Department for Environment, Food and Rural Affairs

Application for consent to release genetically modified higher plants for non-marketing purposes

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

Part I 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

Persons associated with this release

Research Scientist (Application Lead)	BSc. PhD. DSc. Plant metabolic engineering. 25 years' experience at Cambridge University, Long Ashton Research Station (Bristol) and Rothamsted. Flagship Leader, Rothamsted Research
Research Scientist	BSc, PhD. Plant Biochemistry. 20 years' experience at Rothamsted

Research Scientist	BSc, PhD. Plant Genetics and Synthetic Biology. 8 years' experience at Rothamsted
Research Scientist	BSc, PhD. Plant Biochemistry. 20 years' experience at Rothamsted, University of Warwick, University of York.
Research Scientist	BSc, PhD. Plant Biochemistry. 20 years' experience at Rothamsted, University of Cambridge, University of Bristol.
Research Scientist	BSc, PhD. Plant Biochemistry. 20 years' experience at Rothamsted, University of Bristol.
Farm Manager	BSc. 20 years' experience with agricultural experimentation.
Head of Health Safety and Biosafety	BSc., PhD. Institute Safety Officer Experience managing clinical containment 1, 2 and 3 laboratories
Director, Rothamsted Research	Director & CEO, Rothamsted Research

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, Suneson

(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 -110 days, with seeds being sown in late 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 in Europe.

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, Czechia, 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 USA.

8. Where the application relates to a plant species which is not normally grown in Europe, 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, Hyaloperonospora 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 strains used were *Agrobacterium* tumefaciens strains GV3101 (Koncz and Schell, 1986) and AGL1 (Lazo et al., 1991).

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 the “open-source” Golden Gate cloning vector pCambia (Sauret-Güeto et al., 2020).

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 (25 bp) [♠]
LB	*Agrobacterium tumefaciens*	T-DNA Left border (25 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	*Glycine max*	Oleosin seed storage protein promoter (579 bp)
pSBP	*Vicia faba*	Sucrose binding protein seed-specific promoter (1799bp) [♠]
pARC	*Phaseolus vulgaris*	Arcelin-5 seed specific promoter (1159 bp) [♠]
pFAE	*Brassica napus*	Fatty acid elongase (FAE) seed specific promoter (1430 bp)
pBetaC	*Glycine max*	Beta-conglycinin seed-specific promoter (622 bp)
pAt2S3	*Arabidopsis thaliana*	Seed storage albumin 3 (AT2S3) promoter (449bp)
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) [♠]
PvArc5t	*Phaseolus vulgaris*	Arcelin-5 gene terminator (600bp) [♠]
CatpAt	*Solanum tuberosum*	Cathepsin D inhibitor terminator sequence (235bp) [♠]
E9t	*Pisum sativum*	Rubisco SSU E9 terminator sequence (558bp) [♠]
FAEt	*Brassica napus*	Fatty acid elongase (FAE) terminator sequence (400bp)
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) [♠]
OLEOt	*Glycine max*	Oleosin seed storage protein terminator (512 bp)
Biosynthetic genes		
Δ6-desaturases (Group A)		
OtD6	Synthetic	Encodes a fatty acid **Δ6-desaturase** from *Ostreococcus tauri* (1371 bp) [♠]
MsqD6	Synthetic	Encodes a fatty acid **Δ6-desaturase** from *Mantoniella squamata* (1350 bp) [♠]
PrimD6	Synthetic	Encodes a fatty acid **Δ6-desaturase** from *Primula spp.* (1362 bp)
Δ6-elongases (Group B)		

FcELO6	Synthetic	Encodes a fatty acid **Δ6-elongase** from *Fragilariopsis cylindrus* (819 bp) [♠]
PpELO6	Synthetic	Encodes a fatty acid **Δ6-elongase** from *Physcomitrella patens* (873 bp) [♠]
Δ5-desaturases (C20) (Group C)		
EmiD5	Synthetic	Encodes a fatty acid **Δ5-desaturase** from *Emiliania huxleyi* (1368 bp) [♠]
ThraD5	Synthetic	Encodes a fatty acid **Δ5-desaturase** from *Thraustochytrium spp.* (1317 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) [♠]
AaELO5	Synthetic	Encodes a fatty acid **Δ5-elongase** from *Aureococcus anophagefferens* (765 bp)
ThalpELO5	Synthetic	Encodes a fatty acid **Δ5-elongase** from *Thalassiosira pseudonana* (879 bp)
MmELOVL1	Synthetic	Encodes a fatty acid **elongase** from *Mus musculus* (840 bp)
MmELOVL2	Synthetic	Encodes a fatty acid **elongase** from *Mus musculus* (828 bp)
MmELOVL3	Synthetic	Encodes a fatty acid **elongase** from *Mus musculus* (816 bp)
MmELOVL4	Synthetic	Encodes a fatty acid **elongase** from *Mus musculus* (939 bp)

Δ4-desaturases (Group E)		
TpD4	Synthetic	Encodes a fatty acid **Δ4-desaturase** from *Thalassiosira pseudonana* (1650 bp) [♠]
O809D4	Synthetic	Encodes a fatty acid **Δ4-desaturase** *Ostreococcus* RCC809 (1377 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) [♠]
w3-desaturases (Group F)		
PythW3	Synthetic	Encodes a fatty acid **w3-desaturase** from *Pythium spp* (1101 bp) [♠]
PiW3	Synthetic	Encodes a fatty acid **w3-desaturase** from *Phytophora infestans* (1086 bp) [♠]
HpW3	Synthetic	Encodes a fatty acid **w3-desaturase** from *Hyaloperonospora parasitica* (1086 bp) [♠]
Δ12/Δ15-desaturases (Group I)		
BoofD12	Synthetic	Encodes a fatty acid **Δ12-desaturase** FAD2 activity from *Borago officinalis* (1149 bp) [♠]
PsD12	Synthetic	Encodes a fatty acid **Δ12-desaturase** activity from *Phytophora sojae* (1197 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)

PerfD15	Synthetic	Encodes a fatty acid **Δ15-desaturase** FAD3 activity from *Perilla frutescens* (1176 bp) [♠]
AtFAD3	Synthetic	Encodes a fatty acid **Δ15-desaturase** FAD3 activity from *Arabidopsis thaliana* (1161 bp)
Acyltransferases (Group M)		
BtDGAT1	Synthetic	Encodes diacylglycerol acyltransferase from *Bos tarus* (1470 bp)
ChDGAT1	Synthetic	Encodes diacylglycerol acyltransferase from *Capra hircus* (1470 bp)
CrLPAAT2	Synthetic	Encodes lysophosphatidic acid acyltransferase from *Chlamydomonas reinhardtii* (978 bp)
LPAT1	*Brassica napus*	Encodes a modified lysophosphatidic acid acyltransferase 1 that localises in the endoplasmic reticulum (768 bp) [♠]
HsAGPAT1	Synthetic	Encodes lysophosphatidic acid acyltransferase from *Homo sapiens* (852 bp)
Other seed-specific transgenes (Group N)		
CsWRI1/2	*Camelina sativa*	Encodes the WRINKLED1 (WRI1/2) 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) [♠]
CvTE	Synthetic	Encodes acyl-ACP thioesterase from *Cuphea viscosissima* (1260 bp)
CpuTE	*Synthetic*	Encodes acyl-ACP thioesterase from *Cuphea pulcherrima* (1251 bp)
CpTE	*Cuphea palustris*	Encodes acyl-ACP thioesterase from *Cuphea palustris* (1236 bp) [♠]

UcTE	Synthetic	Encodes acyl-ACP thioesterase from *Umbellularia californica* (1149 bp)
ChTE	*Cuphea hookeriana*	Encodes acyl-ACP thioesterase from *Cuphea hookeriana* (1248 bp) [♠]
BtTE	Synthetic	Encodes acyl-ACP thioesterase from *Bacteroides thetaiotaomicron* with plastid targeting sequence from Rubisco small subunit of *Pisum sativum* (978 bp)
MfTE	Synthetic	Encodes acyl-ACP thioesterase from *Marvinbryantia formatexigens* with plastid targeting sequence from Rubisco small subunit of *Pisum sativum* (951 bp)
ScFAS	Synthetic	Encodes fusion of type 1 fatty acid synthase genes from *Saccharomyces cerevisiae* (11814 bp)
PDCT amiRNA	Synthetic	Arabidopsis thaliana artificial microRNA mi319a adapted to target *Camelina sativa* phosphatidylcholine:diacylglycerol cholinephosphotransferase (404 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) [♠]
CBFD2	*Adonis aestivalis*	Encodes carotenoid β-ring 4-dehydrogenase (930 bp) [♠]
HBFD1	*Adonis aestivalis*	Encodes carotenoid 4-hydroxy-β-ring 4-dehydrogenase (1224 bp) [♠]
ZmPYS	*Zea mays*	Encodes phytoene synthase (1233 bp) [♠]
CsARF2	*Camelina sativa*	Encodes a negative regulator of seed coat cell number (5075 bp)

OLEO-Nfus	Synthetic	Encodes an oleosin protein from Arabidopsis thaliana (519bp) N-terminally fused to a hypervariable domain (330-3621 bp) by a prochymosin cleavage site (45bp).
CcCSAD	*Synthetic*	Encodes cysteine sulfinic acid decarboxylase from *Cyprinus carpio*
CcCDO	*Synthetic*	Encodes cysteine dioxygenase from *Cyprinus carpio*
Regulatory elements used to direct Cas9 expression (Group X)		
Cas9	Synthetic	RNA-guided DNA endonuclease from *Streptococcus pyogenes* (4104 bp) [♠]
pPcUbi4-2	*Petroselinum crispum*	Ubiquitin4–2 promoter to direct expression of Cas9 (971 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) [♠]
	Synthetic	Target-specific templates for guide RNAs (<24bp)
Marker genes and associate regulatory elements (Group Z)		
DsRed	Synthetic	Encodes a red florescent protein from *Discosoma* 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 N herbicides such as glufosinate. (552 bp) [♠]
AHAS	Camelina	Encodes acetohydroxy acid synthase isoform providing resistance to imidazolinone herbicides (3500bp)

*p*Z	*Cassava vein mosaic virus*	CsVMV promoter sequence (528 bp) [♠]
hpt	Synthetic	Encodes a hygromycin-B phosphotransferase (hpt) enzyme, allows selection for resistance to the antibiotic hygromycin B. (1026 bp) [♠]
nptII	Synthetic	Encodes a neomycin phosphotransferase II enzyme, allows transformed plants to metabolize neomycin and kanamycin antibiotics during selection. (795 bp) [♠]
*p*NOS	*Agrobacterium tumefaciens*	Nopaline synthase gene promoter (288 bp) [♠]
Zt	*Agrobacterium tumefaciens*	Nopaline synthase gene terminator sequence (256 bp) [♠]
p35S	*Cauliflower mosaic virus*	35S promoter from the Cauliflower Mosaic Virus (CaMV) (699 bp) [♠]
RUBY	Synthetic	Encodes fusion of a P450 oxygenase CYP76AD1 (1491 bp) from *Beta vulgaris*, a L-DOPA 4,5-dioxygenase (825 bp) from *Beta vulgaris*, and a cyclo-DOPA 5-O-glucosyltransferase (1509 bp) from *Mirabilis jalapa*. The three genes are linked by two 2A self-cleaving peptides (each 66 bp) from *Porcine teschovirus-1 2A*.
[♠] denotes a genetic element that has been included in previous Consents (14/R8/01, 16/R8/01, 18/R8/01, 19/R8/01).		

Part IV Information relating to 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; $20{:}5\Delta^{5,8,11,14,17}$), docosapentaenoic acid (abbreviated to DPA; $22{:}5\Delta^{7,10,13,16,19}$) and docosahexaenoic acid (abbreviated to DHA;

22:6$\Delta^{4,7,10,13,16,19}$) are C20 and C22 fatty acid components of fish oil, and known to help protect against cardiovascular disease. No higher plants contain EPA, DPA or DHA in their seed oils, although very many vegetable oils are rich in the simpler/shorter (C18) 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 DPA and 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 (Han et al., 2020; https://doi.org/10.1111/pbi.13385).

Ultralong PUFAs

Ultra-long chain (ULC) omega-3 PUFAs with chain length comprised between C28 and C36 are found in specific animal organs such as the retina and testis tissues where they play essential physiological roles and are derived from EPA and DHA. Emerging evidence suggest that these ULCs can be taken orally as food supplement and may provide an efficient way to treat and/or prevent a number of pathologies linked to macular degeneration and possibly fertility issues. Such ULC-PUFAs can be produced through the seed-specific expression of a specific class of fatty acid elongase which use omega-3 PUFAs like EPA and DHA as substrates to produce omega-3 ULC-PUFAs by elongating the C20/22 fatty acid to a C28+ form.

Astaxanthin

The ketocarotenoid astaxanthin is found in a range of marine microorganisms, and the accumulation of this compound in marine foodwebs results in the distinctive pink pigmentation found in many organisms. Astaxanthin is also included in the diets of farmed fish such as salmon. Natural sources of astaxanthin include some marine species of krill and shrimp, and the microalga *Haematococcus pluvialis.* There are very few examples of higher plants which accumulate astaxanthin, the most notable example being the bright red flowers of the Adonis family. However, there are no reports of higher plants accumulating astaxanthin as a seed storage reserve. Astaxanthin biosynthetic genes from *Adonis aestivalis* were placed under the control of seed-specific promoters, resulting in the accumulation of this ketocarotenoid in C. sativa seeds. No accumulation of this compound was observed in any vegetative part of the plant.

Both omega-3 LC-PUFAs and astaxanthin are currently sourced from the marine environment. Thus, there is interest in producing new sources of these compounds to reduce reliance on oceanic stocks. Both compounds are routinely used in aquaculture as feed ingredients.

Milk fat substitutes

Animal fats (including milk fats) contain higher proportions of long-chain saturated fatty acids (mainly C16) esterified to the stereospecific numbering (sn)-2 position on the glycerol backbone of the triacylglycerol molecule than are found in fats (and oils)

from plants. Bovine milk fat also characteristically contains significant amounts of short to medium chain saturated fatty acids esterified to the sn-3 position (C4 to C12 ~15% of total fatty acids by weight). These features contribute to the physical, sensory and nutritive properties of the animal fats. There is significant interest in producing more sustainable and affordable alternatives to animal fats and we have previously shown it is possible to make transgenic plants produce the main type of triacylglycerol molecule found in human milk (van Erp et al. (2019) https://doi.org/10.1073/pnas.1907915116; van Erp et al. (2021) https://doi.org/10.1016/j.ymben.2021.05.009). The aim of these current studies is to determine the performance of *Camelina sativa* plants that accumulate triacylglycerol molecules in their seeds that have higher levels of saturated fatty acids and are more similar to those found in human and bovine milk.

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

(a) 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,

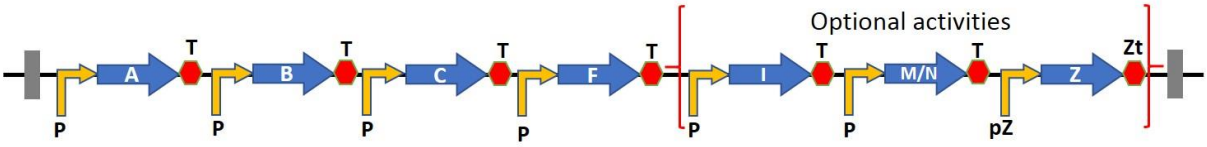
(b) the size and function of the deleted region or regions,
(c) the copy number of the insert, and
(d) the location or locations of the insert or inserts in the plant cells (whether it is integrated in the chromosome, chloroplasts, mitochondria, or maintained in a non-integrated form) and the methods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

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

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, 18/R8/01 and 19/R8/01. In particular, the formulaic description of constructs is identical to that used in 19/R8/01.

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 pGATEWAY or pCAMBIA binary vectors, 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-XXX_aa).

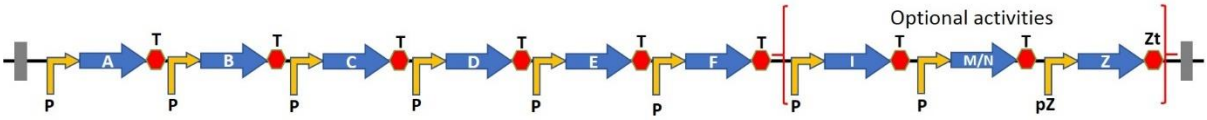
EPA-Super6-4



EPA-XXX constructs optimally 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 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) or Group N (Trans-acting factors and other seed metabolic activities). Equally, the presence of the Group F cassette could be despensed with, to alter the n-3:n-6 ratio. The optional presence of a selectable marker cassette(s) (Group Z) is also indicated. 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. Please note that the order of the Groups presented here is purely for ease of comprehension – there is no requirement to array them in a particular order, nor is any order implied. 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**).

The results of the field release of these lines have been reported in Usher et al., 2017; https://doi.org/10.1038/s41598-017-06838-0 , Han et al., 2020; https://doi.org/10.1111/pbi.13385). Specfically, exchanging some of the biosyntheitc activities for orthologous sequences derived from different sources resulted in variation in the accumulation of the target fatty acids such as EPA. For example, EPA2015.4 made less EPA than EPA2016.1, as a consequence of changing the Δ5-desaturase [activites represented in Group C]. New configurations of the pathway will focus on optimising promoter selection (avoiding repetition which could trigger silencing) as well as enhancing the flux towards the conversion of omega-6 to omega-3.

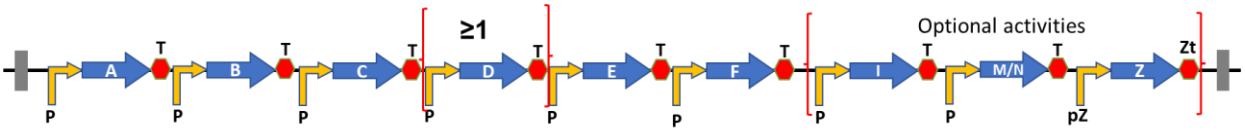
DHA-Super6-6



DHA-XXX optimally 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 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) or Group N (Trans-acting factors and other seed metabolic activities). Equally, the presence of the Group F cassette could be despensed with, to alter the n-3:n-6 ratio. The optional presence of a selectable marker cassette(s) (Group Z) is also indicated. In a further iteration, the presense of the Group E activity could be ommitted, leading to the synthesis of EPA and docosapentaenoic acid (DPA) but not DHA. 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. Please note that the order of the Groups presented here is purely for ease of comprehension – there is no requirement to array them in a particular order, nor is any order implied.

We have previously been given approval to carry out field release of GM Camelina plants containing this configuration of transgenes (i.e. DHA2015 series in **18/R8/01**; B7.2 in **16/R8/01**; DHA5_33 in **14/R8/01**). The results of the field release of these lines have been reported in Usher et al., 2015; DOI: 10.1016/j.meteno.2015.04.002, Usher et al., 2017; Ibid., Han et al., 2020; Ibid.). Similar to as described for the EPA constructs above, exchanging some of the activities for orthologous sequences derived from different sources resulted in modest variation in the accumulation of the target fatty acids such as EPA and DHA. For example, DHA2015.3 and DHA2015.4 made less DHA than DHA2015.1, as a consequence of changing the D4-desaturase and ELO5 for closely related sequences. New configurations of the pathway will focus on optimising promoter selection (avoiding repetition which could trigger silencing, which was previously observed and reported Han et al. 2020; Ibid.) as well as enhancing the flux towards the conversion of omega-6 to omega-3.

UltraPUFAs-Super6-7



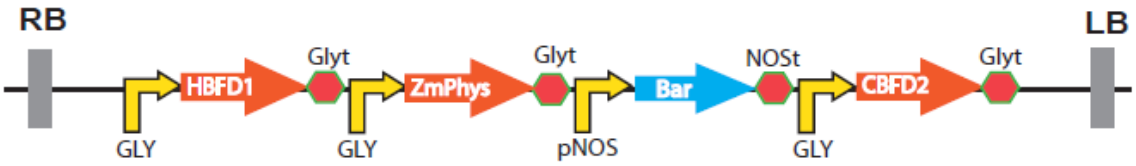
UltraPUFA-Δ6 optimally contains a minimum of seven expression cassettes. Each cassette is an independent unit containing a promoter element (P), open reading frame (ORF) encoding biosynthetic activity of interest (A-J) and transcription regulatory element (T). In the example shown, (reading from left 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 and DHA in transgenic Camelina. In this specific iteration, more than one Δ5-elongase from Group D is used to further elongate EPA and DHA to C22+ forms. 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) or Group N (Trans-acting factors and other seed metabolic activities). Equally, the presence of the Group F cassette could be despensed with, to alter the n-3:n-6 ratio. The optional presence of a selectable marker cassette(s) (Group Z) is also indicated. 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. Please note that the order of the Groups presented here is purely for ease of comprehension – there is no requirement to array them in a particular order, nor is any order implied.

Although we have not previously evaluated such a configuration in the field, we have previously been given approval to carry out field release of GM Camelina plants containing very similar configurations of transgenes (i.e. DHA2015 series in **18/R8/01**; B7.2 in **16/R8/01**; DHA5_33 in **14/R8/01** – details above). The only difference is the presence of an additional elongating activity which converts EPA and DHA to longer chain forms.

Additional seed quality modification traits

Astaxanthin

ASX-A2

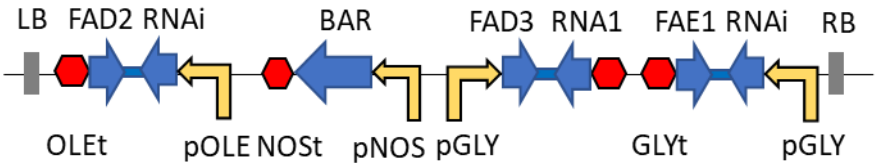


In Construct ASX-A2, three heterologous genes under the control of seed-specific promoters were assembled to direct the synthesis of the ketocarotenoid astaxanthin in the seeds of transgenic *C. sativa*. A phytoene synthase from maize (ZmPhys) was introduced to enhance the accumulation of phytoene (by the conversion of geranylgeranyl-diphosphate to phytoene) and subsequently to b-carotene, the latter

to provide substrate for the carotenoid β-ring 4-dehydrogenase (CBFD2) from *Adonis aestivalis.* The product of that enzyme, a 4-hydroxy-β-ring, is then converted to astaxanthin by the 4-hydroxy-β-ring 4-dehydrogenase (HBFD1) from *Adonis aestivalis.* All three genes are synthetic, being codon-optimised from their native sequences (derived either from maize or *Adonis aestivalis*). Each synthetic coding sequence is regulated by an individual seed-specific promoter, and also defined by a transcription termination sequence. Within the T-DNA there is also the selectable marker, BAR, which is constituitively expressed under the control of the nopaline synthase promoter and terminator. In a second iteration, the ZmPhys cassette was omitted from the construct to yield ASX-NG2. Genetic crosses between ASX-X and EPA_XXX or DHA_XXX are envisaged, as previously described in **18/R8/01**, which also defines the precise molecular organisation of this construct. The 3-gene iteration has been trialled in the field since 2018.

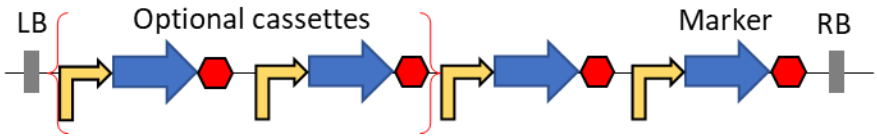
Milk fat substitutes

HO construct



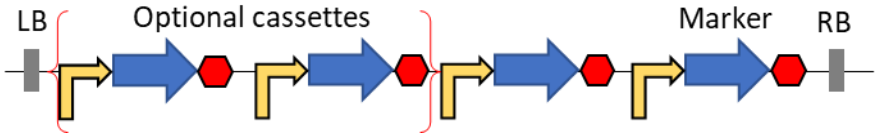
The HO construct is designed to increase seed oleic acid (C18:1) content and reduce polyunsaturated and very long chain fatty acid content. *Camelina sativa* transgenic lines containing this construct provide an appropriate background for engineering to produce substitutes for animal/milk fats by transformation with HS and SN2 constructs described below, or by crossing to lines that contain them. HO contains four expression cassettes. Each cassette is an independent unit containing a promoter sequence (yellow from Group P or Z), a transcribed sequence (blue from Group N or Z), and a transcriptional terminator (red from Group T or Z). Three of the cassettes are designed for hairpin RNA interference (RNAi) and contain transcribed sequences that consist of inverted repeats of gene fragments from *Arabidopsis thaliana* fatty acid desaturase 3 and *Camelina sativa* fatty acid desaturase 2 and fatty acid elongase 1 (Group N) set either side of *Flaveria trinervia* pyruvate orthophosphate dikinase intron 3 (Group T). The promoters used in these three cassettes are seed specific (Group P). The fourth cassette contains the selectable marker gene BAR (Group Z) as the transcribed sequence. The details of this construct have been published (Chen, G. Q., Johnson, K., Nazarenus, T. J., Ponciano, G., Morales, E., & Cahoon, E. B. (2021). Genetic Engineering of Lesquerella with Increased Ricinoleic Acid Content in Seed Oil. Plants (Basel, Switzerland), 10(6), 1093. https://doi.org/10.3390/plants10061093).

HS constructs



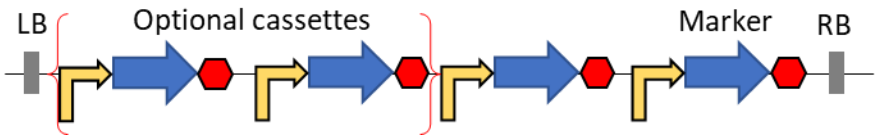
HS constructs each have a specific identifier and are designed to increase seed short to long-chain saturated fatty acid content (C4:0 to C18:0). They contain up to four expression cassettes. Each cassette is an independent unit containing a promoter sequence (yellow from Group P or Z), a transcribed sequence (blue from Group N or Z), and a transcriptional terminator (red from Group T or Z). One cassette contains the selectable marker gene (Group Z) as the transcribed sequence. The other cassettes contain acyl-ACP thioesterases, diacylglycerol acyltransferases or type 1 fatty acid synthase, either individually or optionally in combinations as the transcribed sequences (Group N) and use seed-specific promoters (Group P).

SN2 constructs



SN2 constructs each have a specific identifier and are designed to incorporate long-chain saturated fatty acyl groups (C16:0) into the stereospecific numbering (sn)-2 position of the glycerol backbone of triacylglycerol in seeds. They contain up to four expression cassettes. Each cassette is an independent unit containing a promoter sequence (yellow from Group P or Z), a transcribed sequence (blue from Group M, N or Z), and a transcriptional terminator (red from Group T or Z). One cassette contains the selectable marker gene (Group Z) as the transcribed sequence. The other cassettes contain lysophosphatidic acid acyltransferases and optionally also artificial microRNA (amiRNA) derived from *Arabidopsis thaliana* mi319a designed to target *Camelina sativa* lysophosphatidic acid acyltransferase 2 or phosphatidylcholine:diacylglycerol cholinephosphotransferase as the transcribed sequences (Group N) and use seed-specific promoters (Group P).

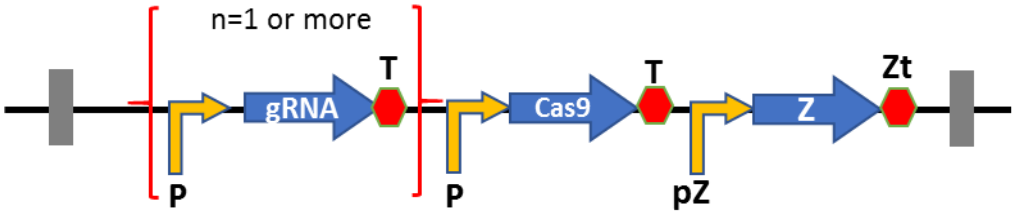
Taurine (TA) construct



The TA construct is designed to improve the amino acid composition of the seed by directing the synthesis of non-proteinogenic amino sulfonic acid taurine, which is used in aquafeed diets and is a metabolite of cysteine. The TA construct contains three expression cassettes. Each cassette is an independent unit containing a promoter sequence (yellow from Group P or Z), a transcribed sequence (blue from Group N or Z), and a transcriptional terminator (red from Group T or Z). One cassette contains the selectable marker gene (Group Z) as the transcribed sequence. The other cassettes contain (in combination) the cysteine sulfinic acid decarboxylase (CSAD) and cysteine dioxygenase (CDO) activities listed in Group N and used in conjunction with seed-specific promoters (Group P).

CRISPR-Cas9-mediated gene-edited lines

It is envisaged that some of the constructs described above could undergo CRISPR-Cas9 gene editing to inactivate (through sequence-specific deletion) endogenous genes. Screening such mutant populations in the field would significantly increase the ability to identify useful mutations, especially when they are at a precursor stage to obtaining HQP/PBO status. 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 more 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. Such an approach has previously been described in **19/R8/01** and the results published in Han et al., 2022 https://doi.org/10.1111/pbi.13876 .

15. The following information on the expression of the insert

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

Transgenes are integrated into the nuclear genome by *Agrobacterium*-mediated transformation, a natural process which generates stable modifications to the genome, inherited in a Mendelian fashion.

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. Thus, phenotypes are only manifest in the seeds of these transgenic plants and not present in any other vegetative tissue. The seed-specific nature of the promoters listed in Group P has been extensively tested and validated in a range of different transgenic hosts – for further details please see https://www.aphis.usda.gov/brs/aphisdocs/17_32101p.pdf and https://www.aphis.usda.gov/brs/aphisdocs/17_23601p.pdf . The use of an expanded palate of seed-specific promoters allows the optimisation of multigene expression whilst avoiding recursive inclusion of homologous sequences via the reliance on the same promoter to drive multiple activities (cf. the use of the CNL promoter in DHA2015.1). Seed-specific promoters are invariably derived from seed-storage protein genes and have a defined window of activity that is maximal during mid-stage seed development. Efficient metabolic engineering requires for the co-ordinate expression of desired activities in a regulated fashion – the promoters selected in Group P are all well-characterised and collectively been authorised by USDA-APHIS for the seed-specific expression of transgenic activities in canola.

b). Conclusions on the molecular characterisation of the genetically modified plant.

Based on the previous Consents (**14/R8/01**, **16/R8/01**, **18/R8/01** and **19/R8/01**), no unexpected differences apart from the presence of the defined transgenes. No unexpected differences were observed for EPA2015.8, DHA2015.1-DHA2015.5 (Han et al., 2020, 2022; Ibid.)

Part IVA Information on specific areas of risk

16. Any change to the persistence or invasiveness of the genetically modified plant and its ability to transfer genetic material to sexually compatible relatives and the adverse environmental effects arising,

Unlikely, given the seed-specific nature of these traits.

17. Any change in the ability of the genetically modified plant to transfer genetic material to microorganisms and the adverse environmental effects arising,

None known or expected.

18. The mechanism of interaction between the genetically modified plant and target organisms, if applicable, and the adverse environmental effects arising,

None known or expected.

19. Potential changes in the interactions of the genetically modified plant with no-target organisms resulting from the genetic modification and the adverse environmental effects arising,

None known or expected.

20. Potential changes in agricultural practices and management of the genetically modified plant resulting from the genetic modification, if applicable, and the adverse environmental effects arising,

None known or expected.

21. Potential interactions with the abiotic environment and the adverse environmental effects arising,

None known or expected.

22. 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 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, as are ketocarotenoids such as astaxanthin. Oleic and palmitic acid are ubiquitous components of all food webs. Short and medium chain saturated fatty acids are found in edible oils from crops (e.g. coconut and palm) and in dairy products (e.g. milk, butter and cheese).

Equally, ULC-PUFAs are minor components of marine foodweb and present in many marine-derived foodstuffs.

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.

23. Conclusions on the specific areas of risk.

There are no significant risks associated with the release of these plants. This conclusion is supported by previous assessments of similar traits and releases (e.g. **14/R8/01**, **16/R8/01**, **18/R8/01** and **19/R8/01**). Equally, lines released under 16/R8/01 and 18/R8/01 have been evaluated and released in both USA and Canada (**APHIS-USDA 16-336-106e-a1**, **APHIS USDA 121-CUA0XEW**; **CFIA: 17-AGQ1-406-CAM).**

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 glufosinate represent additional methods.

It is equally possible to use fatty acid profiling to identify plants associated with the omega-3 PUFAs, 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 through the same methodology (GC-FID of fatty acid methyl-esters). Analogously, plants accumulating the ketocarotenoid astaxanthin can be identified by HPLC profiling of carotenoids. Collectively, these methods allow for the unambiguous detection of any GM material containing the transgenes described in this document.

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 and related compounds such as astaxanthin were previously approved in the UK for experimental field release (DEFRA Consents **19/R8/01**, **18/R8/01**, **16/R8/01** and **14/R8/01**). The details of these releases are reported in Usher et al., 2015, Usher et al., 2017, Han et al., 2020, Han et al., 2022a, Han et al., 2022b. Some lines (DHA2015.1, EPA2015.8) have been released in the USA and Canada under the appropriate permits from USDA-APHIS and CFIA (see above for details).

Part V Information relating to the site of release

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 **TL122134**, and also at the experimental farm at Rothamsted Research, Brooms Barn, at grid reference **TL762655**.

For the area for the proposed Harpenden field trial, the GM plot will cover up to a maximum area of 650m^2. This is a small decrease in the size of release compared to previous Consents.

In the case of Brooms Barn field trial, the GM plot will cover up to a maximum area of ~4,000m^2, identical to the previous releases carried out 2019-2023 under **19/R8/01** and **18/R8/01**.

Thus, the total area proposed for field releases is less than previously approved by ACRE and carried out under Ministerial Consent **19/R8/01**.

Both field releases will, as previously defined under successive Consents, enforce a 50m separation zone out from the edge of the release, ensuring the absence of any cultivated Camelina species in that area. Although the use of a pollen barrier is not envisaged, the option to deploy one (at least 4m wide) is requested.

The whole area of each trial site will be enclosed by 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 actively-managed agricultural land in the South East of England.

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 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). There are no trials of any Camelina species planned on the Rothamsted Farm in the vicinity of these GM trial locations.

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, ketocarotenoids) in their seed oils, or variation in the accumulation of native fatty acids such as oleic and palmitic acid and short chain fatty acids.

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

The GM *C. sativa* will be planted within the four-year period of this application (2024-2027). The plants will be sown in April/May and harvested in Aug/Sept/Oct. Alternatively, a winter-sowing could be carried out (in Oct/Nov), to be harvested in the following June/July.

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 GM 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 autoclaving 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, 19/R8/01. Practically, we will use 1.8m x 6m strips, replicated within the GM trial area. The area containing the GM trial plots will have the option of being surrounded by a non-GM pollen barrier of *C. sativa* “Celine” (WT), of at least 4m width. 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 650m^2 being used for the cultivation of GM lines (including WT controls) on the Pastures site (Harpenden) – thus at a density of 250 plants/m^2, the release would be 162,500 GM plants. In the case of the Marlpit site (Brooms Barn), the maximum area of GM is 4,000m^2, meaning the release would be 1,000,000 GM plants. The total annual maximum release (assuming a density of 250 plants/m^2) is therefore 1,162,500 GM plants, although this entire capacity may not be required every year. This total area of release is very similar to that previously carried out under Consents **18/R8/01** and **19/R8/01** – for example, the total number of plant released under **18/R8/01** in 2022 was 1,397,639.

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

35-(1) A description of any precautions to maintain spatial and, as the case may be, temporal separation of the genetically modified plant from sexually compatible plant species.

(2) In sub-paragraph (1) "plant species" means-

(a) Wild and weedy relatives, or
(b) Crops

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

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

The trial will receive standard agronomic practise as regard to herbicide, fungicides and nitrogen fertiliser in conjunction with advice from the scientific co-ordinator and the Farm Manager. The site will be regularly monitored from sowing to harvest and during the following cropping year, as per the Trial Management protocols developed in conjunction 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 combine (plot or conventional) 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 recorded. All samples removed from the trial site will be under appropriate containment and eventually destroyed by an approved technique (such as autoclaving). The remainder of the site (pollen barrier, if used) will be harvested by the plot combine or similar. The grain obtained will be disposed to deep landfill using an approved contractor or autoclaving. 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-Sept) and monthly after the completion 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, control plots and any 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 fences and CCTV.

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 Gateway as previously described (Ruiz-Lopez et al., 2014)

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.

References

Plant and Biotechnology Risk Assessment Unit, Canadian Food Inspection Agency

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