNNDG
Mouse (AF288525)	60 (78)	65 GNGGNNG	226 LNTANTERY	277 NGSPQNTFVPGI	331 SYNHLGNNDG
Human (AF220530)	60 (77)	65 GNGGNNG	226 LNTANTERY	277 NGSPQNTFVPGI	331 SYNHLGNNDG
<i>Leishmania</i> (U91965)	56 (72)	63 GNGGNNG	226 LNTANTERY	278 NGSPQNTFVPGI	332 SYNHLGNNDG
<i>Entamoeba</i> (Y11270)	54 (74)	61 GNGGNNG	222 LNTANTERY	274 NGSPQNTFVPGI	328 SYNHLGNNDG
<i>Plectia</i> (AF078915)	53 (71)	68 GVDGNNG	239 LNTANTERY	291 NGSPQNTFVPGI	345 SYNHLGNNDG

^a Numbers in parentheses denote the Genbank accession numbers.

^b The numbers on the left of the sequences indicate the residue position

► Yeast-based complementation assay

A yeast-based complementation assay system confirmed that the *SeMIPS* gene encodes a *myo*-inositol-1-phosphate synthase (MIPS) of sesame by showing functional expression of the *SeMIPS1* cDNA in yeast mutants containing the disrupted *INO1* gene for yeast MIPS (Fig. 2).

► Organ-specific expression of *SeMIPS* gene

Northern blot indicated that the expression of the *SeMIPS1* gene might be organ-specifically regulated (Fig. 3).

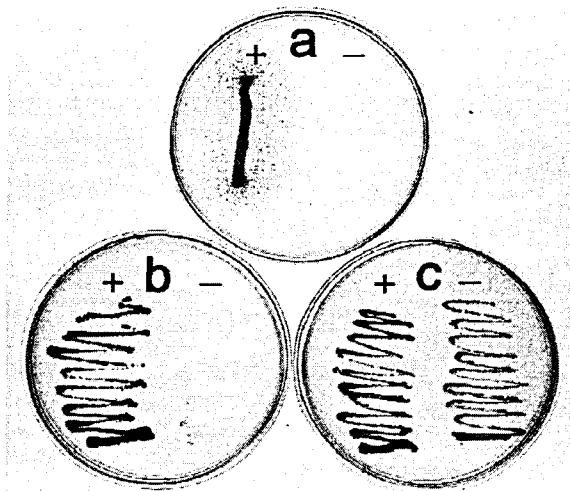


Fig. 2. Complementation analysis of the inositol auxotrophic yeast mutants, SH227 strain and SH306 transformants. a: Growth (red colonies) of the SH227 strain surrounding the SH306 transformants (a rod-like shape) transformed with the *SeMIPS* cDNA vector (pRS426GPD-*SeMIPS*) on the inositol lacking medium (plus sign) and no growth of both the SH227 strain and SH306 transformants with the control vector (pRS426GPD) on the same medium (minus sign). b: Growth of the SH306 transformants harboring the *SeMIPS* cDNA vector on the inositol lacking medium (plus sign). c: Growth of two types of transformants each transformed with the *SeMIPS* cDNA vector (plus sign) and with control vector (minus sign) on the inositol containing medium.

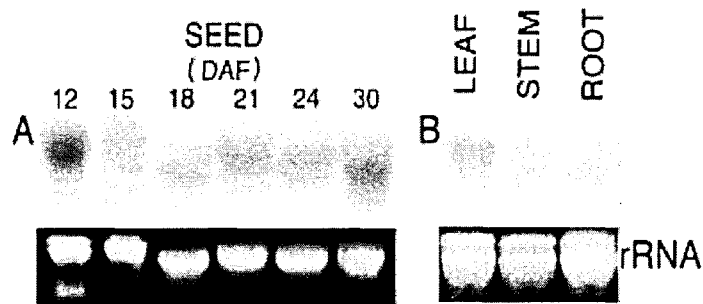


Fig. 3. Differential transcription of the *SeMIPS* gene in different organs of sesame. The full-length *SeMIPS* cDNA was used as a probe to hybridize total RNAs extracted from each tissue. A: Transcription patterns of the *SeMIPS* gene in developing sesame seeds from 13 DAF to maturity (M). B: Transcription patterns of the *SeMIPS* gene in leaf, stem and root of sesame.

► Effect of salt stress on transcription of the *SeMIPS* during sesame seed germination

Salt stress during sesame seed germination had adverse influence on transcription of the *SeMIPS*

gene and greatly reduced its transcript levels as the time exposed to saline environment lengthened and NaCl concentration increased. Germination initiation of sesame seeds was severely delayed as NaCl level increased. These results suggested that the expression of the *SeMIPS* gene is down-regulated by salt stress during sesame seed germination (Fig. 4).

► Antisense technique for application of *SeMIPS* gene to create low phytic acid sesame crop

Antisense *SeMIPS* RNA can be synthesized by the general method. If the coding region of the

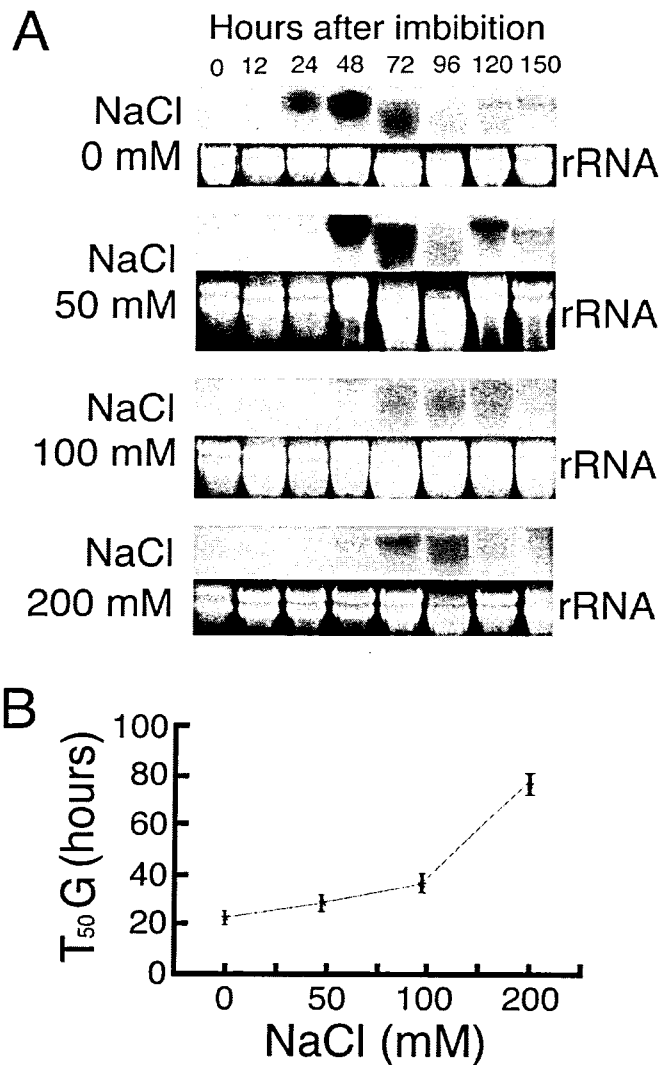


Fig. 4. Effects of NaCl stress on transcription of the *SeMIPS* gene during sesame seed germination and on germination rates of sesame seeds. A: Effect of NaCl treatment at different concentrations of 50, 100 and 200 mM on transcription of the *SeMIPS* gene from 0 to 150 HAI during sesame seed germination. B:: Effect of salt stress at the same levels of NaCl on germination rates of sesame seeds. Three replications were applied for the tests and their mean values (mean±SE) are indicated.

full-length *SeMIPS* cDNA is fused to its promoter in reverse orientation (Fig. 5), the antisense strand of the DNA will be transcribed to produce an antisense RNA. Transformation of sesame with antisense *SeMIPS* cDNA will specifically block the translation of the mRNA corresponding to the *SeMIPS* gene. This expression construct will be contributed to reduction of phytic acid in sesame seeds.

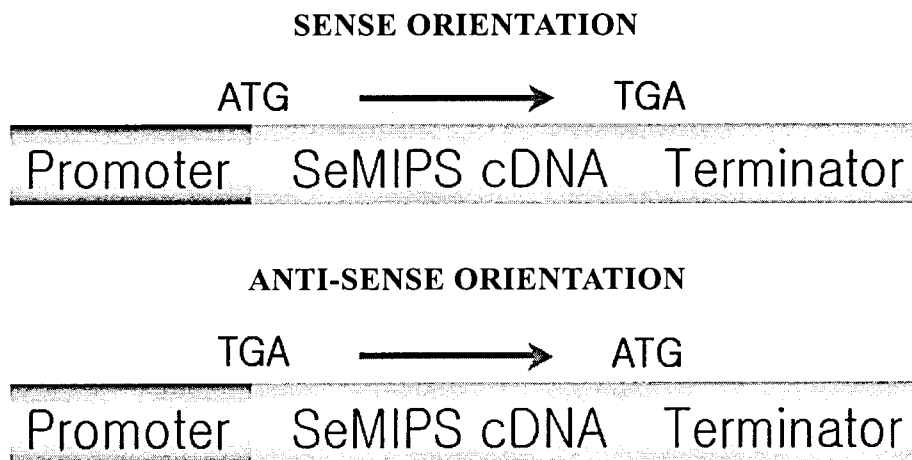


Fig. 5. Structure of *SeMIPS* gene expression vectors in sense and antisense orientation.

◆ Over-expression of the target genes in sesame seeds: Isolation of a promoter expressed only in sesame seeds

In general, expression of a foreign gene is dependent on the gene being surrounded by a collection of signals that can be recognized by the host organisms. These signals advertise the presence of the gene and provide instructions for the transcriptional and translational apparatus of the organisms. The three most important signals are; promoter, terminator and ribosome binding site. The promoter is the critical component of an expression vector because it controls the very first stage of gene expression and determines the rate at which mRNA is synthesized (Brown, 1995). So, we have isolated a sesame seed-specific promoter which controls the expression of ω -6 desaturase gene of sesame seeds and characterized the gene (Jin et al., 2001).

► Seed specificity of ω -6 fatty acid desaturase expression in sesame seeds

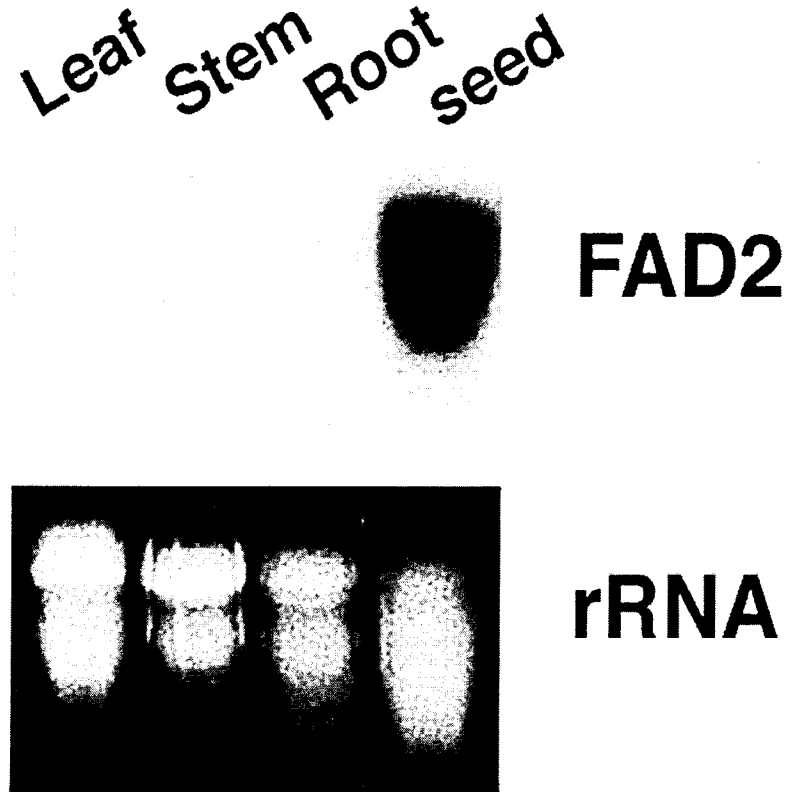


Fig. 6. Northern hybridization analysis of total RNAs. Total RNAs prepared from seeds, roots, stems and leaves of sesame were resolved on a 0.8% agarose gel containing 15% formaldehyde and transferred to a nylon membrane. Then the membrane was hybridized with the radiolabeled cDNA probe as performed generally.

► Structure of seed-specific sesame ω -6 fatty acid desaturase promoter (Fig. 7)

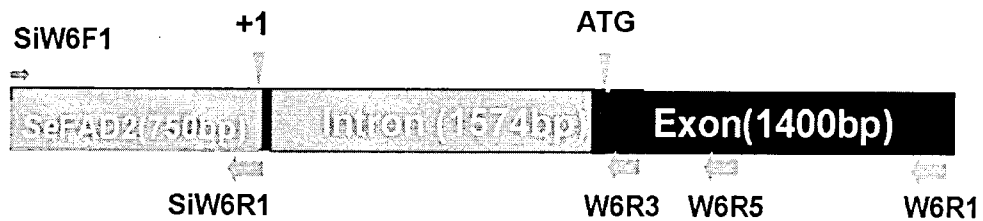


Fig. 7. Structure of sesame ω -6 fatty acid desaturase promoter. The size of the promoter and intron are ca. 750 bp and 1574 bp, respectively.

► Seed-specific expression of GUS by control of sesame ω -6 fatty acid desaturase promoter (Fig. 8)

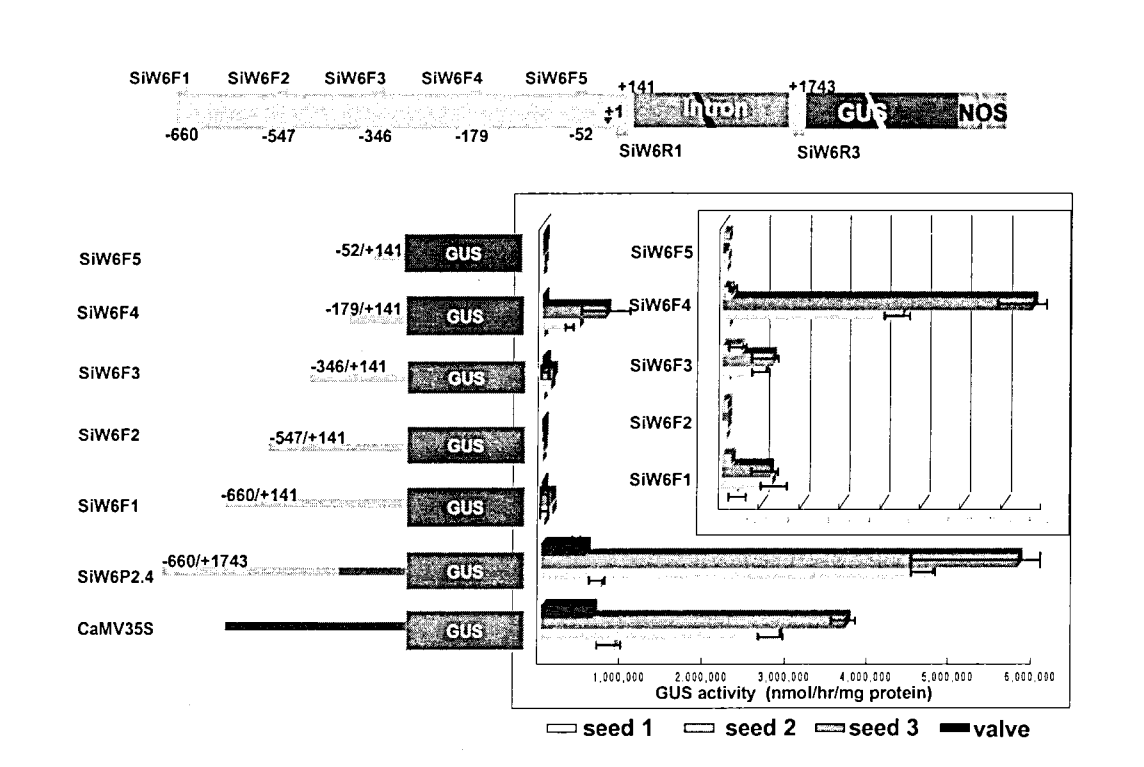
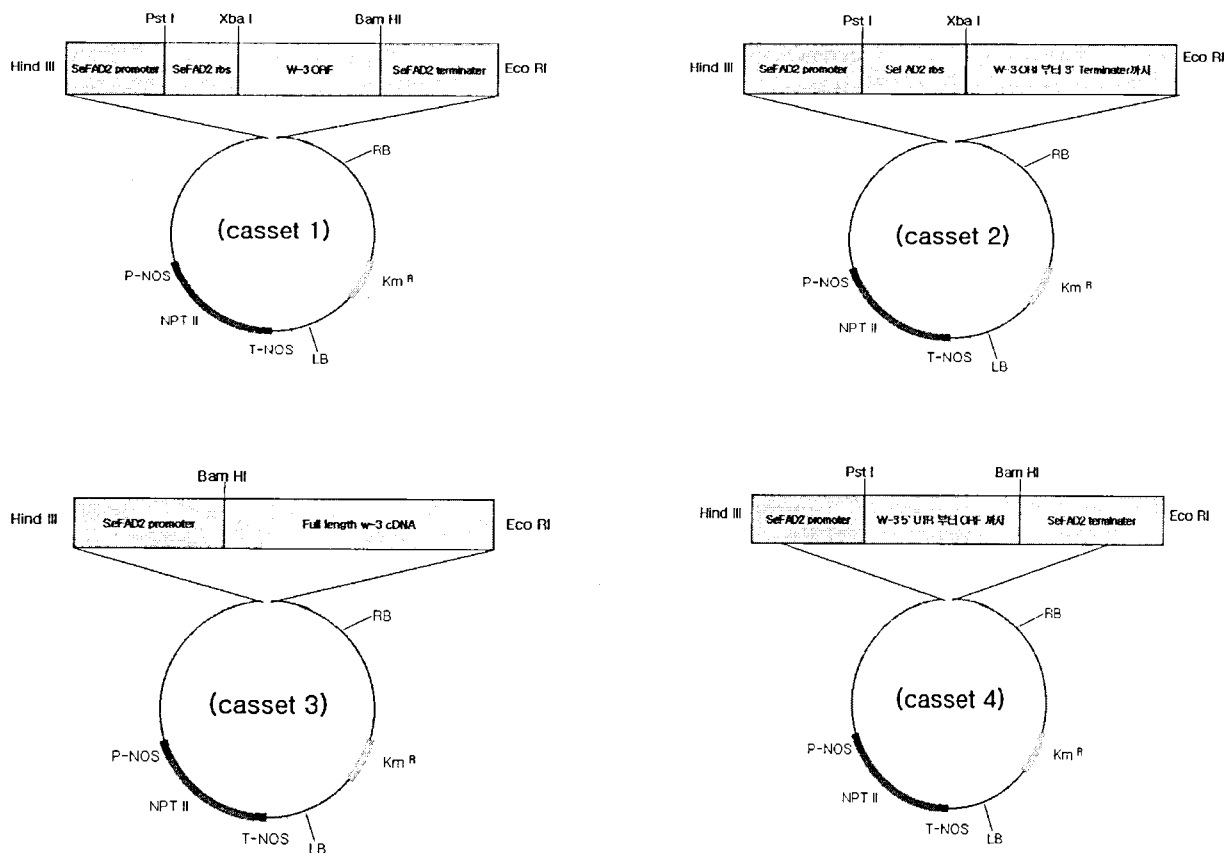


Fig. 8. GUS expression with sesame ω -6 fatty acid desaturase promoter. This figure shows that the intron of the 5'-flanking region may be essential for full expression of the sesame ω -6 fatty acid desaturase.

► Four expression cassettes were constructed using sesame ω -6 fatty acid desaturase promoter for efficient overexpression of perilla ω -3 fatty acid desaturase in sesame seeds



◆ Transformation of sesame crop with *Agrobacterium tumefaciens* EHA105 strain harboring plant vectors containing four different ω -3 fatty acid desaturase expression cassettes

A variety of techniques for plant transformation are available. Of them *Agrobacterium*-mediated transformation is probably the most widely used. We developed a method for regenerating some sesame varieties and now developing an *Agrobacterium*-mediated transformation method with GUS and ω -3 desaturase constructs. Some important results were obtained as follows.

► **Regeneration of some sesame varieties**

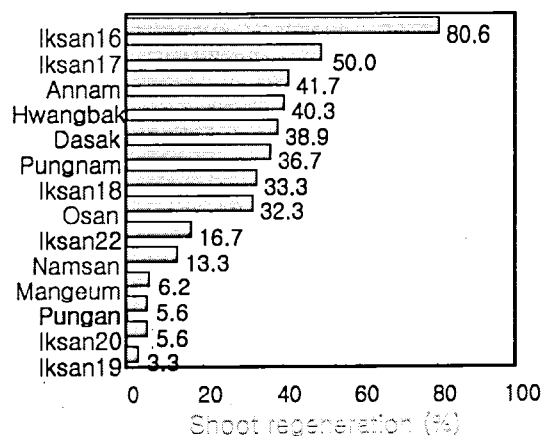


Fig. 9. Frequency of shoot formation

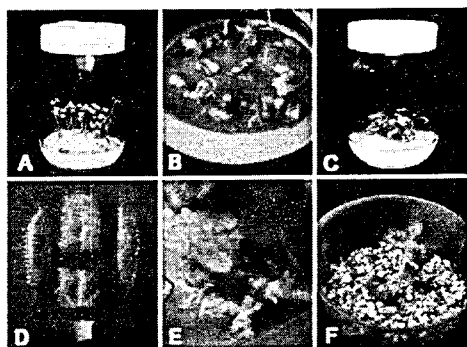


Fig. 11. Regeneration from sesame cotyledon explants

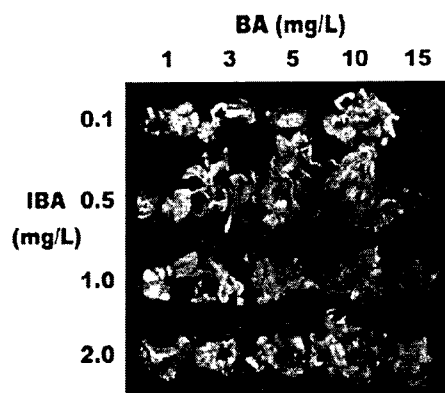


Fig. 10. Shoot formation of sesame plants

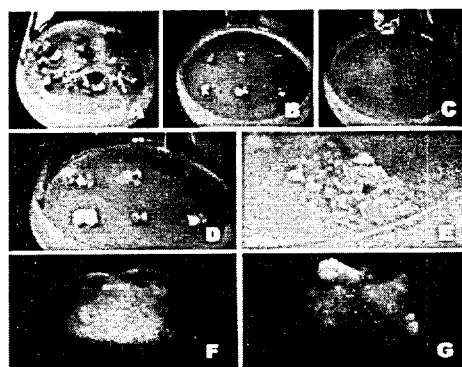


Fig. 12. GUS transformation of sesame cotyledon explants

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