

()

***fae* Antisense
(*Brassica napus*)**

*

(// : // :)

(C22:1)

β -ketoacyl-CoA synthase(KCS)
fae (Fatty Acid Elongase)

C22 C20 C18

PCR (fae) CTAB DNA

(HindIII EcoRV)
pSK

fae

pBI121

pBI121

LBA4404

Agrobacterium tumefaciens

PCR

fae
B. napus

fae

KCS

:

... fae Antisense :

)

(

DNA

(HEAR)

PCR

)

(

DNA

fae

()

)

DNA

PCR

()

PCR

(B A

pBluescript II SK (pSK⁺)

fae

PCR

SacI

(pSK⁺)

Cfr9I

) *fae*

(AF274750

E. coli

(*fae*)

MWG

(DH5α)

SacI

Cfr9I

()

(

)

ZSJ7:5'- CACGAGCTC ATGACGTCCGTTAACGTA (27mer)

SacI

ZSJ9: 5'- CATCCCCGGGTTAGGACCGACCGTTTTG (27 mer)

Cfr9I

) LB

(

PCR

BglI

Cfr9I, *SacI*

Oligo

fae

%

fae

(Dideoxy-Chain Termination)

PCR

(

)

ZSJ9 ZSJ7

) DNA

PCR

(

/) Mg²⁺

(

3. Templet
4. *Taq* DNA polymerase
5. *pfu* polymerase
6. Proof reading
7. Ligation
8. Competent Cell
9. Sequencing

1. Dellaporta
2. CTAB

PCR

MWG

ZSJ9 ZSJ7

PCR

T7 T3

fae

()

pBI121

(/ / /)

PCR

fae

()

ZSJ9 ZSJ7

*Sma*I

*Cfr*9I

) *Cfr*9I *Sac*I

LBA4404)

)

(C58pGV3101

pBI121

(sticky

()

() GUS

fae

(B)

(*fae*)

HEAR

E. coli

)

PCR

(

MS

()

/ MS

LBA4404

)

BAP

() *npt*II

pBI121

)

rpm (pBIZSJ

-
- 3. Cotyledonary leaves
 - 4. 6-Banzylaminopurine

-
- 1. Construct
 - 2. Freeze and thaw

... fae Antisense :

DNA

DNA T₀

PCR

PCR DG Probe Synthesis Kit (Cat. No. 1 636

Roche 090)

Nos terminator

/ MS)

() Sambrook and Russell

(

)

(IBA MS)

(Southern Blotting)

()

T₀

DNA

EcoRV

DNA

PCR

DNA

()

(fix)

(Gas chromatography)

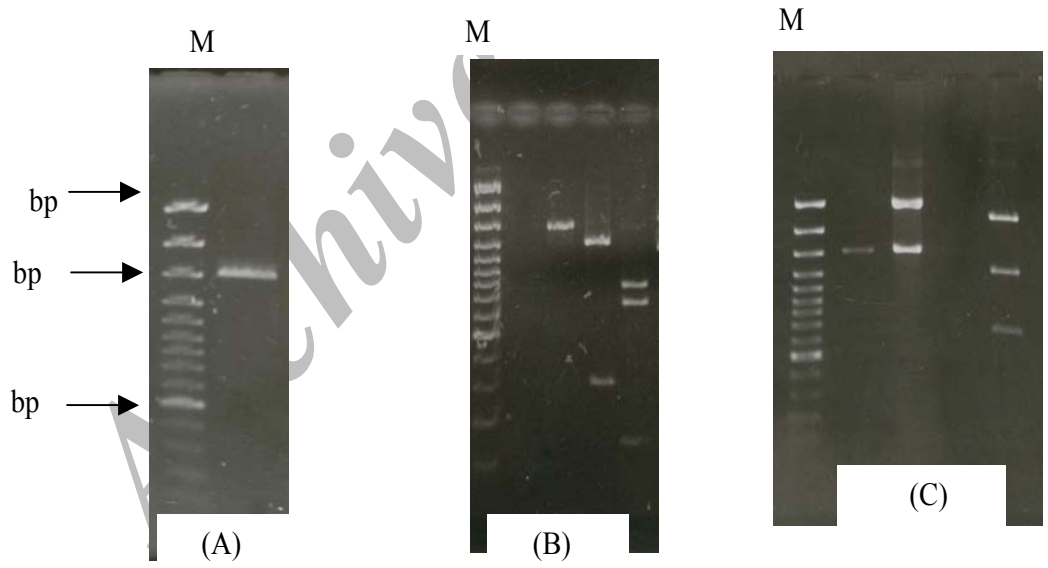
()

(Dot Blotting)

(GC)

-
1. Infection
 2. Co-cultivation
 3. Shoot Induction Medium
 4. Dot blot
 5. Southern hybridization

Hind III *EcoRV* / ()
EcoRV *fae*
Hind III
 %
 pSK+ (B)
 pSK+ *fae*
) PCR
Bgl II *Sac I* *Cfr9I* (PCR
 (C) Mg^{2+} / /
fae ()
 A
 PCR



M ()
EcoRV *fae* () *FAE* : A . *FAE*
 () *FAE* () : B
HindIII ()
 () ZSJ9 ZSJ7 : C ()
 ()
 () *Sac I* *Cfr9I* *fae* pSK+
 () *Bgl I* ()

... fae Antisense :

pBI121 fae () fae
Hind III PCR
pBI121 () (AF274750
fae
HindIII ()
()

(BLAST and Alignment)

SacI Cfr9I

PCR

BAP

B.napus *B.napus*(AF 274750)
B. juncea (BJU 558197) (AF 490462)
B. oleracea (AF 490460) *B. rapa* (AF 490461)
Arabidopsis thaliana (NM- 119617)
% *Simmondsia chinensis* (SCU 37088)
% / % / % / % / % / % /
()

() BAP

B. oleracea fae
Simmondsia *Arabidopsis thaliana* *B. juncea*
() % % % *chinensis*
() fae

BAP

Map

% /

PF

Clustal

() (% / % /)

B. napus
(% /) % / (AF 274750)
Simmondsia chinensis (SCU 37088)

B. napus

- 1. *Brassica oleracea*
- 2. *B. campestris*
- 3. Partial

()

atgacgtccgtaaacgtaaagctccttaccattacgcataaccaacctttcaacctttgcttcttccgtaaacggc
gatcgtcgcggaaaagcctatcggcttaccatagacgatcttaccacttatactattcctatctccaacacaacctcat
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gaagaacctagccctagcaccgatcgtatgtagggatcaagatcaacgttacatagatttggaaacacttcatctagc

fae

		Percent Identity									
		1	2	3	4	5	6	7	8		
Divergence	1	█	85.9	85.7	85.6	85.5	85.6	85.9	45.1	1	<i>Arabidopsis thaliana (NM)</i>
	2	15.5	█	99.7	99.7	98.6	99.7	100.0	46.4	2	<i>Brassica napus (askari)</i>
	3	15.8	0.3	█	99.9	98.6	99.6	99.7	46.4	3	<i>B. juncea</i>
	4	15.9	0.3	0.1	█	98.6	99.5	99.7	46.3	4	<i>B. rapa (AF)</i>
	5	16.0	1.5	1.4	1.5	█	98.6	98.6	45.8	5	<i>B. oleracea (AF)</i>
	6	15.9	0.3	0.4	0.5	1.5	█	99.7	46.2	6	<i>B. napus (westar)</i>
	7	15.5	0.0	0.3	0.3	1.5	0.3	█	46.4	7	<i>Gene complete1 (FAE)</i>
	8	64.0	61.2	61.2	61.4	60.9	61.8	61.2	█	8	<i>Simmondsia chinensis</i>
		1	2	3	4	5	6	7	8		

(Gene complete) fae (Homology)
(DNA)

		Percent Identity									
		1	2	3	4	5	6	7	8		
Divergence	1	█	99.4	99.8	99.0	98.6	99.2	76.3	4.3	1	Gene Complete 1 (FAE)
	2	0.4	█	99.4	99.0	99.0	99.6	75.9	4.3	2	<i>B. juncea</i>
	3	0.0	0.4	█	99.0	98.6	99.2	76.3	4.3	3	<i>Brassica napus (askari)</i>
	4	0.8	0.8	0.8	█	98.6	98.8	75.5	4.3	4	<i>B. napus (westar)</i>
	5	1.2	0.8	1.2	1.2	█	98.8	75.5	4.3	5	<i>B. (oleracea) AF</i>
	6	0.6	0.2	0.6	1.0	1.0	█	75.7	4.3	6	<i>B. rapa AF</i>
	7	28.3	28.8	28.3	29.4	29.4	29.1	█	4.7	7	<i>Arabidopsis thaliana NM</i>
	8	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	█	8	<i>Simmondsia chinensis</i>
		1	2	3	4	5	6	7	8		

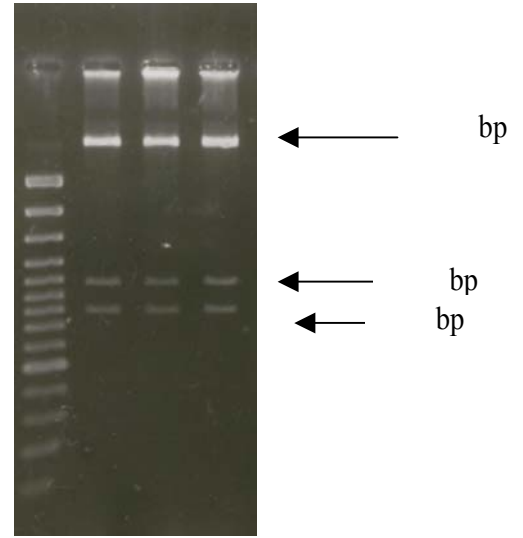
(Gene complete) fae (Homology)
(DNAstar)

... fae Antisense :

)
LBA4404) (C58pGV3101

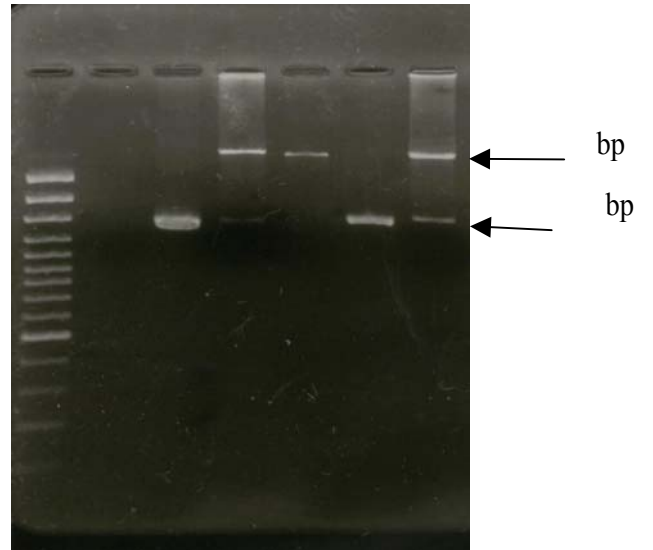
% / Map
% / PF

(% /)
(% /) LBA4404
(% /) C58pGV3101



fae pBI121
HindIII

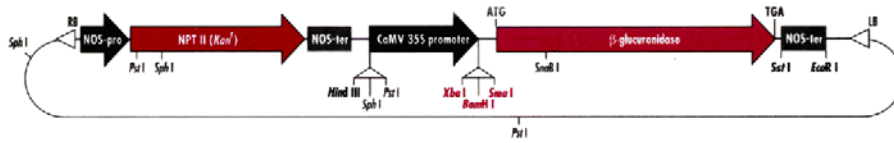
westar
RI25
()



BAP LBA4404 /

ZSJ7 PCR ZSJ9
Hind III pBI121
Cfr9 I Sac I

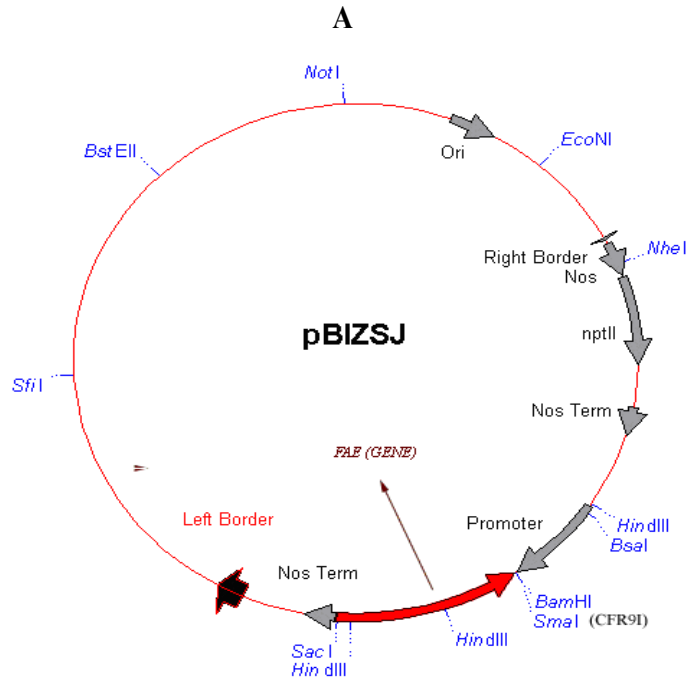
C22:1 T-DNA
() pBI121



pBI121

Sma I (CFR91)
TCT AGA GGA TCC CCG GGT GGT CAG TCC CTT ATG
Xba I BamHI

from Clontech catalogue 1996 / 97



T-DNA pBI121 :A.
 pBI121 GUS

B
fae (pBIZSJ) pBI121 :B



C

D

A

B

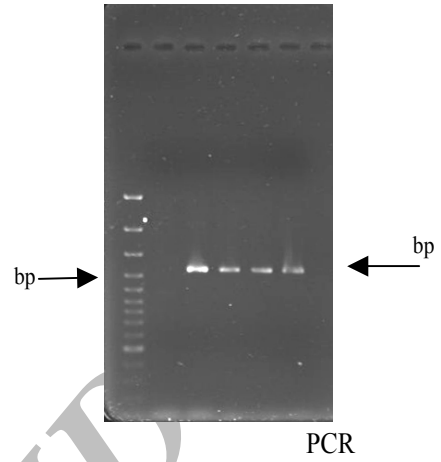
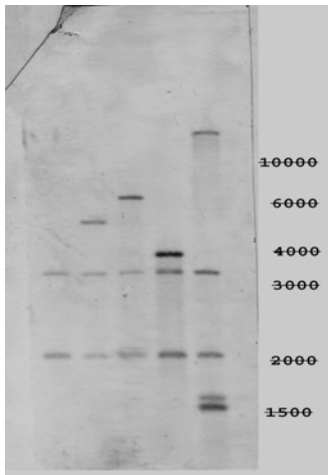
A.

:C.

:B.

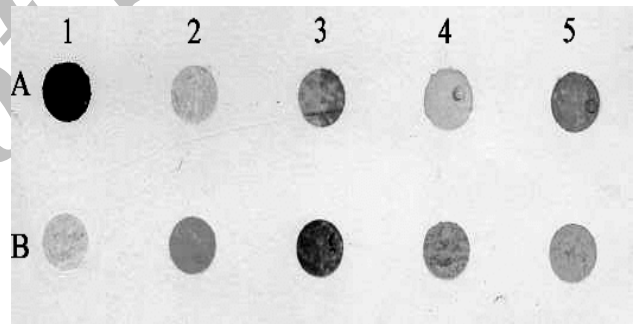
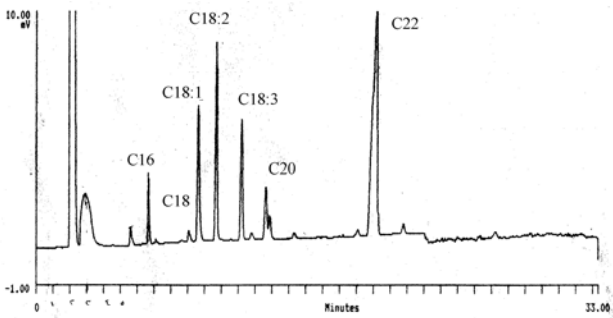
:D ()

... fae Antisense :

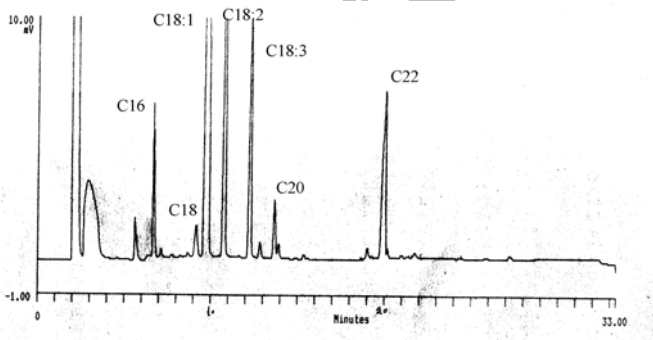


EcoRV DNA .Nos *fae*
() ()

pBI121) : (



fae
pBI121 : A₁
B₁ A₂



() GC
() (C22)

DNA PCR .
fae

()

(Dot blotting)

()

()

fae

(GC)

(0.1% SDS 2X SSC)

RNA

RNA

(0.1% SDS 0.5X SSC)

fae

()

)

()

()

fae

(MapI)

()

EcoRV

()

fae

(%)

(C18)

()

XbaI

% /

% /

(.)

(0.1% SDS 2X SSC)

) high stringency

(

()

fae

()

()

ω 3 ω 6 desaturase

()

α

1. High stringency

... fae Antisense :

() %

fae *B. napus*

()

%

Oleate desaturase

(*B. juncea*)

%

(*B. napus*)

()

GUS GFP

REFERENCES

5. Bao, X., Pollard, M. & Ohlrogge, J. 1998. The biosynthesis of erucic acid in developing embryos of *Brassica rapa*. *Plant Physiol.* 118: 183-190.
6. Barret, P., R. Delourme, M. Renard, F. Dmurgue, L. Lessire, M. Delseny, & T. J. Roscoe. 1998. A rapeseed *fad1* gene linked to the E1 locus associated with variation in the content of erucic acid. *Theor. Appl. Genet.* 96: 177-186.
7. Christey, M. C. & Earle, E. D. 1991. Regeneration of *Brassica oleracea* from peduncle explants. *Hortscience.* 26: 1069-1072.
8. Christof Stoll, M., K. Zarhloul, A. Syring-Ehemann, L. Hausmann, F. Spener, R. Tofer, W. Friedt, & W. Lush. 1999. Genetic modification of medium-chain fatty acids in oilseed rape (*Brassica napus* L.) for industrial purposes. New Horizons for an old crop. Proceeding of 10th International Rapeseed Congress, Canberra, Australia.
9. Das, S., T.J. Roscoe, M. Delseny, P.S. Srivastava, & M. Lakschmikumar. 2002. Cloning and molecular characterization of the Fatty Acid Elongase 1 (*fae1*) gene from high and low erucic acid lines of *Brassica campestris* and *Brassica oleracea*. *Plant Sci.* 162: 245-250.
10. Doyle, J. J. & J. L. Doyle. 1990. Isolation of plant DNA from fresh tissue. *Focus.* 12: 13-15.
11. Fourmann, M., P. Barret, M. Rendar, G. Pelletier, R. Delourme, & D. Brunel. 1998. The two genes homologous to *Arabidopsis fae1* co-segregate with the two loci governing erucic acid content in *Brassica napus*. *Theor. Appl. Genet.* 96: 852-858.
12. Gopalan, G. D., D. Krisnamurthy, I. S. Shenolikar, & K. A. V. R. Krisnamurthy. 1974. Myocardial changes in monkeys fed on mustard oil. *Nutr. Metab.* 16: 352-365.
13. Hall, L. N., G. A. Tucker, C. J. S. Smith, C. F. Watson, G. B. Seymour, & D. Grierson. 1993. Antisense inhabitation of pectinesterase gene expression in transgenic tomatoes. *The Plant J.* 3: 121-129.
14. Han, J., W. Lush, K. Sonntag, U. Zahringer, S. D. Borchardt, F. P. Wolter, E. Heinz., & M. Frentzen. 2001. Functional characterization of β -ketoacyl-CoA synthase genes from *Brassica napus* L. *Plant Mol. Biol.* 46: 229-239.

15. Harwood, J. L. 1988. Fatty acid metabolism. *Annual Review of Plant Physiology and Plant Mol. Biol.* 39: 101-188.
16. Harwood, J. L. 1996. Recent advances in the biosynthesis of plant fatty acids. *Biochemica et Biophysica Acta*, 1301: 7-56.
17. Helliwell, C. & P. Waterhouse. 2003. Constructs and methods for high-throughput gene silencing in plants. *Method.* 30: 289-295.
18. James, D. W., L. E. Keller, I. Plooy., & H. K. Donner. 1995. Directed tagging of the *Arabidopsis* fatty acid elongation 1 (*fae1*) gene with the maize transposon activator. *Plant Cell.* 7: 309-319.
19. Khehra, G. S. & R. J. Mathias. 1992. The interaction of genotype, explant and media on the regeneration of shoot from complex explants of *Brassica napus* L. *J. Exp. Bot.* 43: 1413-1418.
20. Kinney, A. J. 1994. *Current Opinon. Biotechnology.* 5: 144-151.
21. Kinney, A.J. 1997. in *Physiology, Biochemistry and Molecular Biology of Plant Lipids.* pp. 298-301, Kluwer Academic Publishers, Dordrecht.
22. Knutzon, DS., G. A. Thompson, S. E. Radke, W. B. Johnson, V. C. Knauf, & J. C. Kridl. 1992. Modification of *Brassica* seed oil by antisense expression of a stearyl-acyl carrier protein desaturase gene. *Proc Natl Acad Sci USA*, 89: 2624-2628.
23. Lassner, M. W., K. Lardizabel., & J. G. Metz. 1996. A Jojoba β -ketoacyl – CoA synthase cDNA complements the Canola fatty acid elongation mutation in transgenic plants. *Plant Cell.* 8: 281-292.
24. Metcalf, L. C., A. A. Schmitz, & J. R. Pelka. 1966. Rapid preparation of methyl esters from lipid for gas chromatography analysis. *Analytical Chemistry.* 38: 514-515.
25. Murphy, D. J. 1995. The use of conventional and molecular genetics to produce new diversity in seed oil composition for the use of plant breeders – progress, problems and future prospects. *Euphytica.* 85: 433-440.
26. Murphy, I. S. & J. G. Sonntag. 1991. Erucic, behenic: feedstocks of the 21st century. *Inform.* 2: 449-463.
27. O'Brien, P. 1994. *Lecture Notes, Laboratory manual. Molecular Biology 1 N305, Section2.* 17-20.
28. Parks, L. C. 1993. *Hand Book of Microbiological Media.* 2nd edition, Ch.2: 120-122.
29. Princen, L. H., & J. A. Rothfus. 1984. Development of new crops for industrial raw materials. *Journal of American Oil Chemistry Society.* 61: 281-289.
30. Puyaubert, J., B. Garbay, P. Costaglioli, W. Dieryck, T. J. Roscoe, M. Renard, C. Cassagne., & R. Lessire. 2001. Acyl-CoA elongase expression during seed development in *Brassica napus*. *Biochemica et Biophysica Acta (BBA).* 1533: 141-152.
31. Sambrook, J. & D. W. Russell. 2001. *Molecular Cloning. A Laboratory manual.* 3rd Edition. Cold Spring Harhor Press, New York.
32. Sheehy, R. E., J. Pearson, C. J. Brady, & W. R. Hiatt. 1988. Molecular characterization of tomato fruit polygalacturonase. *Mol. Gen. Genet.* 208: 30-36.
33. Sindelar, L., M. Sindelarova, & Burketova. 1998. Hexokinases of tobacco leaves: influence of plant age on particulate and soluble isozyme composition. *Plant Biol.* 40: 469-474.
34. Somerville, C. & J. Browse. 1991. Plant lipids: metabolism, mutants and memberanes. *Science.* 252: 80-87.
35. Sonntag NOV. 1995. Industrial utilization of long-chain fatty acids and their derivatives. In: Kimber D, McGregor DI (eds) *Brassica* oilseeds. CAB International, Oxon, UK. pp 339-352.
36. Southern, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol,* 98: 503.
37. Stoutjesdijk, P. A., C. Hurlestone, S. P. Singh. & A. G. Green. 2000. High – oleic acid Australian *Brassica napus* and *B. juncea* varieties produced by co-suppression of endogenous $\Delta 12$ – desaturases. *Biochemical Society Transactions.* 28: 938-940.

38. Tenllado, F., Barajas, D., Vargas, M., Atencio, F.A., Gonzalez-Jara, P. & D Iaz-Ruiz, J.R. 2003. Transient expression of homologous hairpin RNA causes interference with plant virus infection and is overcome by a virus encoded suppressor of gene silencing. *Mol. Plant-Microbe Interact.* 16: 149-158.
39. Todd, J., D. Post-Beittenmiller., & J. G. Jarowski. 1999. KCS encodes a fatty acid elongase 3- ketoacyl-CoA synthase affecting wax biosynthesis in *Arabidopsis thaliana*, *the Plant. J.* 17: 119-130.
40. Tofer, R., N. Martini., & J. Schell. 1995. Modification of plant lipid synthesis. *Science.* 268: 681-686.
41. Venkateswari, J., S. Kanrar, P.B. Kirti, V. G. Malathi, & V. L. Chopra. 1999. Molecular cloning and characterization of Fatty Acid Elongation1 (*Bjfae1*) gene of *Brassica juncea*. *J. Plant Biochem. Biotech.* 8: 53-55.
42. Voelker, T. A., A. C. Worrel, L. Anderson, J. Bleibaum, C. Fan, D. J. Hawkins, & H. M. Davies. 1992. Engineering laurate production in oilseed. In: *Proceeding of the Miami Biotechnology Winter Symposium 2: IRL Press, Oxford.* P. 102.
43. Von Wettstein-Knowles, P. M. 1993. Waxes, cutin and suberin, in: T.S. Moore Jr (Ed.), *Lipid metabolism in plants*, CRC Press, Boca Raton. pp. 127-166.
44. Wang, M.B., N. M. Upadhyaya, R. I. S. Brettell, & P. M. Waterhouse. 1997. Intron-mediated improvement of a selectable marker gene for plant transformation using *Agrobacterium tumefaciens*. *J. Genet. Breed.* 51: 325-334.
45. Wenderoth, I. & A. V. Schaewen. 2000. Isolation and characterization of plant N-Acetyl Glucosaminyl transferaseI(*GntI*) cDNA sequences. Functional analyses in the *Arabidopsis* *cg1* mutant and in antisense plants. *Plant Physiol.* 123: 1097-1108.
46. Zhang, Y. & P. L. Bhalla. 1999. Shoot regeneration potential from seedling explants of Australian cultivars of oil seed rape (*Brassica napus* L.). *New Horizons for an old crop. Proceeding of 10th International Rapeseed Congress, Canberra, Australia.*
47. Zhang, Y., M. B. Singh, & P. L. Bhalla. 1999. Genetic transformation of Australian cultivars of oil seed rape (*Brassica napus* L.). *New Horizons for an old crop. Proceeding of 10th International Rapeseed Congress, Canberra, Australia.*