Department for Environment, Food and Rural Affairs

Application for consent to release a GMO – Higher plants

Part A1: Information require under Schedule 1 of the Genetically Modified Organisms (Deliberate Release) Regulations 2002

Part 1 General information

1. The name and address of the applicant and the name, qualifications and experience of the scientist and of every other person who will be responsible for planning and carrying out the release of the organisms and for the supervision, monitoring and safety of the release.

The application is made on behalf of Rothamsted Research, at the following address:

Rothamsted Research,
West Common, Harpenden
Hertfordshire,
AL5 2JQ
UK

2. The title of the project.

Synthesis and accumulation of seed storage compounds in ***Camelina sativa***

Part II Information relating to the parental or recipient plant

3. The full name of the plant –

(a) family name	Brassicaceae
(b) genus	*Camelina*
(c) species	*Camelina sativa*
(d) subspecies	-
(e) cultivar/breeding line	Celine

(f) common name Gold-of-pleasure, false flax

4. Information concerning -

(a) the reproduction of the plant:

(i) the mode or modes of reproduction,

Reproduction is sexual leading to formation of seeds. *C. sativa* is reported to be primarily, but not exclusively, self-pollinating. *C. sativa* may also be pollinated by insects.

(ii) any specific factors affecting reproduction

Pollination, seed-set and grain filling are dependent on temperature, weather conditions, agronomic practice and biotic stresses.

(iii) generation time; and

The generation time is 85 -100 days, with seeds being sown in Spring.

(b) the sexual compatibility of the plant with other cultivated or wild plant species, including the distribution in Europe of the compatible species.

It has been reported that *Camelina sativa* can intercross with other members of the Camelina genus, in particular *Camelina microcarpa* and *Camelina alyssum* (Seguin-Swartz et al., 2013). Within the larger Camelineae tribe, species such as *Arabidopsis lyrata*, *Capsella bursa pastoris* and *Neslia paniculata* are believed not to cross-hybridise with *C. sativa*, or result in viable seed (Julie-Galau et al., 2013). No cross pollination, either natural or forced, has been observed between *C. sativa* and members of the Brassica genus, such as *B. napus*, *B. juncea*, *B. rapa* and *B. nigra*. Artificial *in vitro* somatic hydrisiation between protoplasts of *C. sativa* and *B. napus*, *B. carinata* and *B. oleracea* has been reported, but with low success and/or sterile hybrids (http://www.inspection.gc.ca/plants/plants-with-novel-traits/applicants/directive-94-08/biology-documents/camelina-sativa-l-/eng/1330971423348/1330971509470 - this dossier also contains many useful details regarding the growth and cultivation of *C. sativa*).

5. Information concerning the survivability of the plant:

(a) its ability to form structures for survival or dormancy,

C. sativa is an annual species and survives from year to year only via seed production. Under agricultural practice, some mature seeds may fall from the plant prior to or at the time of harvest and not be collected. If not managed, these seeds could potentially over-winter in the soil and germinate the following spring as 'volunteers'. However, little empirical data are available regarding the over-wintering capacity of *C. sativa* grown in the UK, its tolerance of low temperatures and frost, photoperiod or dormancy.

(b) any specific factors affecting survivability.

None of note.

6. Information concerning the dissemination of the plant:

(a) the means and extent (such as an estimation of how viable pollen and/or seeds decline with distance where applicable) of dissemination; and

Pollen can be disseminated by the wind but is also likely to be transmitted by insects.

(b) any specific factors affecting dissemination.

C. sativa is reported to be as attractive to bees as other species such as flax and canola. However, the flowers of *C. sativa* are much smaller than canola or flax, so may be less apparent to pollinators. Seed is usually retained by the plant within the seed capsules until harvest, but a small proportion can be spilt to the ground at that time. Dispersal of seed prior to harvest by wind is unlikely, but possible by wildlife. http://www.inspection.gc.ca/plants/plants-with-novel-traits/applicants/directive-94-08/biology-documents/camelina-sativa-l-/eng/1330971423348/1330971509470.

7. The geographical distribution of the plant.

C. sativa originated in Europe and was historically grown across South-Eastern Europe and South-Western Asia. It is a native species in many European countries, including Albania, Austria, Belarus, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France (including Corsica), Germany, Greece (including Crete), Hungary, Italy (including Sardinia, Sicily), Latvia, Lithuania, Moldova, The Netherlands, Norway, Poland, Romania, Russian Federation, Slovakia, Slovenia, Sweden, Switzerland, Ukraine and the United Kingdom.

C. sativa is grown as a crop in Canada and the Great Plains states (e.g. Montana, Nebraska) of the USA.

8. Where the application relates to a plant species which is not normally grown in the United Kingdom, a description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts.

Not applicable

9. Any other potential interactions, relevant to the genetically modified organism, of the plant with organisms in the ecosystem where it is usually grown, or elsewhere, including information on toxic effects on humans, animals and other organisms.

C. sativa is known to have a range of pests and fungal pathogens. The main insect pests in the UK are likely to be Crucifer Flea Beetle (*Phyllotreta cruciferae*) and pollen beetle (*Meligethes aeneus*). Other potential pests include cabbage root fly (*Delia radicum*) and the diamondback moth (*Plutella xylostella*).

Fungal pathogens are likely to include the following: *Albugo candida, Botrytis cinere, Fusarium* spp, *Mycosphaerella brassicicola, Peronospora parasitica, Pythium debaryanu, Rhizoctonia solani, Sclerotinia sclerotiorum, Ustilago* spp., *Verticillium longisporum.*

Part III Information relating to the genetic modification

10. A description of the methods used for the genetic modification.

Transgenic *C. sativa* plants were produced via *Agrobacterium*-mediated transformation ("floral dip") using published methods (Lu and Kang, 2008; Sayanova et al, 2012). No tissue culture step is involved in this method.

The Agrobacterium strain used was *Agrobacterium tumefaciens* strain GV3101 (Koncz and Schell, 1986).

11. The nature and source of the vector used.

The genes of interest were carried on a binary vector pRS-3GSeed or pSUN2 (Ruiz-Lopez et al, 2012; Sayanova et al, 2012), a derivative of pBIN19 (Frisch et al, 1995). Alternatively, the genes of interest were carried on pGREEN (Hellens et al., 2000), in conjunction with the pSOUP helper plasmid.

12. The size, intended function and name of the donor organism or organisms of each constituent fragment of the region intended for insertion.

Element	Donor Organism	Description, size, and Intended Function
RB	*Agrobacterium tumefaciens*	T-DNA Right border (24 bp)
LB	*Agrobacterium tumefaciens*	T-DNA Left border (23 bp)
Seed-specific promoters (Group P)		
pUSP	*Vicia faba*	Unknown Seed Protein Seed-specific promoter (684 bp)
pNP	*Brassica napus*	Napin seed specific promoter (664 bp)
pCNL	*Linum usitatissimum*	2S seed storage protein (Conlinin) promoter (1064 bp)
pGLY	*Glycine max*	11S Seed storage protein (Glycinin) promoter (702 bp)

pOLEO	*Arabidopsis thaliana*	*Oleosin seed storage protein promoter (579 bp)*
Regulatory elements (Group T)		
OCS*t*	*Agrobacterium tumefaciens*	octopine synthase gene terminator sequence (192 bp)
Gly*t*	*Glycine max*	11S storage protein (Glycinin) terminator (451 bp)
HSP*t*	*Arabidopsis thaliana*	Heat shock protein 18.2 (HSP) gene terminator sequence (245 bp)
Phas*t*	*Phaseolus vulgaris*	Alpha-phaseolin gene terminator sequence (183 bp)
Fad2*t*	*Camelina sativa*	Microsomal oleate desaturases (FAD2) gene terminator sequence (182 bp)
PDK-i3	*Flaveria trinervia*	Pyruvate orthophosphate dikinase intron 3 (i3) (1597 bp)
Regulatory elements used to direct Cas9 expression (Group X)		
Cas9	Synthetic	RNA-guided DNA endonuclease from *Streptococcus pyogenes* (4104 bp)
pEC1.2	*Arabidopsis thaliana*	Egg-cell specific promoter to direct restricted expression of Cas9 (1000 bp)
pPcUbi4-2	*Petroselinum crispum*	Ubiquitin4–2 promoter to direct expression of Cas9 (971 bp)
pU3-26	*Arabidopsis thaliana*	Promoter to express gRNAs for Cas9 targeting (400 bp)
pU6-26	*Arabidopsis thaliana*	Promoter to express gRNAs for Cas9 targeting (400 bp)
U6-26*t*	Synthetic	Terminator sequence behind gRNAs for Cas9 targeting (120 bp)
Pea3A*t*	*Pisum sativa*	Terminator sequence behind Cas9 (470 bp)

	Synthetic	Target-specific templates for guide RNAs (<24bp)
Biosynthetic genes		
Δ6-desaturases (Group A)		
OtD6	Synthetic	Encodes a fatty acid **Δ6-desaturase** from *Ostreococcus tauri* (1665 bp)
AaD6	Synthetic	Encodes a fatty acid **Δ6-desaturase** from *Aureococcus anophagefferens* (1350 bp)
MsqD6	Synthetic	Encodes a fatty acid **Δ6-desaturase** from *Mantoniella squamata* (1350 bp)
O809D6	Synthetic	Encodes a fatty acid **Δ6-desaturase** from *Ostreococcus* RCC809 (1662 bp)
PvD6	Synthetic	Encodes a fatty acid **Δ6-desaturase** from *Primula vialii* (1360 bp)
PfD6	Synthetic	Encodes a fatty acid **Δ6-desaturase** from *Primula farinosa* (1360 bp
ThalpD6	Synthetic	Encodes a fatty acid **Δ6-desaturase** from *Thalassiosira pseudonana* (1455 bp)
AnelD6	Synthetic	Encodes a fatty acid **Δ6-desaturase** from *Anemone leveillei* (1341 bp)
MnnD6	Synthetic	Encodes a fatty acid **Δ6-desaturase** from Mamiellales spp. (1456bp)
Δ6-elongases (Group B)		
AaELO6	Synthetic	Encodes a fatty acid **Δ6-elongase** from *Aureococcus anophagefferens* (900 bp)
BpELO6	Synthetic	Encodes a fatty acid **Δ6-elongase** from *Bathycoccus prasinos* (900 bp)

FcELO6	Synthetic	Encodes a fatty acid **Δ6-elongase** from *Fragilariopsis cylindrus* (825 bp)
MsqELO6	Synthetic	Encodes a fatty acid **Δ6-elongase** from *Mantoniella squamata* (900 bp)
MnnELO6	Synthetic	Encodes a fatty acid **Δ6-elongase** from Mamiellales spp. (903 bp)
PpELO6	Synthetic	Encodes a fatty acid **Δ6-elongase** from *Physcomitrella patens* (873 bp)
MoalELO6	Synthetic	Encodes a fatty acid **Δ6-elongase** from *Mortierella alpine* (954 bp)
ThalpELO6	Synthetic	Encodes a fatty acid **Δ6-elongase** from *Thalassiosira pseudonana* (819 bp)
Δ5-desaturases (C20) (Group C)		
EmiD5	Synthetic	Encodes a fatty acid **Δ5-desaturase** from *Emiliania huxleyi* (1368 bp)
AaD5	Synthetic	Encodes a fatty acid **Δ5-desaturase** from microalga *Aureococcus anophagefferens* (1380 bp)
MsqD5	Synthetic	Encodes a fatty acid **Δ5-desaturase** from *Mantoniella squamata (*1380 bp)
O809D5	Synthetic	Encodes a fatty acid **Δ5-desaturase** from picoalgae *Ostreococcus* RCC809 (1380 bp)
ThraD5		Encodes a fatty acid **Δ5-desaturase** from *Thraustochytrium spp.* (1317 bp)
MnnD5	Synthetic	Encodes a fatty acid **Δ5-desaturase** from Mamiellales spp. (1450 bp)
ThalpD5	Synthetic	Encodes a fatty acid **Δ5-desaturase** from *Thalassiosira pseudonana* (1449 bp)
PhaeoD5	Synthetic	Encodes a fatty acid **Δ5-desaturase** from *Phaeodactylum tricornutum* (1410 bp)

Δ5-elongases (Group D)		
OtElo5	Synthetic	Encodes a fatty acid **Δ5-elongase** from ***O****streococcus* ***t****auri* (903 bp)
BpELO5	Synthetic	Encodes a fatty acid **Δ5-elongase** from *Bathycoccus prasinos* (900 bp)
AaELO5	Synthetic	Encodes a fatty acid **Δ5-elongase** from *Aureococcus anophagefferens* (900 bp)
MsqELO5	Synthetic	Encodes a fatty acid **Δ5-elongase** from *Mantoniella squamata* (900 bp)
O809E5	Synthetic	Encodes a fatty acid **Δ5-elongase** from *Ostreococcus RCC809* (900 bp)
ThalpELO5	Synthetic	Encodes a fatty acid **Δ5-elongase** from *Thalassiosira pseudonana* (879 bp)
MnnELO5	Synthetic	Encodes a fatty acid **Δ5-elongase** from Mamiellales spp. (900 bp)
PhaeoELO5	Synthetic	Encodes a fatty acid **Δ5-elongase** from *Phaeodactylum tricornutum* (909 bp)
Δ4-desaturases (Group E)		
BpD4	Synthetic	Encodes a fatty acid **Δ4-desaturase** from *Bathycoccus prasinos* (1500 bp)
TpD4	Synthetic	Encodes a fatty acid **Δ4-desaturase** from *Thalassiosira pseudonana* (1650 bp)
O809D4	Synthetic	Encodes a fatty acid **Δ4-desaturase** *Ostreococcus* RCC809 (1480 bp)
EmiD4	Synthetic	Encodes a fatty acid **Δ4-desaturase** *Emiliania huxleyi* (1467 bp)
OtD4	Synthetic	Encodes a fatty acid **Δ4-desaturase** from *Ostreococcus tauri* (1611 bp)
OlD4	Synthetic	Encodes a fatty acid **Δ4-desaturase** from *Ostreococcus lucimarinus* (1398 bp)

w3-desaturases (Group F)		
PveW3	Synthetic	Encodes a fatty acid **w3-desaturase** from *Pythium spp* (1092 bp)
PolW3	Synthetic	Encodes a fatty acid **w3-desaturase** from *Pythium spp* (1080 bp)
ParW3	Synthetic	Encodes a fatty acid **w3-desaturase** from *Pythium spp* (1074 bp)
PiW3	Synthetic	Encodes a fatty acid **w3-desaturase** from *Phytophora infestans* (1086 bp)
AdvaW3	Synthetic	Encodes a fatty acid **w3-desaturase** from *Adineta vaga* (1122 bp)
PlduW3	Synthetic	Encodes a fatty acid **w3-desaturase** from *Platynereis dumerilii* (1167 bp)
PlvuW3	Synthetic	Encodes a fatty acid **w3-desaturase** from *Patella vulgate* (1263 bp)
AcmiW3	Synthetic	Encodes a fatty acid **w3-desaturase** from *Caenorhabditis elegans* (1179 bp)
Δ12/Δ15-desaturases (Group I)		
BoofD12	Synthetic	Encodes a fatty acid **Δ12-desaturase** FAD2 activity from *Borago officinalis* (1149 bp)
TpD12	Synthetic	Encodes a fatty acid **Δ12-desaturase** from *Thalassiosira pseudonana* (1182bp)
PsD12	Synthetic	Encodes a fatty acid **Δ12-desaturase** activity from *Phytophora sojae* (1143 bp)
CsD12	Synthetic	Encodes a fatty acid **Δ12-desaturase** FAD2 activity from *Camelina sativa* (1149 bp)
CsD15	Synthetic	Encodes a fatty acid **Δ15-desaturase** FAD3 activity from *Camelina sativa* (1158 bp)

Δ9-elongases (Group J)		
EhuxELO9	Synthetic	Encodes a fatty acid **Δ9-elongase** from *Emiliania huxleyi* (300 bp)
IsocrELO9	Synthetic	Encodes a fatty acid **Δ9-elongase** from *Isochrysis galbana* (300 bp)
PmarFAE9	Synthetic	Encodes a fatty acid **Δ9-elongase** from *Perkinsus marinus* (1320 bp)
Δ8-desaturases (Group K)		
PsD8	Synthetic	Encodes a fatty acid **Δ8-desaturase** from *Pavlova salina* (1440 bp)
EhD8	Synthetic	Encodes a fatty acid **Δ8-desaturase** from *Emiliania huxleyi* (1440 bp)
AcD8	Synthetic	Encodes a fatty acid **Δ8-desaturase** from *Acanthamoeba castellanii* (1440 bp)
Δ5-desaturases (C18) (Group L)		
AqvuD5	Synthetic	Encodes a fatty acid **Δ5-desaturase** from *Aquilegia vulgaris* (990 bp)
AnleD5-Al10	Synthetic	Encodes a fatty acid **Δ5-desaturase** from *Anemone leveillei* (AL10) (990 bp)
AnleD5-Al21	Synthetic	Encodes a fatty acid **Δ5-desaturase** from *Anemone leveillei* (AL21) (990 bp)
ChlamyD5	Synthetic	Encodes a fatty acid **Δ5-desaturase** from *Chlamydomonas reinhardtii* (1800 bp)
Acyltransferases (Group M)		
DGAT1	Synthetic	Encodes acyl-CoA:diacylglycerol

		acyltransferase (DGAT) catalyzes the final step of the triacylglycerol synthesis pathway – isoform 1 (1563 bp)
DGAT2	Synthetic	Encodes acyl-CoA:diacylglycerol acyltransferase (DGAT) catalyzes the final step of the triacylglycerol synthesis pathway – isoform 2 (1330 bp)
PDAT	Synthetic	Encodes the phospholipid:diacylglycerol acyltransferase involved in the biosynthesis of triacylglycerol (2016 bp)
GPAT	Synthetic	Encodes the glycerol-3-phosphate acyltransferase involved in the first committed step on the Kennedy pathway for the biosynthesis of glycerolipids. (1131 bp)
LPAAT	Synthetic	Encodes the acyl-CoA sn-2 acyltransferase involved in the second step on the Kennedy pathway for the biosynthesis of glycerolipids. (1071 bp)
LPCAT	Synthetic	Encodes the acyltransferase responsible for the conversion 1-acyl-sn-glycero-3-phosphocholine into phosphatidylcholine via the Lands cycle. (1389 bp)
LPAT1	*Brassica napus*	Encodes a modified lysophosphatidic acid acyltransferase 1 that localises in the endoplasmic reticulum (768 bp)
Other transgenes (Group N)		
AtWRI1	*Arabidopsis thaliana*	Encodes the WRINKLED1 (WRI1) AP2/ERWEBP transcription factor involved in the control of storage compound biosynthesis – Arabidopsis homolog (1317 bp)
CsWRI1	*Camelina sativa*	Encodes the WRINKLED1 (WRI1) AP2/ERWEBP transcription factor involved in the control of storage compound biosynthesis – Camelina homolog (1317 bp)

AtFATb	*Arabidopsis thaliana*	Encodes a plastidial palmitoyl-acyl carrier protein thioesterase (1239 bp)
CsFAD2-AS	*Camelina sativa*	Arabidopsis fatty acid desaturase 2 (FAD2) DNA fragment for antisense expression (299 bp)
CsFAD3-AS	*Camelina sativa*	Arabidopsis fatty acid desaturase 3 (FAD3) DNA fragment for antisense expression (323 bp)
CsFAE1-AS	*Camelina sativa*	Camelina fatty acid elongase 1 (FAE1) DNA fragment for antisense expression (251 bp)
LPAT2 amiRNA	Synthetic	Arabidopsis artificial microRNA mi319a adapted to target Camelina lysophosphatidic acid acyltransferase 2 (404 bp)
SAD	Synthetic	Encodes an engineered sinapic acid decarboxylase derived from *Bacillus pumilis* strain UI-670 (481 bp)
Marker genes (Group Z)		
DsRed	Synthetic	Encodes a red florescent protein from ***D****isco****s****oma* spp. (684 bp)
EGFP	Synthetic	Encodes a green fluorescent protein from *Aequorea victoria* (759 bp)
bar	Synthetic	Encodes phosphinothricin acetyl transferase, providing to resistance to Class H herbicides such as Basta ™, in which bialaphos is the active ingredient. (548 bp)
*p*Z	*Cassava vein mosaic virus*	CsVMV promoter sequence (528 bp)
*p*NOS	*Agrobacterium tumefaciens*	Nopaline synthase gene promoter (288 bp)
Zt	*Agrobacterium tumefaciens*	Nopaline synthase gene terminator sequence (256 bp)

Part IV Information relating the genetically modified plant

13. A description of the trait or traits and characteristics of the genetically modified plant which have been introduced or modified.

Polyunsaturated fatty acids

The omega-3 long chain polyunsaturated fatty acids (LC-PUFA) eicosapentaenoic acid (abbreviated to EPA; $20{:}5\Delta^{5,8,11,14,17}$) and docosahexaenoic acid (abbreviated to DHA; $22{:}6\Delta^{4,7,10,13,16,19}$) are components of fish oil, and known to help protect against cardiovascular disease (CVD). No higher plants contain EPA or DHA in their seed oils, although very many vegetable oils are rich in the simpler/shorter omega-3 α-linolenic acid (abbreviated to ALA; $18{:}3\Delta^{9,12,15}$). Through the seed-specific expression of algal and fungal genes involved in the biosynthesis of omega-3 LC-PUFAs, ALA is converted to EPA and thence DHA. This conversion only occurs in the seed, and results in a seed oil in which the fatty acid profile is modified. No alteration to fatty acid composition or lipid profile is observed in any other vegetative part of the plant. Similarly, omega-3 and omega-6 NMI-PUFAs such as sciadonic acid (abbreviated to SA; $20{:}4\Delta^{5,11,14}$; n-6) and juniperonic acid (abbreviated to JA; $20{:}4\Delta^{5,11,14,17}$; n-3) have very limited distribution in Angiosperm plants but are also emerging as useful fatty acids in the treatment of CVD. Such NMI-PUFAs can be produced through the seed-specific expression of "front-end" Δ5-desaturases which insert a double bond at the Δ5-position, irrespective of the position of pre-existing double bonds, generating the non-methylene interrupted polyunsaturated fatty acid.

Increased oil production

Vegetable oils make a substantial contribution to human and livestock nutrition and provide feedstock for a wide range of industrial products. The global demand for vegetable oils is continually increasing and so there is a need to improve the yield of oilseed crops, as well as specific attributes of their oil such as the types of fatty acids they contain. While exploring how enzymes control seed fatty acid composition in *Camelina sativa* seeds, we discovered a combination of transgenic modifications that increases seed oil content in containment glasshouse conditions. The approach uses seed-specific gene promoters to express an acyl-acyl carrier protein thioesterase gene from *Arabidopsis thaliana* and a modified form of a lysophosphatidic acid acyltransferase (LPAT) gene from *Brassica napus* in the developing seeds of *Camelina sativa*, while also suppressing expression of endogenous genes coding for the enzymes LPAT2, fatty acid desaturase 2 (FAD2), FAD3 and fatty acid elongase 1. The reason for including these plants in the trial is to determine whether they produce seeds with increase oil content under field conditions.

Sinapoyl ester reduction

Brassicaceous plants accumulate significant amounts of sinapine, which is derived from a branch of the phenylpropanoid pathway. Unfortunately, the presence of sinapine (and other sinapoyl esters) in Brassicaceous oilseeds reduces the suitability of their protein-rich seed meal for use as animal feed. Sinapine has a bitter taste and can reduce protein digestibility. To lower seed sinapine (and total sinapoyl ester) content in *Camelina sativa* we expressed a modified bacterial sinapic acid decarboxylase (SAD) transgene under the control of a seed specific promoter. This depletes the hydroxycinnamic acid precursor pools for sinapoyl ester biosynthesis and reduces sinapine content in seeds of plants grown in containment glasshouse conditions. The reason for including these plants in the trial is to determine whether they produce seeds with low sinapine content under field conditions.

CRISPR-Cas9 gene-editing of genes involved in fatty acid metabolism

C. sativa plants have been generated in which particular enzyme activities have been ablated as a result of the targeted disruption of some or all of the homeologues which encode a given gene – since *C. sativa* is hexaploid, this usually means three homeologues for any given gene. For example, the microsomal oleate ∆12-desaturase FAD2 has been targeted to increase the levels of oleic acid present within the seed oil (Moreineau et al., 2017). Similarly, the fatty acid elongase FAE1, responsible for the elongation of oleic acid to C20+ forms, has been inactivated (Ozseyhan et al., 2018) to generate a seed oil profile effectively devoid of long chain fatty acids >C18. Both the CRISPR-Cas9-generated *fad2* and *fae1* mutants are devoid of transgenes.

14. The following information on the sequences actually inserted or deleted:

the size and structure of the insert and methods used for its characterisation, including information on any parts of the vector introduced into the genetically modified plant or any carrier or foreign DNA remaining in the genetically modified plant,

(b) the size and function of the deleted region or regions,
(c) the copy number of the insert, and
(d) the location or locations of the insert or inserts in the plant cells (whether it is integrated in the chromosome, chloroplasts, mitochondria, or maintained in a non-integrated form) and the methods for its determination.

It is proposed to evaluate the performance of multiple individual events derived from constructs generated from the elements listed above in **Section 12**, with the objective of determining performance of different combinations of biosynthetic activities and regulatory elements which are most efficient for the production of omega-3 LC-PUFAs such as EPA and DHA in transgenic *C. sativa*. The representation of the different biosynthetic routes is shown below in Fig. 1.

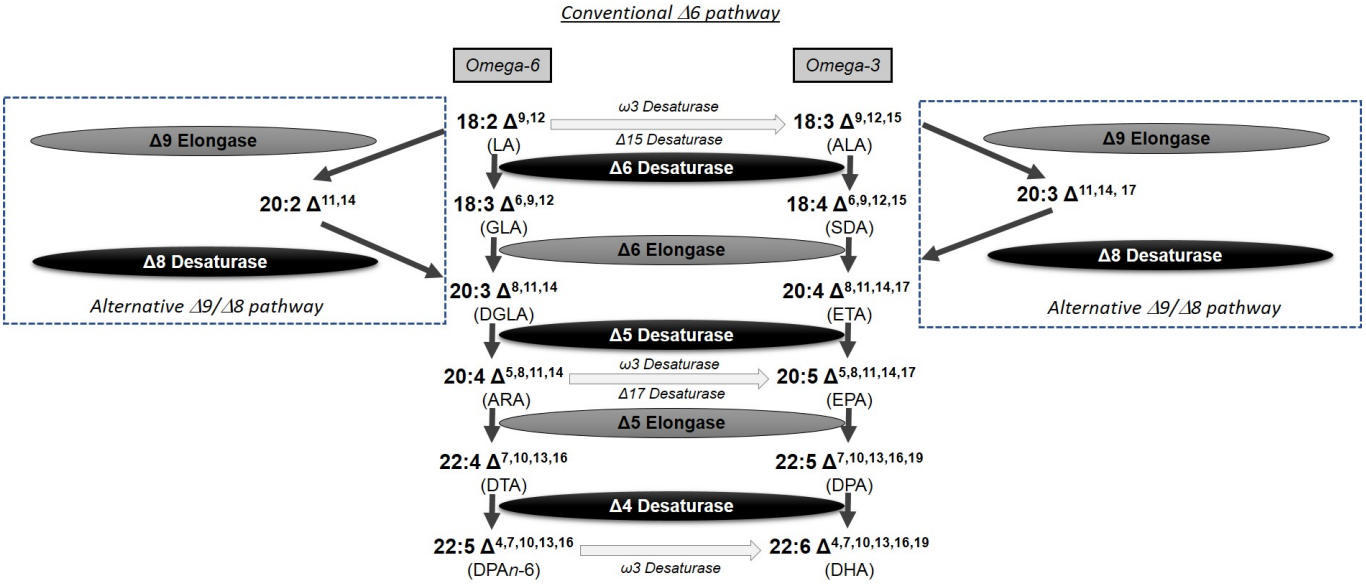


Fig 1. Schematic representation of long chain polyunsaturated fatty acid biosynthesis

It is also proposed to evaluate the performance of genes involved in the synthesis of a related class of fatty acids, the non-methylene-interrupted PUFAs (NMI-PUFAs). A schematic representation of the pathway, showing their common activities, is shown below (Figure 2).

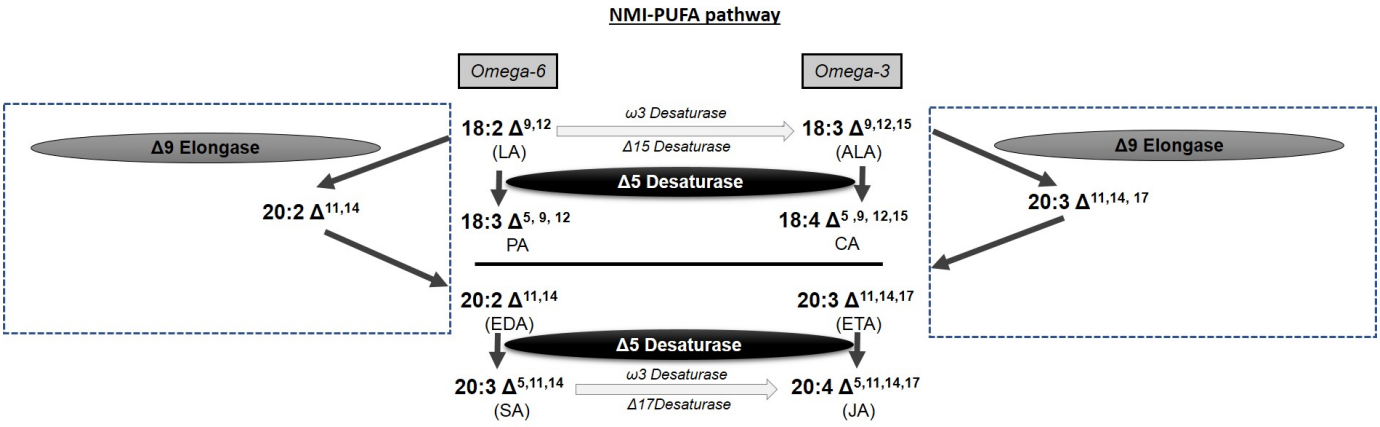


Fig 2. Schematic representation of non-methylene interupted polyunsaturated fatty acid biosynthesis. NMI-PUFAs are pinoleic acid (PA), conferonic acid (CA), sciadonic acid (SA) and juniperonic acid (JA).

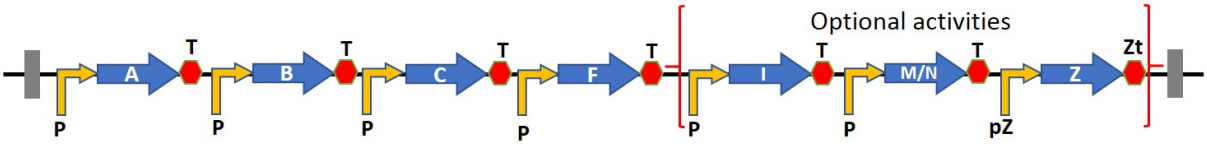
Transgenic C. sativa accumulating Omega-3 Long Chain Polyunsaturated Fatty Acids

As illustrated above in Figure 1, there are two biosynthetic routes by which EPA (and hence metabolites such as DPA and DHA) can be produced – the so-called "conventional" Δ6-pathway, where precursor fatty acids linoleic acid (18:2$\Delta^{9,12}$; LA) and a-linolenic acid (18:3$\Delta^{9,12,15}$; ALA) are modified by the action of the Δ6-desaturase and susbequent elongation, or the "alternative" Δ8-pathway, where the precursor fatty acids LA and ALA undergo 2-carbon elongation to C20 forms, prior to the introduction of a double bond at the Δ8-position. Both pathways converge at the third step in the pathway, the introduction of a double bond at the Δ5 position in the C20 fatty acid. All enzyme in both pathways recognise both omega-6 (sometimes also known as n-6) and omega-3 (sometimes known as n-3) substrates [see Fig. 1 for full details].

Here we wish to evaluate the performance of various combinations of biosynthetic activities for the synthesis of EPA and DHA in transgenic Camelina, using a panel of genes from different organisms which naturally accumulate these fatty acids. In that respect, this approach is the same as previously carried out in 14/R8/01, 16/R8/01 and 18/R8/01, only on a more comprehensive scale.

As identified in Table 1 of section 12, genes encoding each step of biosynthetic pathway(s) have been identified and characterised in yeast. A panel of constructs for Agrobacterium-mediated plant transformation have been generated in the pSOUP binary vector, in which these activities are under control of seed-specific promoters (as previously described – 18/R8/01). Schematic representation of constructs for the production of different fatty acids are shown below. Each particular combination of genes and regulatory elements would be given a unique identifier (e.g EPA-Δ6-XXX_aa)

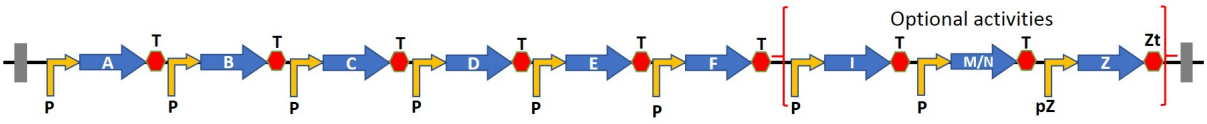
EPA-Δ6



EPA-Δ6 contains a minimum of **four** expression cassettes. Each cassette is an independent unit containing a promoter element (P), open reading frame (ORF) encoding biosynthetic activity of interest (A-J) and transcription regulatory element (T). In the example shown, (reading from left to right), an ORF from Group A (Δ6-desaturases) is under the control of a seed-specific promoter selected from Group P and a transcriptional terminator from Group T. Similarly, ORFs from Group B (Δ6-elongase), Group C (C20 Δ5-desaturase) and Group F (w3-desaturase) are also under the control of a seed-specific promoter selected from Group P and a transcriptional terminator from Group T. Thus, such a construct has all the necessary activities for the seed-specific synthesis of EPA via the conventional Δ6-pathway in transgenic Camelina. The construct can also optionally contain additional cassettes for the seed-specific expression of further genes of interest – in this example, taken

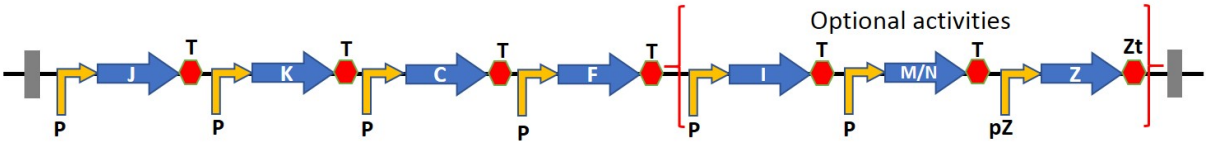
from Group I (Δ12/15-desaturases), Groups M (acyltransferases) and/or Group N (Trans-acting factors). The optional presence of a selectable marker cassette (Group Z) is also indicated. We have previously been given approval to carry out field release of GM Camelina plants containing this configuration of transgenes (i.e. EPA2015.4 and EPA2015.8 -18/R8/01; B4.1 – 16/R8/01). Intergration of the region spanning between the T-DNA left and right borders (LB and RB, indicated by the terminal grey blocks), is into the nuclear genome.

DHA-Δ6



DHA-Δ6 contains a minimum of **six** expression cassettes. Each cassette is an independent unit containing a promoter element (P), open reading frame (ORF) encoding biosynthetic activity of interest (A-J) and transcription regulatory element (T). In the example shown, (reading from left to right), an ORF from Group A (Δ6-desaturases) is under the control of a seed-specific promoter selected from Group P and a transcriptional terminator from Group T. Similarly, ORFs from Group B (Δ6-elongase), Group C (C20 Δ5-desaturase), Group D (Δ5-elongase), Group E (Δ4-desaturase) and Group F (w3-desaturase) are also under the control of a seed-specific promoter selected from Group P and a transcriptional terminator from Group T. Thus, such a construct has all the necessary activities for the seed-specific synthesis of both EPA (via the conventional Δ6-pathway) and DHA in transgenic Camelina. The construct can also optionally contain additional cassettes for the seed-specific expression of further genes of interest – in this example, taken from Group I (Δ12/15-desaturases), Groups M (acyltransferases) and/or Group N (Trans-acting factors). The optional presence of a selectable marker cassette (Group Z) is also indicated. We have previously been given approval to carry out field release of GM Camelina plants containing this configuration of transgenes (i.e. DHA2015.x series in 18/R8/01; B7.2 in16/R8/01; DHA5_33 in 14/R8/01). Intergration of the region spanning between the T-DNA left and right borders (LB and RB, indicated by the terminal grey blocks), is into the nuclear genome.

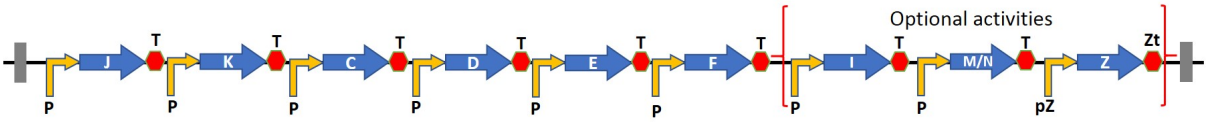
EPAΔ9



EPA-Δ9 contains a minimum of **four** expression cassettes. Each cassette is an independent unit containing a promoter element (P), open reading frame (ORF) encoding biosynthetic activity of interest (A-J) and transcription regulatory element

(T). In the example shown, (reading from left to right), an ORF from Group J (Δ9-elongase) is under the control of a seed-specific promoter selected from Group P and a transcriptional terminator from Group T. Similarly, ORFs from Group K (Δ8-elongase), Group C (C20 Δ5-desaturase) and Group F (w3-desaturase) are also under the control of a seed-specific promoter selected from Group P and a transcriptional terminator from Group T. Thus, such a construct has all the necessary activities for the seed-specific synthesis of EPA via the alternative Δ9-pathway in transgenic Camelina. The construct can also optionally contain additional cassettes for the seed-specific expression of further genes of interest – in this example, taken from Group I (Δ12/15-desaturases), Groups M (acyltransferases) and/or Group N (Trans-acting factors). The optional presence of a selectable marker cassette (Group Z) is also indicated. Although this particular configuration of the EPA biosynthetic pathway has not been considered for release, we have previously been given approval to carry out field release of GM Camelina plants containing the EPA trait (i.e. EPA2015.4 and EPA2015.8 -18/R8/01; B4.1 – 16/R8/01). Intergration of the region spanning between the T-DNA left and right borders (LB and RB, indicated by the terminal grey blocks), is into the nuclear genome.

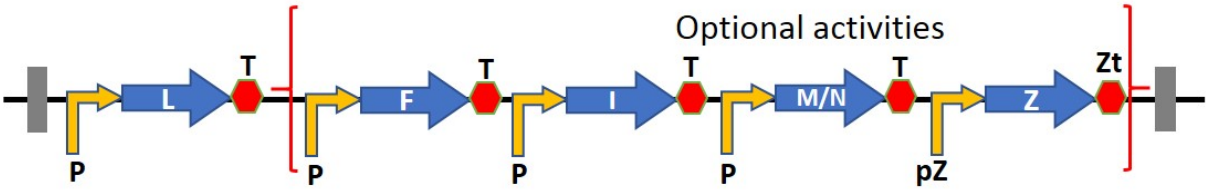
DHA-Δ9



DHA-Δ9 contains a minimum of **six** expression cassettes. Each cassette is an independent unit containing a promoter element (P), open reading frame (ORF) encoding biosynthetic activity of interest (A-J) and transcription regulatory element (T). In the example shown, (reading from left to right), an ORF from Group J (Δ9-elongase) is under the control of a seed-specific promoter selected from Group P and a transcriptional terminator from Group T. Similarly, ORFs from Group K (Δ8-elongase), Group C (C20 Δ5-desaturase) and Group F (w3-desaturase) are also under the control of a seed-specific promoter selected from Group P and a transcriptional terminator from Group T. Thus, such a construct has all the necessary activities for the seed-specific synthesis of EPA via the alternative Δ9-pathway in transgenic Camelina. The construct can also optionally contain additional cassettes for the seed-specific expression of further genes of interest – in this example, taken from Group I (Δ12/15-desaturases), Groups M (acyltransferases) and/or Group N (Trans-acting factors). The optional presence of a selectable marker cassette (Group Z) is also indicated. Although this particular configuration of the EPA & DHA biosynthetic pathway has not been considered for release, we have previously been given approval to carry out field release of GM Camelina plants containing the EPA & DHA trait (i.e. DHA2015.x series in 18/R8/01; B7.2 in16/R8/01; DHA5_33 in 14/R8/01). Intergration of the region spanning between the T-DNA left and right borders (LB and RB, indicated by the terminal grey blocks), is into the nuclear genome.

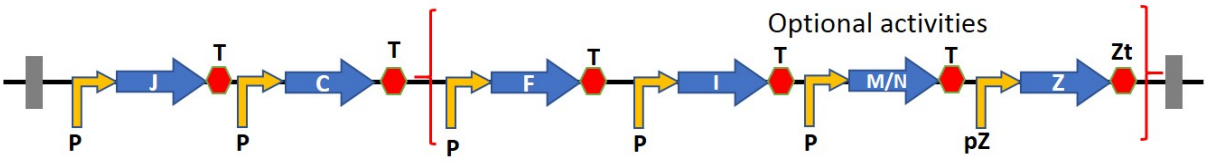
Transgenic C. sativa accumulating Non-Methylene-Interupted Polyunsaturated Fatty Acids (NMI-PUFAs)

C18 NMI-PUFA



C18 NMI-PUFA contains a minimum of **one** expression cassette, but can contain more than these two unit. Each cassette is an independent unit containing a promoter element (P), open reading frame (ORF) encoding biosynthetic activity of interest (A-J) and transcription regulatory element (T). In the example shown, (reading from left to right), an ORF from Group L (C18 Δ5-desaturase) is under the control of a seed-specific promoter selected from Group P and a transcriptional terminator from Group T. The construct can also optionally contain additional cassettes for the seed-specific expression of further genes of interest – in this example, taken from Group F (w3-desaturase), Group I (Δ12/15-desaturases), Groups M (acyltransferases) and/or Group N (Trans-acting factors). The optional presence of a selectable marker cassette (Group Z) is also indicated. Thus, such a construct has all the necessary activities for the seed-specific synthesis of C18 non-methylene interupted fatty acids such as pinoleic acid (18:3$\Delta^{5,9,12}$; PA), conferonic acid (18:4$\Delta^{5,9,12,15}$; CA) and columbinic acid (18:3$\Delta^{5t,9,12}$). Intergration of the region spanning between the T-DNA left and right borders (LB and RB, indicated by the terminal grey blocks), is into the nuclear genome. In a different iteration, it is proposed to replace ORFs from Group L with ORFs from Group A.

C20 NMI-PUFA

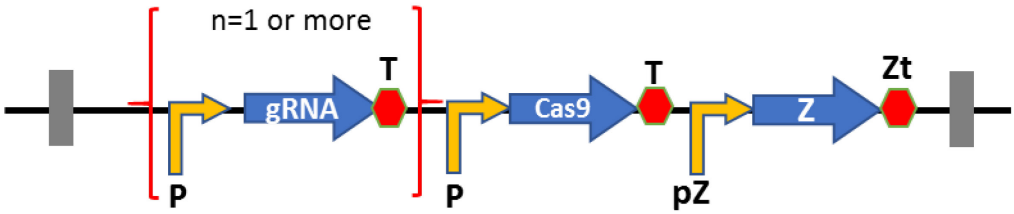


C20 NMI-PUFA contains a minimum of **two** expression cassettes, but can contain more than this two units. Each cassette is an independent unit containing a promoter element (P), open reading frame (ORF) encoding biosynthetic activity of interest (A-J) and transcription regulatory element (T). In the example shown, (reading from left to

right), an ORF from Group J (Δ9-elongase) is under the control of a seed-specific promoter selected from Group P and a transcriptional terminator from Group T. Similarly, an ORF from Group C (C20 Δ5-desaturase) is also under the control of a seed-specific promoter selected from Group P and a transcriptional terminator from Group T. The construct can also optionally contain additional cassettes for the seed-specific expression of further genes of interest – in this example, taken from Group F (w3-desaturase), Group I (Δ12/15-desaturases), Groups M (acyltransferases) and/or Group N (Trans-acting factors). The optional presence of a selectable marker cassette (Group Z) is also indicated. Thus, such a construct has all the necessary activities for the seed-specific synthesis of C20 non-methylene interupted fatty acids such as sciadonic acid (20:3$\Delta^{5,11,14}$; SA) and juniperonic acid 20:4 ($\Delta^{5,11,14,17}$; JA). Intergration of the region spanning between the T-DNA left and right borders (LB and RB, indicated by the terminal grey blocks), is into the nuclear genome.

CRISPR-Cas9-mediated gene-edited lines

It is envisaged that some of the constructs described above could not only be expressed in WT *C. saliva*, but also in lines that have undergone CRISPR-Cas9 gene editing to inactivate (through sequence-specific deletion) endogenous genes. Although such gene-edited plants do not ultimately contain any transgenes, they have undergone *Agrobacterium*-mediated transformation to deliver the Cas9 programmable nuclease and associated guide RNAs (gRNAs). Such a construct is represented below.



In this construct, the promoter (P) and transcriptional terminator (T) sequences are selected from Group X (Section 12) , as is the Cas9 activity (or similar – e.g. dCas9, Cas12, Cas14). The guide RNA sequence is a target-specific template of 20 nucleotides (known as the spacer), and varies from construct to construct. This synthetic sequence (which also contains an additional 3 nucleotides as the so-called PAM domain) is fused to the scaffold domain. The gRNA unit (spacer and scafold) can be represented one or times in the cassette, each time with a different sequence-specific spacer. Thus, a particular gene (or gene family) can be the target for multiple gRNAs and hence, cleavage by Cas9. The optional presence of a selectable marker cassette (Group Z) is also indicated.

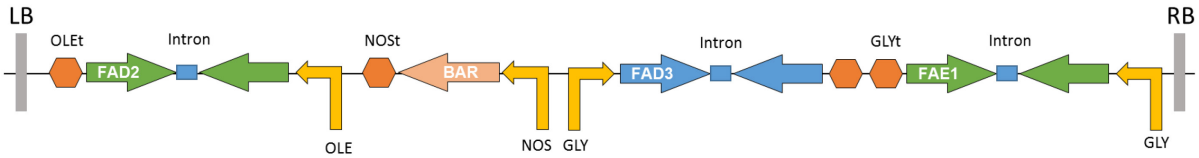
An example is the disruption of the FAD2 loci in *C. sativa*, described in Morineau et al (2017), where CRISPR-Cas9 was used to disrupt two or three of the homeologues of FAD2 (Δ12 desaturase; see also Group I). Gene-edited *C. sativa* plants were generated using two gRNAs against FAD2, and the resulting plants were screened both for the disruption of FAD2 homeologues but also (after backcrossing to WT C. sativa) the absence of the transgene cassette – this was verified by the lack of the DsRed marker (Group Z) and also via gene-specific PCR against the Cas9 cassette. It is envisaged that **two *C. sativa* lines A7** and **F4-24** would be released, varying only in the number of disrupted FAD homeologues – A7 is mutated in all three (designated *fad2* -/-/-) whereas F14-24 is disrupted in two of the three homeologues (designated *fad2* +/-/-) (Faure and Napier, 2018). These two lines were grown on the Appletree site in 2018, after evaluation by DEFRA and ACRE. The precise nature of the mutations present in the A7 and F4-24 lines is described in in Morineau et al (2017).

Another example is the disruption of the FAE1 loci in *C. sativa*, described in Ozseyhan et al. (2018) where CRISPR-Cas9 was used to disrupt all three of the homeologues of fatty acid elongase FAE1. Gene-edited *C. sativa* plants were generated using two gRNAs against FAE1, and the resulting plants were screened both for the disruption of FAE1 homeologues but also (after backcrossing to WT *C. sativa*) the absence of the transgene cassette – this was verified by the lack of the DsRed marker (Group Z) and also via gene-specific PCR against the Cas9 cassette. It is envisaged that **one *C. sativa* line (3-3)** would be released, disrupted in all three *fae1* homeologues, both as an individual line and also crossed with a line accumulating EPA and DHA, such as described in DHA-Δ6. The precise nature of the mutations present in the 3-3 line is described in Ozseyhan et al. (2018).

Additional seed quality modification traits

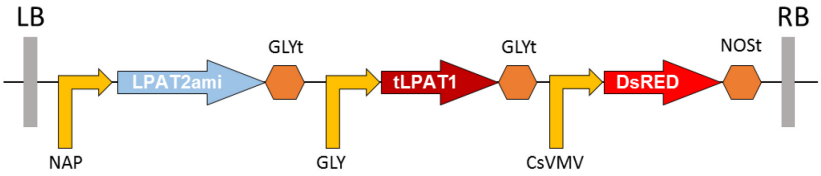
Construct HO

In construct HO, three RNA interference constructs under the control of seed-specific promoters are used to increase oleic acid content by reducing the polyunsaturated and very long chain fatty acid content of triacylglycerol. Specifically, three hairpin cassettes were used containing inverted repeats of *Camelina sativa* fatty acid desaturase 2, fatty acid desaturase 3 and fatty acid elongase 1 gene sequences, respectively placed either side of introns such as *Flaveria trinervia* pyruvate orthophosphate dikinase intron 3.



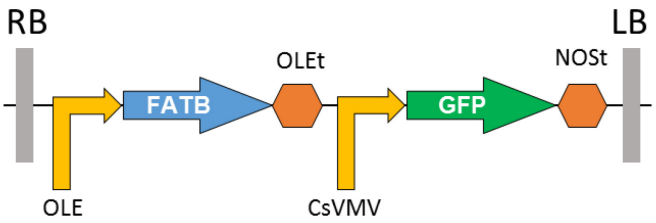
Construct LPAT

In construct LPAT, two heterologous genes under the control of a seed-specific promoters were assembled to direct incorporation of saturated fatty acyl groups into the stereospecific numbering (sn)-2 position of the glycerol backbone of triacylglycerol. Specifically, a sequence encoding a modified version of *Brassica napus* lysophosphatidic acid acyltransferase 1 (LPAT1) that localises to the endoplasmic reticulum and an artificial microRNA derived from *Arabidopsis thaliana* mi319a designed to target *Camelina sativa* LPAT2 were used. Construct LPAT was transformed into a homozygous line containing construct HO.



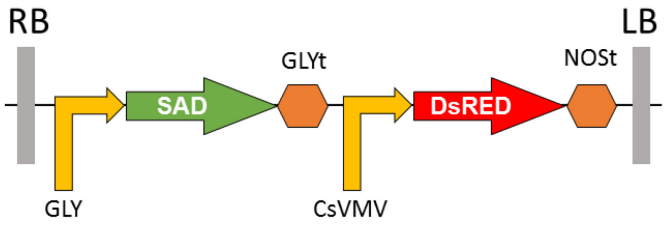
Construct FAT

In construct FAT, a single heterologous gene under the control of a seed-specific promoter was used to increase the production of saturated fatty acyl groups. Specifically, a sequence encoding the *Arabidopsis thaliana* acyl-acyl carrier protein thioesterase B was placed under the control of a seed-specific oleosin promoter. Construct FAT was transformed into homozygous lines containing Construct HO and Construct LPAT.



Construct SAD

In construct SAD, a single heterologous gene under the control of a seed-specific promoter was used to redirect metabolic flux away from sinapoyl ester biosynthesis. Specifically, a sequence encoding a bacterial (*Bacillus pumilus*) phenolic acid decarboxylase that has been engineered to metabolize sinapic acid was codon optimised for *Camelina sativa* expression and placed under the control of a seed-specific glycinin promoter. This results in the decarboxylation of sinapic acid and other hydroxycinnamic acid precursors that would otherwise be used for sinapoyl ester production.



15. The following information on the expression of the insert -

(a) information on the developmental expression of the insert during the lifecycle of the plant and methods used for its characterisation,

Seed-specific promoters (Group P) are active in the mid-stages of seed development but are otherwise not expressed in any other vegetative part of the plant.

(b) the parts of the plant where the insert is expressed, such as roots, stem or pollen.

The transgenes encoding the omega-3 LC-PUFA, NMI-PUFA biosynthetic pathways and other seed quality traits are all under the control of seed-specific promoters which are maximally active during the mid-stage of seed development. Expression is not observed in any other vegetative tissue during the life cycle of the plant. If a selectable or visible marker is used, this is expressed constitutively throughout the plant.

16. Information on how the genetically modified plant differs from the parental or recipient plant in the following respects -

(a) mode or modes and/or the rate of reproduction,
(b) dissemination,
(c) survivability.

In the case of plants derived from the lines described in **Section 14**, except for the accumulation of non-native omega-3 LC-PUFAs, NMI-PUFAs or other seed quality traits, the transgenic *C. sativa* plants are visually indistinguishable from the untransformed controls. No difference in seed set, seed size or germination has previously been observed for these traits in transgenic *C. sativa* or related species, not would be expected. Equally, no difference in fertility was previously observed or expected, and vegetative performance of the transgenic plants was found to be unaltered.

17. The genetic stability of the insert and phenotypic stability of the genetically modified plant.

Based on previous experiences, as described in earlier applications, plants where the transgenes encoding lipid biosynthetic pathways (such as omega-3 LC-PUFAs) are expressed in a seed-specific manner, all plants are morphologically indistinguishable from untransformed controls. Where already known, the inheritance of the transgene over 3+ generations follows normal rules of Mendelian genetics.

18. Any change to the ability of the genetically modified plant to transfer genetic material to other organisms.

None known or expected

19. Information on any toxic, allergenic or other harmful effects on human health arising from the genetic modification.

There are no known toxic, allergenic or harmful effects known to be associated with omega-3 LC-PUFAs or NMI-PUFAs, nor with variations in the accumulation of endogenous fatty acids such as oleic and palmitic acids in seed oils. Omega-3 LC-PUFAs fatty acids are very widely consumed by humans, being recognised as important and beneficial nutrients, and are widely dispersed in the marine foodweb. NMI-PUFAs are also a minor component of the human diet, being present in many animal-derived foodstuffs at low levels. Oleic and palmitic acid are ubiquitous components of all food webs.

The visual markers DsRed (Jach et al., 2001) encoding the red fluorescent protein from reef coral *Discosoma* sp has no known toxic, allergenic or harmful effects known to be associated with the DsRed protein, similarly for GFP a green fluorescent protein from *Aequorea victoria*.

The selectable marker *bar* (bialaphos resistance) encoding a phosphinothricin acetyl transferase; (PAT) activity from *Streptomyces,* which provides resistance to herbicides which act as inhibitors of glutamine synthase, a key enzyme in the nitrogen assimilation pathway of plants. There are no known toxic, allergenic or harmful effects known to be associated with the presence of this optional gene

20. Information on the safety of the genetically modified plant to animal health, particularly regarding any toxic, allergenic or other harmful effects arising from the genetic modification, where the genetically modified plant is intended to be used in animal feeding stuffs.

There are no planned feeding studies of genetically modified plants associated with this trial.

21. The mechanism of interaction between the genetically modified plant and target organisms, if applicable.

Not applicable

22. The potential changes in the interactions of the genetically modified plant with non-target organisms resulting from the genetic modification.

There are no obvious mechanisms that could result in a change in behaviour of non-target organisms as a result of exposure to omega-3 LC-PUFAs or NMI-PUFAs. Accumulation of such fatty acids in the seeds of camelina is not predicted to affect non-target organisms, neither is variation in endogenous components of the seed nor with the proteins associated with the marker genes (e.g. DsRed, *bar*). Thus, the likelihood of this potential hazard ever being realised is extremely low.

23. The potential interactions with the abiotic environment.

There are no known or predicted interactions associated with these traits and abiotic stress.

24. A description of detection and identification techniques for the genetically modified plant.

PCR using primers specific for synthesised & recoded (i.e. unique) transgenes can be used to detect GM Camelina. All constructs contain synthetic (i.e. non-native and not-existing in nature) sequences which allow for the design of highly specific PCR primers. Similarly, the optional presence of the florescent protein DsRed which acts a visual reporter on excitation at 555nm, or the presence of the *bar* gene and associated resistance to bialaphos represent additional methods.

It is equally possible to use fatty acid profiling to identify plants associated with the omega-3 or NMI-PUFA, since these lipids are not native to higher plants such as Camelina. By the same methods, it is possible to identify plants with altered fatty acid compositions (such as high oleic acid) through the same methodology (GC-FID of fatty acid methyl-esters). Such an approach can also be used to distinguish CRISPR-Cas9 gene-edited plants carrying mutations in *fad2* or *fae1.*

25. Information about previous releases of the genetically modified plant, if applicable.

Transgenic *C. sativa* containing related constructs for the seed-specific expression of omega-3 LC-PUFA were previously approved in the UK for experimental field

release (DEFRA Consents 18/R8/01, 16/R8/01 and 14/R8/01).

Part V Information relating to the site of release

(Applications for consent to release only)

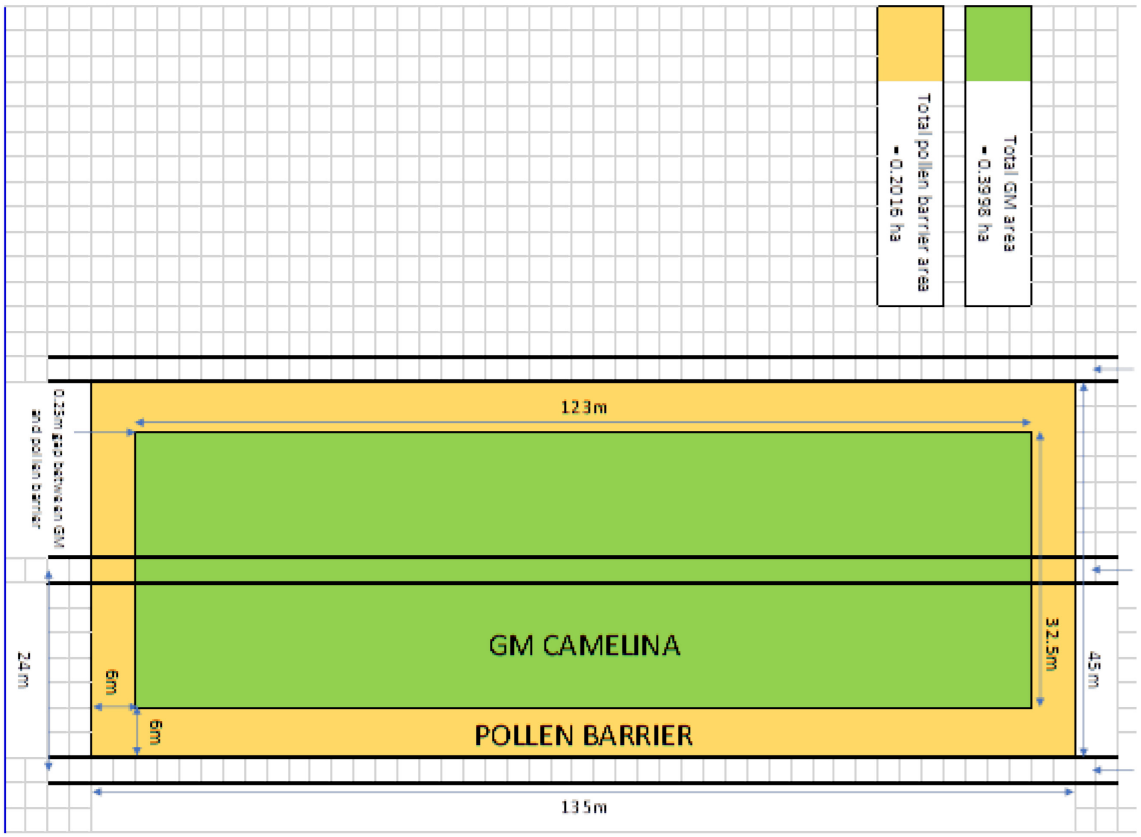
26. The location and size of the release site or sites.

We propose to carry out trials on both sites in consecutive seasons and will avoid reusing the same plots in subsequent years. It will be sited on the experimental farm at Rothamsted Research, Harpenden, at grid reference TL120130, and also at the experimental farm at Rothamsted Research, Brooms Barn, at grid reference TL756654.

For the area for the proposed Harpenden field trial, including controls, and spacing between GM plots will cover ~2900m^2, of which ~ 675m^2 will be GM.

Indicative plot layout for Appletree field trial site (Harpenden). One hundred and thirty-two plots of 4.25m x 1.2m indicated, allowing for the evaluation of up to 66 duplicated lines.

In the case of Brooms Barn field trial, the GM plot will cover ~4000m^2 with a surrounding pollen barrier of ~2000m^2



Enclosing the whole site will be a deer-proof fence also with lockable gates to prevent the entry of mammals.

27. A description of the release site ecosystem, including climate, flora and fauna.

The release sites are in arable agricultural areas forming part of the Rothamsted experimental farm. The flora and fauna are typical of agricultural land in the South East.

28. Details of any sexually compatible wild relatives or cultivated plant species present at the release sites.

C. sativa is known to cross-pollinate with other close members of the Camelina tribe.

Hybridisation with more distantly related Brassicaceae such as members of the Capsella tribe may be possible (Julie-Galau et al., 2013; Seguin-Swartz et al., 2013). In the cases of Camelina species which readily cross-hybridises with *C. sativa* (such as *C. alyssum, C. microcarpa*), there are no observed or reported cases of these species present on the Rothamsted farms. Querying the National Biodiversity Network database (www.nbn.org.uk) for the presence of *C. alyssum*, *C. microcarpa* and *C. rumelica*, returns no reports of these species being present within 15 km of the Rothamsted farms. Whilst potential cross-hybridising species such as *Capsella bursa-pastoris* are widely distributed across the UK and commonly found in the TL Ordnance survey 100km grid which contains both the Harpenden and Brooms Barn sites, the ability of *C. sativa* and *C. bursa-pastoris* to form viable offspring has experimentally been demonstrated to be very limited (Julie-Galau et al., 2013).

29. The proximity of the release sites to officially recognised biotopes or protected areas which may be affected.

There are no protected areas near the trial sites.

Part VI Information relating to the release

30. The purpose of the release of the genetically modified plant, including its initial use and any intention to use it as or in a product in the future.

This is a research trial to determine the agronomic performance and seed oil yield of transgenic *C. sativa* plants that have been engineered to accumulate non-native lipids (such as omega-3 LC-PUFAs, NMI-PUFAs etc) in their seed oils, or variation in the accumulation of native fatty acids such as oleic and palmitic acid.

31. The foreseen date or dates and duration of the release.

The GM C. sativa will be planted within the five-year period of this application (2019-2023). The plants will be sown in April/May and harvested in Aug/Sept.

32. The method by which the genetically modified plants will be released.

Seeds will be drilled using conventional drilling equipment, plot-scale farm equipment or hand-sowing. All drills will be cleaned within the designated trial areas.

33. The method for preparing and managing the release site, prior to, during and after the release, including cultivation practices and harvesting methods.

The sites will be prepared according to standard agronomic practices for spring Brassicaceae cultivation. The release will be monitored regularly during all stages of development and harvested at maturity. Some seeds from the GM and control plots will be conditioned, threshed and analysed in appropriate GM facilities to determine oil composition and oil quantity. All other material, including that from any pollen barrier will be harvested, either manually or via combining, and disposed of by incineration or deep burial at a local authority-approved landfill site using an approved contractor. Transportation of waste materials will be in secure containers.

34. The approximate number of genetically modified plants (or plants per square metre) to be released.

Trial design will be finalised depending on the number of lines to be evaluated in the field. We will adopt a plot design based on previous *C. sativa trials* e.g. 18/R8/01, although we envisage a greater number of lines to be tested in this instance. Practically, we will use 1.2m x 4.25m strips, duplicated within the GM trial area. The area containing the GM trial plots will be surrounded by a non-GM pollen barrier of C. sativa “Celine” (WT), of at least 6m width, sown to a density of at least 300 plants/m^2. GM plots will be sown to achieve a density of at least 250 plants/m^2.

As indicated in section 26, we envisage a maximum area of 675m^2 being used for the cultivation of GM lines (including WT controls) on the Appletree site (Harpenden) – thus at a density of 250 plants/m^2, the release would be 168,750 GM plants. In the case of the MarlPit site (Brooms Barn), the maximum area of GM is 4000m^2, meaning the release would be 1,000,000 GM plants. The total annual release (assuming a density of 250 plants/m^2) is therefore 1,168,750 GM plants.

Part VII Information on control, monitoring, post-release and waste treatment plans

35. A description of any precautions to -

(a) maintain the genetically modified plant at a distance from sexually compatible plant species, both wild relatives and crops.

There are no sexually compatible species liable to give rise to viable progeny present within the Rothamsted farms and associated estate.

(b) any measures to minimise or prevent dispersal of any reproductive organ of the genetically modified plant (such as pollen, seeds, tuber).

The trial has a strip of non-GM *C. sativa* to function as a pollen barrier – this will serve as a pollen-trap for pollen released from the GM C. sativa.

The drills will be filled on the trial area and will be thoroughly cleaned before leaving the trial area. All straw will be chopped and left on site.

Bird scaring measures such as; suspending wires across the area, deployment of gas guns and hawk kites to deter birds off the site. At drilling all care will be taken to ensure that no seed remains on the surface after drilling because this encourages pigeons in to try and find seeds. However, the risk is minimal at sowing and the bird scaring measures specified above will be enough to mitigate the risk. Appropriate husbandry steps will be taken to minimise the potential for seed dispersal by molluscs such as slugs.

36. A description of the methods for post-release treatment of the site or sites.

The trial will receive standard farm practise as regard to herbicide, fungicides and nitrogen in conjunction with the scientific co-ordinator. The site will be regularly monitored from sowing to harvest and during the following cropping year, as per the protocols developed with the GM Inspectorate.

37. A description of the post-release treatment methods for the genetically modified plant material including wastes.

At harvest, all seeds from the GM plots will be collected with a plot combine to obtain yield measurements. The seed sampled will be analysed in the contained laboratory facilities at Rothamsted Research, all samples taken from the field will be closely monitored and records kept of weights and movements of seeds. All samples removed from the trial site will eventually be destroyed by an approved technique. The remainder of the site will be harvested by the plot combine or similar. The grain obtained will be disposed to deep landfill using an approved contractor. The plot combine will be cleaned on the trial site prior to leaving the site so that all traces of GM plant material will remain in the trial area. The trial area will remain in stubble for the following year to enable monitoring of volunteers and a broad-spectrum herbicide such as glyphosate will be applied as required.

38. A description of monitoring plans and techniques.

As defined by the GM Inspectorate, the trial sites will be monitored regularly (at least weekly) during the growing period (May-Aug) and after the termination of the trial during the following year. Records will be kept of each visit.

39. A description of any emergency plans.

In the unlikely event that the integrity of the site is seriously compromised, the trial will be terminated and all plants, (including GM and control plots and pollen barrier) will be destroyed using a suitable herbicide or harvesting, as deemed appropriate. All harvested material will be removed from the site and disposed of by incineration or deep burial at a local authority-approved landfill site using an approved contractor. Transportation of waste materials will be in secure containers. The phone numbers of all key staff will be available to site security and farm.

40. Methods and procedures to protect the site.

The Hertfordshire Constabulary have been notified that we are seeking permission to carry out further GM field trials at Rothamsted Research. Internal security measures will continue as before. The trial sites are surrounded by secure fences.

Part VIII Information on methodology

41. A description of the methods used or a reference to standardised or internationally recognised methods used to compile the information required by this Schedule, and the name of the body or bodies responsible for carrying out the studies.

1. DNA synthesis was carried out by GenScript Inc. USA (http://www.genscript.com/index.html).

2. Standard molecular biology reagents and methods were used following Sambrook et al, (1989). Modular assemble of constructs was carried out using the LOOP protocols described in Pollak et al, (2018).

3. *C. sativa* transformation via floral infiltration was performed using *Agrobacterium* as described in Lu and Kang, (2008). This method avoids the use of any tissue culture steps in the generation of the transgenic *C. sativa* lines.

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