

# 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 *C. sativa*

(d) subspecies -

(e) cultivar/breeding line 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* is also pollinated by insects.

##### **(ii) any specific factors affecting reproduction**

Pollination, seed set and grain filling are dependent on temperature, weather conditions, agronomic practice and pressure applied by pests and disease.

##### **(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 hybridisation 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. <http://www.inspection.gc.ca/plants/plants-with-novel-traits/applicants/directive-94-08/biology-documents/camelina-sativa-l-eng/1330971423348/1330971509470>. However, the flowers of *C. sativa* are much smaller than canola or flax, so may be less apparent.

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.

**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 vectors pSUN2 or pRS-3GSeed (Ruiz-Lopez et al, 2012; Sayanova et al, 2012) both of which are derivatives of pBIN19 (Frisch et al, 1995).

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

<b>Element</b>	<b>Size</b>	<b>Donor Organism</b>	<b>Description and Intended Function</b>
RB	24bp	<i>Agrobacterium tumefaciens</i>	T-DNA Right border
LB	23bp	<i>Agrobacterium tumefaciens</i>	T-DNA Left border

<b>Seed-specific promoters</b>			
USP	684bp	<i>Vicia faba</i>	Unknown Seed Protein Seed-specific promoter
NP	664bp	<i>Brassica napus</i>	Napin seed specific promoter
CNL	1064bp	<i>Linum usitatissimum</i>	2S seed storage protein (Conlinin) promoter
GLY	702bp	<i>Glycine max</i>	11S Seed storage protein (Glycinin) promoter
SBP	1800bp	<i>Arabidopsis</i>	Sucrose-binding protein promoter (seed-specific)
PvArc	1138bp	<i>Phaseolus vulgaris</i>	<i>Arcelin-5</i> seed storage protein promoter
<b>Omega-3 biosynthetic genes</b>			
Ot $\Delta$ 6	1665bp	Synthetic	Encodes a fatty acid $\Delta$ 6- <b>desaturase</b> from the marine picoalga <i>Ostreococcus tauri</i>
PSE1	873bp	Synthetic	Encodes an acyl-CoA-dependent $\Delta$ 6- <b>elongase</b> from the moss <i>Physcomitrella patens</i>
Tc $\Delta$ 5	1320bp	Synthetic	Encodes a fatty acid $\Delta$ 5- <b>desaturase</b> from the marine species <i>Thraustochytrium</i>
Piw3	1086bp	Synthetic	Encodes a fatty acid <b>w3-desaturase</b> from <i>Phytophthora infestans</i>
Ps $\Delta$ 12	1197bp	Synthetic	Encodes a fatty acid $\Delta$ 12- <b>desaturase</b> FAD2 activity from <i>Phytophthora sojae</i>

OtElo5	903bp	Synthetic	Encodes an acyl-CoA dependent $\square$ 5-elongase from the marine picoalgae <i>Ostreococcus tauri</i>
Tp $\square$ 4	1653bp	Synthetic	Encodes a fatty acid $\square$ 4- <b>desaturase</b> from the marine diatom <i>Thalassiosira pseudonana</i>
O809 $\square$ 4	1480bp	Synthetic	Encodes a fatty acid $\square$ 4- <b>desaturase</b> from the marine picoalgae <i>Ostreococcus</i> RCC809
Msq $\Delta$ 6	1350bp	Synthetic	Encodes a fatty acid $\Delta$ 6- <b>desaturase</b> from the marine algae <i>Mantoniella squamata</i>
EmiD5	1368bp	Synthetic	Encodes a fatty acid $\Delta$ 5-desaturase from the marine algae <i>Emiliana huxleyi</i>
Hpw3	1086bp	Synthetic	Encodes a fatty acid $\omega$ 3-desaturase from <i>Hyaloperonospora parasitica</i>
PerfD15	1176bp	Synthetic	Encodes a fatty acid $\Delta$ 15-desaturase from <i>Perilla frutescens</i>
809E5	906bp	Synthetic	Encodes a fatty acid $\Delta$ 5-elongase from the marine picoalgae <i>Ostreococcus</i> RCC809
<b>Astaxanthin biosynthetic genes</b>			
ZmPhys	1232bp	Synthetic	Encodes phytoene synthase from maize
HBFD1	1226bp	Synthetic	Encodes carotenoid 4-hydroxy- $\beta$ -ring 4-dehydrogenase from <i>Adonis</i>

			<i>aestivalis</i>
CBFD2	687bp	Synthetic	Encodes carotenoid $\beta$ -ring 4-dehydrogenase from <i>Adonis aestivalis</i>
<b>Wax Ester biosynthetic genes</b>			
CpThio14	1270bp	Synthetic	Encodes an acyl-ACP C14 thioesterase from <i>Cuphea palustris</i>
MhWS	1427bp	Synthetic	Encodes a wax synthase from <i>Marinobacter hydrocarbonoclasticus</i>
MaFAR	1539bp	Synthetic	Encodes a fatty acyl-CoA reductase form <i>Marinobacter aquaeolei</i>
<b>MAP protein</b>			
MAP22	1455bp	cDNA	Encodes a microtubule associated protein with a calmodulin binding domain from <i>Arabidopsis thaliana</i> At4g23060
<b>Photorespiration bypass</b>			
GlcD	1497bp	Synthetic	Encodes Subunit D of the Glycolate Dehydrogenase from <i>Escherichia coli</i>
GlcE	1050bp	Synthetic	Encodes Subunit E of the Glycolate Dehydrogenase from

			<i>Escherichia coli</i>
GlcF	1221bp	Synthetic	Encodes Subunit F of the Glycolate Dehydrogenase from <i>Escherichia coli</i>
<b>Marker genes</b>			
DsRed	684bp	Synthetic	Encodes a florescent protein from <i>Discosoma</i> spp.
<i>bar</i>	548bp	Synthetic	Encodes phosphinothricin acetyl transferase, providing to resistance to Class H herbicides such as Basta <sup>™</sup> , in which bialaphos is the active ingredient.
<b>Regulatory elements</b>			
<i>p35S</i>	446 bp	Cauliflower mosaic virus	35S promoter
<i>pNOS</i>	288bp	<i>Agrobacterium tumefaciens</i>	Nopaline synthase gene promoter
<i>NOS<sub>t</sub></i>	256bp	<i>Agrobacterium tumefaciens</i>	Nopaline synthase gene terminator sequence
<i>OC<sub>t</sub></i>	192bp	<i>Agrobacterium tumefaciens</i>	octopine synthase gene terminator sequence
<i>35S<sub>t</sub></i>	216bp	Cauliflower mosaic virus	35S transcript terminator sequence
<i>CatpAt</i>	235bp	<i>Arabidopsis thaliana</i>	Cathepsin A gene terminator sequence
<i>Gly<sub>t</sub></i>	451bp	<i>Glycine max</i>	11S storage protein (Glycinin) terminator
<i>E9<sub>t</sub></i>	558bp	<i>Arabidopsis</i>	Ubiquitin E9 ligase gene

		<i>thaliana</i>	terminator sequence
pCsVMV	528bp	<i>Cassava vein mosaic virus</i>	CsVMV promoter sequence
PvARCT	600bp	<i>Phaseolus vulgaris</i>	Arcelin-5 gene terminator sequence
HSPt	245bp	<i>Arabidopsis thaliana</i>	Heat shock protein 18.2 (HSP) gene terminator sequence
Phast	183bp	<i>Phaseolus vulgaris</i>	Alpha-phaseolin gene terminator sequence
Fad2t	182bp	<i>Camelina sativa</i>	Microsomal oleate desaturases (FAD2) gene terminator sequence
<b>Additional elements</b>			
ChITP	240bp	Synthetic	Chloroplast targeting sequence of the small chain of RUBISCO from <i>Arabidopsis thaliana</i>
2A-Pept	60bp	Synthetic	2A self-cleaving peptide from Teschovirus A

## 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.

The omega-3 long chain polyunsaturated fatty acids (LC-PUFA) eicosapentaenoic acid (abbreviated to EPA; 20:5 $\omega$ <sup>3,8,11,14,17</sup>) and docosahexaenoic acid (abbreviated to DHA; 22:6 $\omega$ <sup>3,7,10,13,16,19</sup>) are components of fish oil, and known to help protect against cardiovascular disease. No higher plants contain EPA or DHA in their seed oils, although very many vegetable oils are rich in the simpler/shorter omega-3  $\omega$ -linolenic

acid (abbreviated to ALA; 18:3 $\omega$ <sup>9,12,15</sup>). 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.

### **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 used in aquaculture as feed ingredients.

### **Wax esters**

Wax esters have potential as natural lubricants, being more stable than traditional triacylglycerol-based formulations. Wax esters are synthesized through the conversion of a fatty acid to a fatty alcohol, followed by the esterification of the fatty alcohol to a fatty acid. The resulting wax ester is highly stable as a consequence of the O-linked bond between the acyl chains. Many organisms including higher plants synthesize and accumulate wax esters. In the case of plants, such WEs are usually found on the surface of leaves, as part of epidermis and cuticle, acting as a natural barrier. In one example, the jojoba plant (*Simmondsia chinensis*) accumulates WEs in its seeds. Transgenic camelina have been developed which accumulate chain wax esters in their seeds (Ruiz-Lopez et al, 2017). No accumulation of these wax esters was observed in any vegetative part of the plant.

### **MAP22**

Improving the architecture of Camelina could result in superior growth and seed yields. Over expression of microtubule binding proteins (MAP) of the IQD family have been shown to regulated organ size and leaf morphology. Overexpression of MAP22 in Camelina has, in glasshouse studies, resulted in plants with improved stem thickness and larger leaves (thus providing increased photosynthetic area). However, this potentially useful trait needs to be validated through field-release. The MAP22 protein is expressed constitutively throughout the transgenic camelina plant.

## GDH - Improved plant productivity through enhanced photosynthesis

Increasing Camelina productivity and seed yield is a desirable trait. Proof of concept studies have shown that this can be achieved by engineering a photorespiration bypass in Camelina resulting in increased photosynthetic efficiency, plant growth and seed yield (Dalal et al., 2015). Photorespiration is caused by the oxygenase activity of ribulose-1,5-bisphosphate carboxylase/Oxygenase (RuBisCO) which competes with the CO<sub>2</sub> fixing activity of this enzyme, and eventually results in a net loss of carbon via respiration. This competing pathway can be bypassed by expressing the glycolate dehydrogenase (GDH) from *E. coli*. GDH is a trimeric enzyme encoded by three genes in *E. coli*: GlcD, GlcE and GlcF. Targeting this enzyme to the chloroplast has two synergistic effects: it decreases photorespiration by converting the photorespiratory substrate glycolate into glyoxylate, and CO<sub>2</sub> is released in the chloroplast as a by-product of a downstream reaction, which increases photosynthesis. The aim of the proposed trial is to validate in the field the increased seed yield observed with GDH transgenic camelina lines in glass houses. The GDH proteins are expressed constitutively throughout the transgenic camelina plant.

### 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.

It is proposed to evaluate the performance of 14 individual events derived from the constructs listed below.

	<b>Construct ID</b>	<b>Phenotype</b>	<b># of genes</b>	<b>Marker</b>
1	DHA2015.1	Omega-3 LC PUFAs seed oil	Seven <sup>1</sup>	DsRed
2	DHA2015.2	Omega-3 LC PUFAs seed oil	Six <sup>1</sup>	DsRed
3	DHA2015.3	Omega-3 LC	Six <sup>1</sup>	DsRed

		PUFAs seed oil		
4	DHA2015.4	Omega-3 LC PUFAs seed oil	Six <sup>1</sup>	DsRed
5	DHA2015.5	Omega-3 LC PUFAs seed oil	Eight <sup>1</sup>	DsRed
6	EPA2015.4	Omega-3 LC PUFAs seed oil	Four <sup>1</sup>	DsRed
7	EPA2015.8	Omega-3 LC PUFAs seed oil	Five <sup>1</sup>	DsRed
8	EPA2016.1	Omega-3 LC PUFAs seed oil	Four <sup>1</sup>	DsRed + BAR
9	ASX-A2	Astaxanthin in seeds	Three <sup>1</sup>	BAR
10	THIO14	Medium chain fatty acids in seeds	One <sup>1</sup>	DsRed
11	MaMa14-6*	Wax esters in seeds	Three <sup>1</sup>	DsRed
12	GDH	Enhanced photosynthesis	One <sup>1</sup>	DsRed
13	GDH-PP	Enhanced photosynthesis	One <sup>1</sup>	DsRed
14	MAP22	Improved architecture	One <sup>2</sup>	DsRed
<p>* = two events from this construct will be tested</p> <p>1 = synthetic, codon-optimised sequence    2= native sequence</p>				

In addition, genetic crosses between **DHA2015.1** and either **ASX-A2** or **MAP22** will be evaluated, to determine the efficacy of stacking the individual traits associated with these constructs.

The identity of the various genes and regulatory elements is shown above in the Table in **Section 12**. The nature of the constructs and the specific event to be

considered for release are described in detail below. For clarity, a simplified representation of the omega-3 long chain polyunsaturated fatty acid biosynthetic pathway is shown below. More details can also be found in Haslam et al 2013).

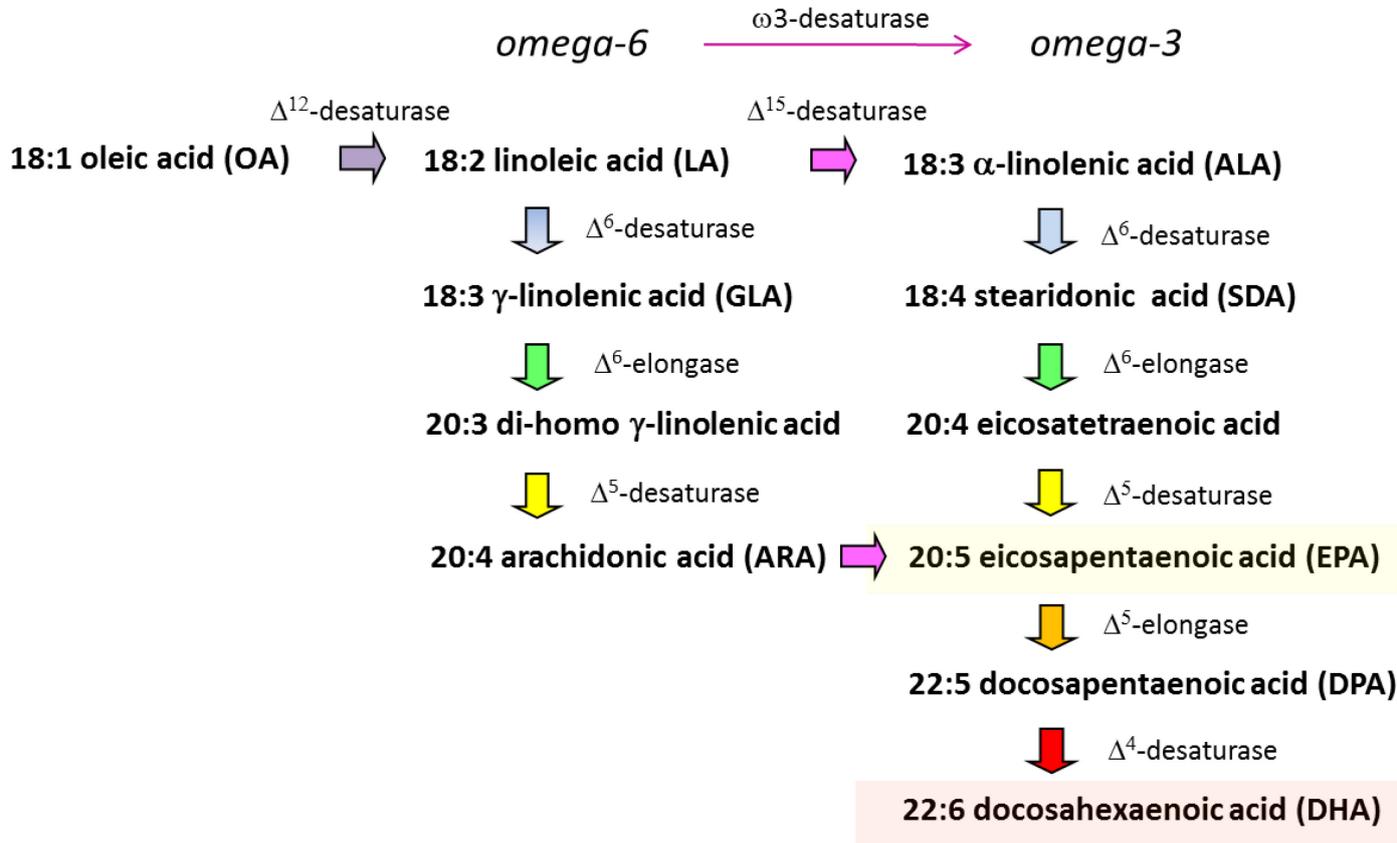


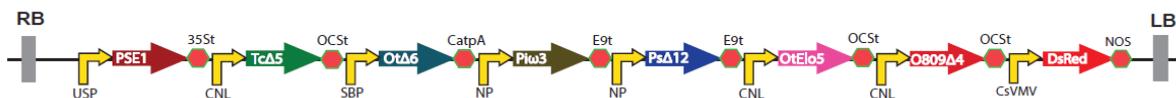
Fig 1. Schematic representation of long chain polyunsaturated fatty acid biosynthesis

## Constructs used to generate transgenic *C. sativa*

### Omega-3 Long Chain Polyunsaturated Fatty Acids

#### Construct DHA2015.1 (Size of T-DNA insert is 20,963 bp)

DHA2015.1

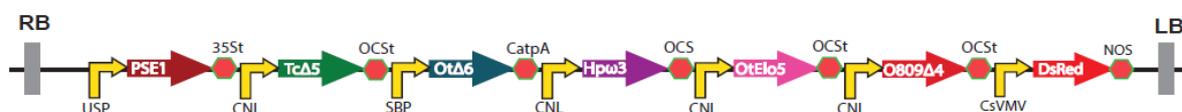


In **Construct DHA2015.1**, seven heterologous genes under the control of seed-specific promoters were assembled to direct the synthesis of EPA and docosahexanoic acid (DHA) in the seeds of transgenic *C. sativa*. The heterologous pathway commenced by the  $\Delta^6$ -desaturation (**Ot $\Delta^6$** ) of endogenous fatty acids such as alpha-linolenic acid (ALA) and linoleic acid (LA). These  $\Delta^6$ -desaturation products (SDA, GLA) then undergo C2-elongation (**PSE1**) to the equivalent C20 fatty acids, which then receive a further double bond at the  $\Delta^5$ -position by the  $\Delta^5$ -desaturase (**Tc $\Delta^5$** ). These products then undergo a second C2-elongation (**OtElo5**) to generate C22 polyunsaturated fatty acids, which are then substrate for the  $\Delta^4$ -desaturase (**O809 $\Delta^4$** ) which is responsible for the direct synthesis of DHA (Fig. 1).

The accumulation of C20 and C22 omega-3 fatty acids such as EPA and DHA is enhanced by the activity of a  $\Delta^3$ -desaturase (**Piw3**), which converts omega-6 fatty acids to the omega-3 form (Fig. 1). Additional substrate for **Ot $\Delta^6$**  was generated through the activity of the  $\Delta^{12}$ -desaturase (**Ps $\Delta^{12}$** ). All seven of these genes are synthetic, being codon-optimised from their native sequences (derived from the picoalgae *Ostreococcus tauri* or *Ostreococcus RCC809*, the moss *Physcomitrella patens*, the Thraustochytriaceae *Thraustochytrium*, the oomycetes *Phytophthora infestans* and *Phytophthora sojae* and the diatom *Thalassiosira pseudonana*) to that of a form that represents the codon-usage of dicotyledonous plants, specifically the Cruciferae. Each synthetic coding sequence is regulated by an individual seed-specific promoter, and also defined by a transcription termination sequence.

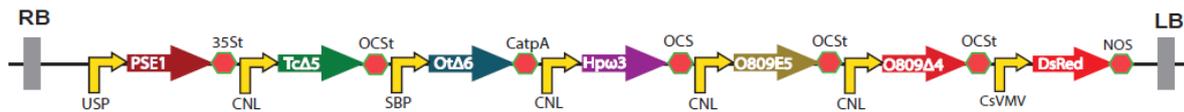
Within the T-DNA there is also the visual selectable marker, DsRed, which is constitutively expressed under the control of the casava vien mosaic virus promoter and nopaline synthase terminator. This T-DNA region has been introduced into transgenic *C. sativa* by *A. tumefaciens*-mediated transformation, and primary transgenic events identified by the florescence of DsRed at 555nm. Individual plants were self-pollinated, and homozygous progeny identified on the basis of strong florescence and fatty acid profile, with homozygosity confirmed by the 100% presence of these transgene-derived traits in the next (selfed) generation.

**Construct DHA2015.2** (Size of T-DNA insert is 18,544 bp)



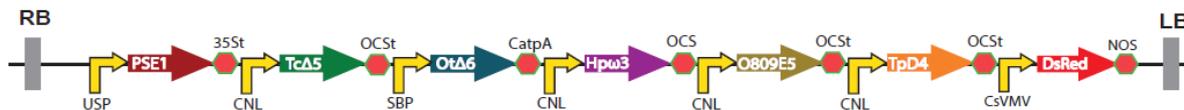
Construct DHA2015.2 contains six heterologous genes under the control of seed-specific promoters were assembled to direct the synthesis of EPA and docosahexanoic acid (DHA) in the seeds of transgenic *C. sativa*. The only differences between this construct and DHA2015.1 are the absence of a  $\Delta^{12}$ -desaturase, and the substitution of a  $\omega^3$ -desaturase from *Hyaloperonospora parasitica* for the same activity encoded by *Phytophthora infestans*. In all other respects, DHA2015.2 and DHA2015.1 are identical.

**Construct DHA2015.3** (Size of T-DNA insert is 18,547 bp)



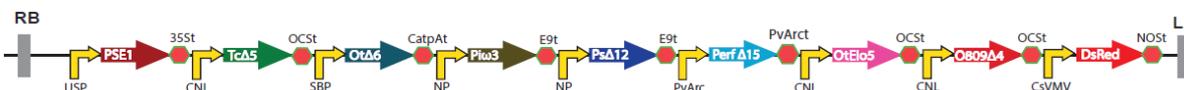
Construct DHA2015.3 contains six heterologous genes under the control of seed-specific promoters were assembled to direct the synthesis of EPA and docosahexanoic acid (DHA) in the seeds of transgenic *C. sativa*. The only differences between this construct and DHA2015.2 is the substitution of a C20 elongase (ELO5) from *Ostreococcus* RCC809 for the same activity encoded by *Ostreococcus tauri*. In all other respects, DHA2015.3 and DHA2015.2 are identical.

**Construct DHA2015.4** (Size of T-DNA insert is 18,720 bp)



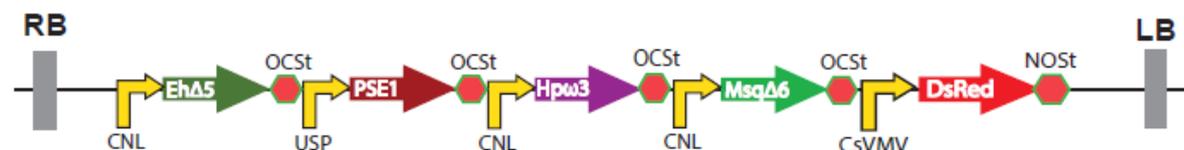
Construct DHA2015.4 contains six heterologous genes under the control of seed-specific promoters were assembled to direct the synthesis of EPA and docosahexanoic acid (DHA) in the seeds of transgenic *C. sativa*. The only differences between this construct and DHA2015.3 is the substitution of a D4-desaturase from *Thalassiosira pseudonana* for the same activity encoded by *Ostreococcus* RCC809. In all other respects, DHA2015.4 and DHA2015.3 are identical.

**Construct DHA2015.5** (Size of T-DNA insert is 23,877 bp)



Construct DHA2015.5 contains eight heterologous genes under the control of seed-specific promoters were assembled to direct the synthesis of EPA and docosahexanoic acid (DHA) in the seeds of transgenic *C. sativa*. The only differences between this construct and DHA2015.1 is the additional presence of a D15-desaturase from *Perilla fructans*. In all other respects, DHA2015.5 and DHA2015.1 are identical.

**Construct EPA2015.4** (Size of T-DNA insert is 13,410 bp)

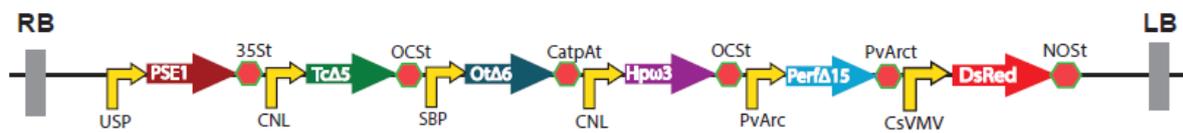


Construct EPA2015.4 contains four heterologous genes under the control of seed-specific promoters were assembled to direct the synthesis of EPA (but not DHA) in the seeds of transgenic *C. sativa*. The heterologus pathway commenced by the  $\Delta 6$  desaturation ( $\Delta 6$ ) of endogenous fatty acids such as alpha-linolenic acid (ALA)

and linoleic acid (LA). These  $\omega$ -6-desaturation products (SDA, GLA) then undergo C2-elongation (**PSE1**) to the equivalent C20 fatty acids, which then receive a further double bond at the  $\omega$ -5-position by the  $\omega$ -5-desaturase (**Eh $\omega$ 5**). The construct also contains a  $\omega$ -3-desaturase (**Hpw3**), which converts omega-6 fatty acids to the omega-3 form

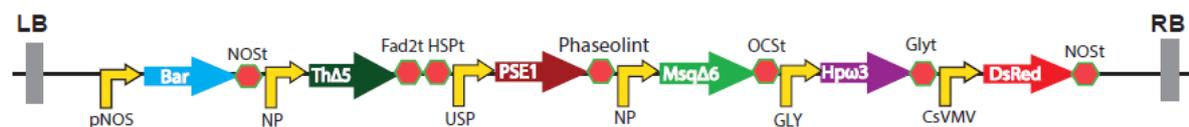
Within the T-DNA there is also the visual selectable marker, DsRed, which is constitutively expressed under the control of the casava vein mosaic virus promoter and nopaline synthase terminator.

### Construct EPA2015.8 (Size of T-DNA insert is 15,924 bp)



Construct EPA2015.8 contains five heterologous genes under the control of seed-specific promoters were assembled to direct the synthesis of EPA (but not DHA) in the seeds of transgenic *C. sativa*. The only differences between this construct and EPA2015.4 is the additional presence of a  $\omega$ -15-desaturase from *Perilla fructans*, and the substitutions of the  $\omega$ -6-desaturase from *Mantonella squamata* (MsD6) with the same activity from *Ostreococcus tauri*, and also the  $\omega$ -5-desaturase from *Emiliana huxleyi* (EhD5) with the same activity from *Thraustochytrium* (TcD5). In all other respects, EPA2015.8 and EPA2015.4 are identical.

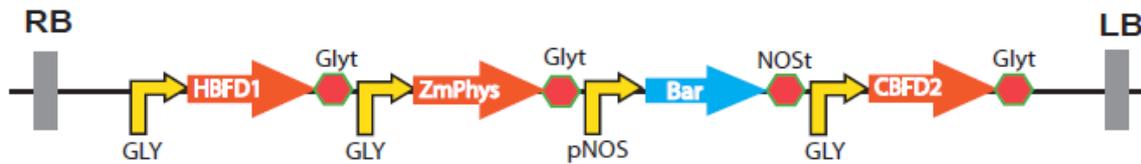
### Construct EPA2016.1 (Size of T-DNA insert is 14,455 bp)



Construct EPA2016.1 contains four heterologous genes under the control of seed-specific promoters were assembled to direct the synthesis of EPA (but not DHA) in the seeds of transgenic *C. sativa*. The only differences between this construct and EPA2015.4 is the substitution of the  $\omega$ -5-desaturase from *Emiliana huxleyi* (EhD5) with the same activity from *Thraustochytrium* (TcD5), and with the addition of transcription termination sequences from the *C. sativa* FAD2 gene and the *Arabidopsis thaliana* HSP18.2 gene. In addition, the *bar* selectable marker encoding the PAT resistance gene is included within the T-DNA.

### Astaxanthin

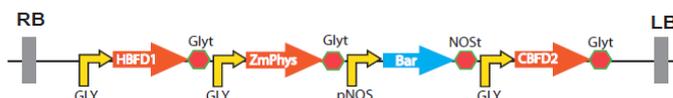
## Construct ASX-A2\_ (Size of T-DNA insert is 9,238 bp)



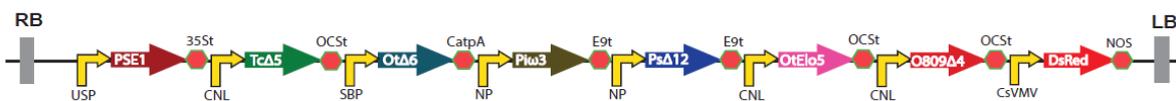
In Construct ASX-A2, three heterologous genes under the control of seed-specific promoters were assembled to direct the synthesis of the ketocarotenoid astaxanthin in the seeds of transgenic *C. sativa*. A phytoene synthase from maize (*ZmPhys*) was introduced to enhance the accumulation of phytoene (by the conversion of geranylgeranyl-diphosphate to phytoene) and subsequently to  $\beta$ -carotene, the latter to provide substrate for the carotenoid  $\beta$ -ring 4-dehydrogenase (*CBFD2*) from *Adonis aestivalis*. The product of that enzyme, a 4-hydroxy- $\beta$ -ring, is then converted to astaxanthin by the 4-hydroxy- $\beta$ -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 constitutively expressed under the control of the nopaline synthase promoter and terminator. This T-DNA region has been introduced into transgenic *C. sativa* by *A. tumefaciens*-mediated transformation, and primary transgenic events identified by their resistance to bialaphos (glufosinate-ammonium). To check for the presence of astaxanthin accumulation, astaxanthin was extracted from seeds with acetone and the absorbance of the extract was measured at 475 nm. This was compared to a standard curve constructed using commercially available astaxanthin. Seeds from lines which show astaxanthin accumulation were then sown on  $\frac{1}{2}$  M+S plates containing 300  $\mu$ g/ml glufosinate-ammonium and survivors were transferred to soil. The traits encoding in the ASX-A2 construct are inherited in a Mendelian fashion indicative of a single insertion, and have been shown to be stable to the T5 generation.

## Genetic cross DHASX-A2

ASX



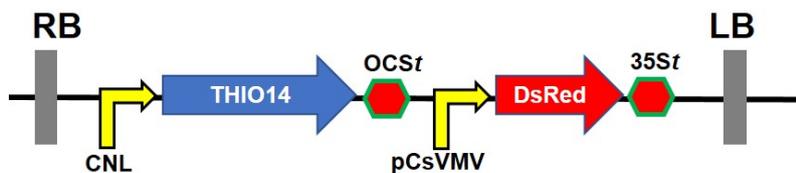
DHA2015.1



Homozygous plants containing **Construct DHA2015.1** were used to cross-pollinate plants containing **Construct ASX-2**, and the resulting seeds used to identify progeny which contained both the omega-3 trait and the astaxanthin trait. Plants from this initial cross were grown up and self-pollinated, and the F2 seed collected. F2 seeds were screened for the PUFA trait by the fluorescence of DsRed at 555nm. DsRed positive seeds are then sown on ½ M+S plates containing 300 ug/ml glufosinate-ammonium to identify the individuals which were also carrying the ASX construct. Survivors of the herbicide treatment were transferred to soil allowed to develop as usual. Plants were bagged prior to flowering to prevent further crossing. F3 seeds were collected and screened as before, for the presence of both constructs. Seedlings displaying both traits were transferred to soil and propagated as before to produce homozygous F4 seeds. The presence of the HBFD1 and the **O809**□4□4 desaturase were determined by PCR to confirm the presence of the two unlinked T-DNA inserts for the ASX and DHA traits, respectively, and shown to be stably inherited across 4 generations.

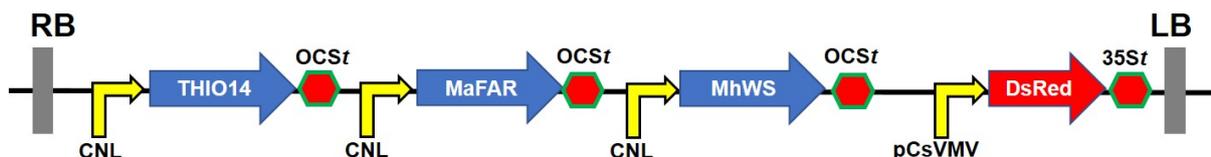
### Wax esters

**Construct THIO14** (Size of T-DNA insert is 6083 bp)



In construct THIO14, a single heterologous gene under the control of seed-specific promoters was used to redirect the synthesis of fatty acids in the seeds of transgenic *C. sativa*. Specifically, a sequence encoding the FATB2 acyl-ACP thioesterase from the higher plant *Cuphea palustris* was placed under the control of a seeds-specific promoter. This results in the accumulation of medium chain fatty acids in the plastids of developing Camelina seeds, suitable for subsequent incorporation into wax esters. These fatty acids (predominantly 14:0 – myristic acid) are significantly in the seeds of this transgenic line, though 14:0 is present at low levels in most plant cells.

**Construct MaMa14** (Size of T-DNA insert is 12,471 bp)



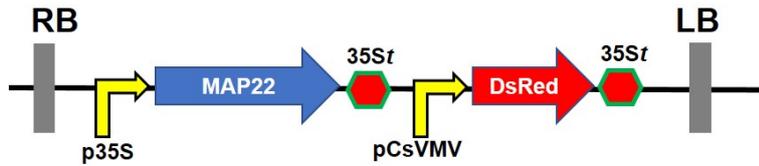
In construct MaMa14-6, in addition to the FATB2 thioesterase from *C. palustris*, two genes from marine bacteria are present, to direct the synthesis of wax esters. These activities are a fatty acyl-CoA reductase (FAR) from *Marinobacter aquaeolei* and a wax synthase (WS) from *Marinobacter hydrocarbonoclasticus*. The *M. aquaeolei* FAR utilises acyl-CoA substrates generated by the FATB2 THIO14 activity to produce fatty alcohols as substrates for the *M. hydrocarbonoclasticus* WS, which links these to an acyl-CoA to generate a wax ester. All three activities (THIO14, FAR, WS) are under the control of seed-specific promoters, with the synthesis and accumulation of wax esters restricted to seeds. This construct has been shown in GH studies to accumulate wax esters with a profile similar to that found in Spermaceti whales, and therefore represents a new plant-based source of these highly functional but protected oils (Ruiz-Lopez et al., 2017). Two lines for this construct will be evaluated under field conditions (MaMa14-6-12, and MaMa14-6-16), accumulating differing levels of WE in their seeds.

**Constructs GDH + GDH-PP** (Size of T-DNA inserts are 7127 and 7798 bp, respectively)



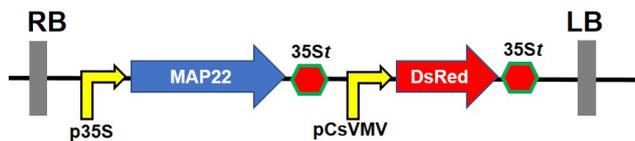
There are two forms of this construct, varying in how three genes derived from *E. coli* are translated. In both cases, the synthetic gene construct containing the three ORFs is expressed as a single transcription unit, under the control of the constitutive 35S promoter from Cauliflower mosaic virus, also with a transcriptional terminator from that same source. The three *E. coli*-derived sequences (GlcD, GlcE and GlcF) encode the D, E and F subunits of the glycolate dehydrogenase complex, which serves to bypass photorespiration when targeted to the plastid of the plant cell. In construct **GDH**, the three activities (GlcD, E & F) are transcribed and translated as a single polypeptide, targeted to the plastid through the addition of the transit sequence from the small subunit of Rubisco from *Arabidopsis thaliana*. In the case of **GDH-PP**, the three polypeptides encoding the GlcD, E and F proteins are co-translationally generated from the single transcript due to the additional presence of the 2A self-processing linker at the terminus of each ORF.

**Construct MAP22** (Size of T-DNA insert is 6,268 bp)

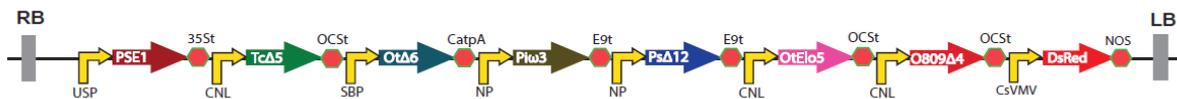


In construct MAP22, the *Arabidopsis thaliana* gene At4g23060 was placed under the control of the constitutive 35S promoter from Cauliflower mosaic virus, also with a transcriptional terminator from that same source. Expression of this microtubule-associate protein (MAP) results in altered plant architecture.

### Genetic cross DHAP22



DHA2015.1



Homozygous plants containing **Construct DHA2015.1** were used to cross-pollinate plants containing **Construct MAP22**, and the resulting seeds used to identify progeny which contained both the omega-3 trait and the MAP22 gene. These were subsequently selfed and homozygous progeny selected on the basis of the presence of DHA in the seeds and transcripts from the Arabisopsis MAP22 gene.

**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,**
- (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, astaxanthin and wax ester biosynthetic pathways 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.

In the case of MAP22 and GDH the transgenes are under the control of cauliflower mosaic virus promoter 35S and are therefore expressed in all tissue-types of the plant to varying degrees.

**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 lines 1-11 listed in the table in **Section 14**, except for the accumulation of non-native omega-3 LC-PUFAs, ketocarotenoids such as astaxanthin, or wax esters in their seeds, the transgenic *C. sativa* plants are visually indistinguishable from the untransformed controls. No difference in seed set, seed size or germination was observed. No difference in fertility was observed. Vegetative performance of the transgenic plants was unaltered.

In the case of lines 12-14 (MAP22 and GDH constructs), the combination of constitutive expression and intended alteration of architecture or assimilation results in some perturbation to plant morphology (such as thicker stems or bigger leaves). However, this does not alter the viability of the plant, nor that of any progeny. It also does not change the fertility of the plant, as indicated by regular Mendelian inheritance patterns.

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

In the case of plants derived from lines 1-11 listed in the table in **Section 14**, where the transgenes are expressed in a seed-specific manner, all plants are morphologically indistinguishable from untransformed controls. The inheritance of the transgene over 3+ generations follows normal rules of Mendelian genetics.

In the case of lines 12-14 (MAP22 and GDH constructs), the combination of constitutive expression results in some perturbation to plant morphology (such as thicker stems or bigger leaves). However, this does not alter the viability of the plant, nor that of any progeny. It also does not change the fertility of the plant, as indicated by regular Mendelian inheritance patterns.

**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 long chain polyunsaturated fatty acids or astaxanthin and associated ketocarotenoids, nor wax esters. The fatty acids are very widely consumed by humans, being recognised as important and beneficial nutrients, and astaxanthin is widely dispersed in the marine foodweb (it is the compound which gives salmon flesh the pinkish/orange hue). Astaxanthin is also consumed as a direct human dietary supplement and also has GRAS status in the USA. Wax esters are also a well-known (albeit minor) component of the human diet, being present in leaf cuticles and also many crustaceans. There are no reports of the *E. coli* Glc proteins being toxic, allergenic or harmful to humans, nor for the Arabidopsis MAP22 protein.

The visual marker DsRed (Jach et al., 2001) encoding the red fluorescent protein from reef coral *Discosoma* sp was used in all constructs apart from **ASX-A2**. There are no known toxic, allergenic or harmful effects known to be associated with the DsRed protein.

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. The *bar* gene is present in **ASX-A2** and **EPA2016.1**. There are no known toxic, allergenic or harmful effects known to be associated with the presence of this 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 long chain polyunsaturated fatty acids and/or ketocarotenoids. Similarly, accumulation of wax esters in the seeds of camelina is not predicted to affect non-target organisms, nor with the proteins associated with the marker genes (*DsRed*, *bar*). Thus, the likelihood of this potential hazard ever being realised is extremely low.

In the case of plants constitutively expressing the MAP22, this sequence is derived from *Arabidopsis thaliana*, and is unlikely to bring about a change in interactions with non-target organisms. In the case of plants expressing the GDH genes derived from *E. coli*, these sequences are targeted to the plastid and compartmentalised therein. Both constructs (MAP22, GDH) exploit the natural plasticity to accommodate changes to form or function, in the case of MAP22, the plants have markedly thicker stem and larger leaves. In the case of the GDH plants, there is a less obvious but still noticeable increase in leaf size and side-branches.

### **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 such as *O. tauri*  $\Delta^6$ -desaturase (**DHA2015.x series**) or the maize phytoene synthase (**ASX-A2**) 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, in most iterations, the presence of the fluorescent protein DsRed also acts a visual reporter on excitation at 555nm, whereas the presence of the *bar* gene and associated resistance to bialaphos is present in **ASX-A2 and EPA2016.1**.

It is equally possible to use fatty acid profiling to identify plants associated with the omega-3 or wax ester trait, since these lipids are not native to higher plants. Similar analytical approaches can be used to identify plants containing non-native ketocarotenoids such as astaxanthin.

**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/or ketocarotenoids were previously approved in the UK for experimental field release (DEFRA Consent 16/R8/01). Transgenic *C. sativa* containing Constructs **ASX-A2 and DHA2015.1** were approved in the US for experimental field release (APHIS Permit # 15-357-101r). Similarly, **DHA2015.1** was released in Canada in 2017 under CFIA permit ICA6-46020

## **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 2 trials in consecutive seasons and will avoid reusing the same plots. 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 ~2200m<sup>2</sup>, of which ~ 310m<sup>2</sup> will be GM (34 plots of 18m<sup>2</sup>; see plan in section 34). In the case of Brooms Barn field trial, where it is proposed to sow only one GM line (DHA2015.1) the GM plot will cover ~4000m<sup>2</sup> with a surrounding pollen barrier of ~2000m<sup>2</sup> (see plan in section 34)

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](http://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, ketocarotenoids, wax esters) in their seed oils.

In addition, the field evaluation of transgene-derived traits which deliver improvements to plant architecture or metabolism will be carried out, with the predominant focus being on enhancement to seed composition.

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

The GM *C. sativa* will be planted consecutively for three years (2018- 20). 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 plot-scale farm equipment or hand-sowing.

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

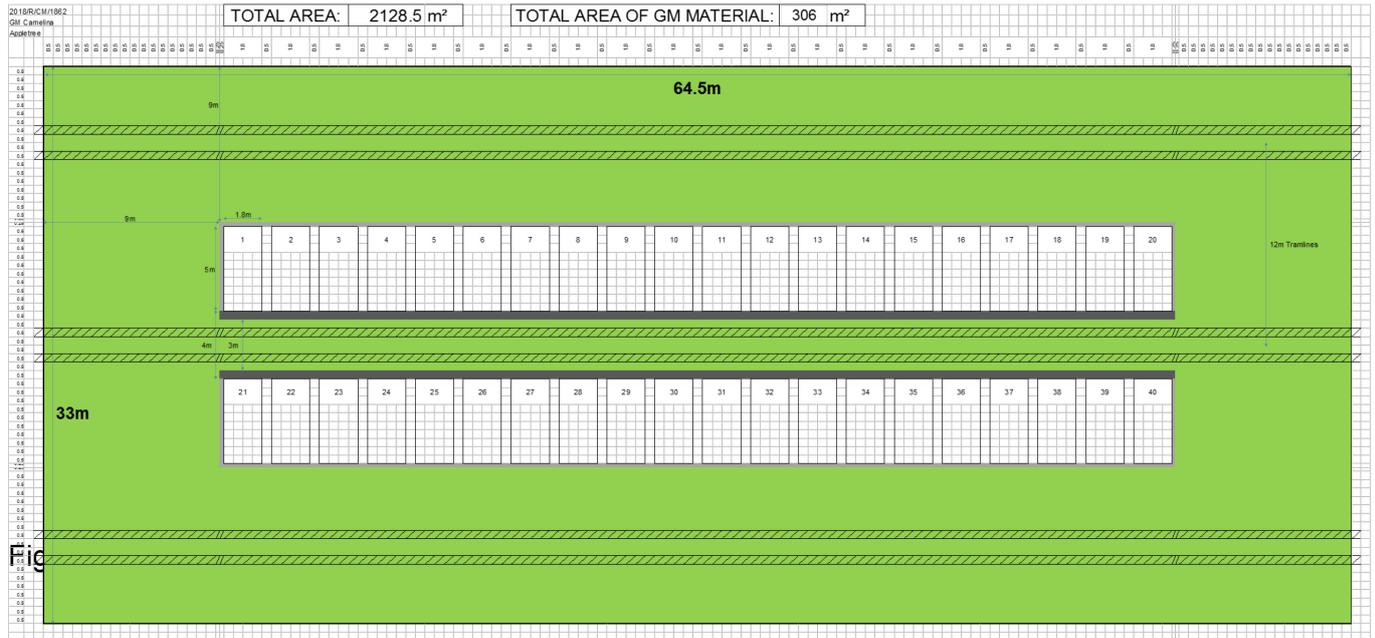
The sites will be prepared according to standard agronomic practices for spring *Brassicaceae* cultivation. The release will be monitored regularly during all stages of development and harvested just prior to full maturity. Some seeds from the GM and control plots will be conditioned, threshed and analysed in appropriate GM facilities to determine oil composition and oil quantity. All other material, including that from any pollen barrier will be harvested 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.**

See trial design below (Fig. 2). GM plants will be sown in duplicated blocks of strips 1.8X 5m (9m<sup>2</sup> per block, thus 18m<sup>2</sup> per line). A total of 17 GM lines (Constructs 1-14 listed in Section 14, of which there are two events for MaMa14-6, and also including two genetic crosses DHASX-A2 and DHAP22), will be sown at Harpenden, thus 306m<sup>2</sup> of GM material will be sown on this site, surrounded by a 9m pollen barrier.

In addition to these 17 GM lines, each block will contain a single WT control Camelina strip, and also 2 mutant alleles in which the FAD2  $\Delta$ 1 $\Delta$ 2-desaturase has been disrupted by CRISPR-Cas9-mediated genome editing (Morineau et al. 2017). These two lines (2F4-24, A7) are mutated in either 2 or 3 homeologs of the FAD2 gene, respectively, meaning that the A7 line is mutated in all three homeologs present in the hexaploid genome of Camelina. In all other respects, the 2F4-24 and A7 lines are genetically identical to the WT plants.

Thus, two rows, each containing 17 GM blocks of 9m<sup>2</sup> each, plus 2 9m<sup>2</sup> blocks of FAD2 mutant lines and 1 9m<sup>2</sup> of WT control.



At Brooms barn, a single GM line (DHA2015.1) will be sown in a single block to a maximum of 4,000m<sup>2</sup>. This block will then be subject to different fertilizer treatments to determine the impact of N-treatments on novel seed oil accumulation.

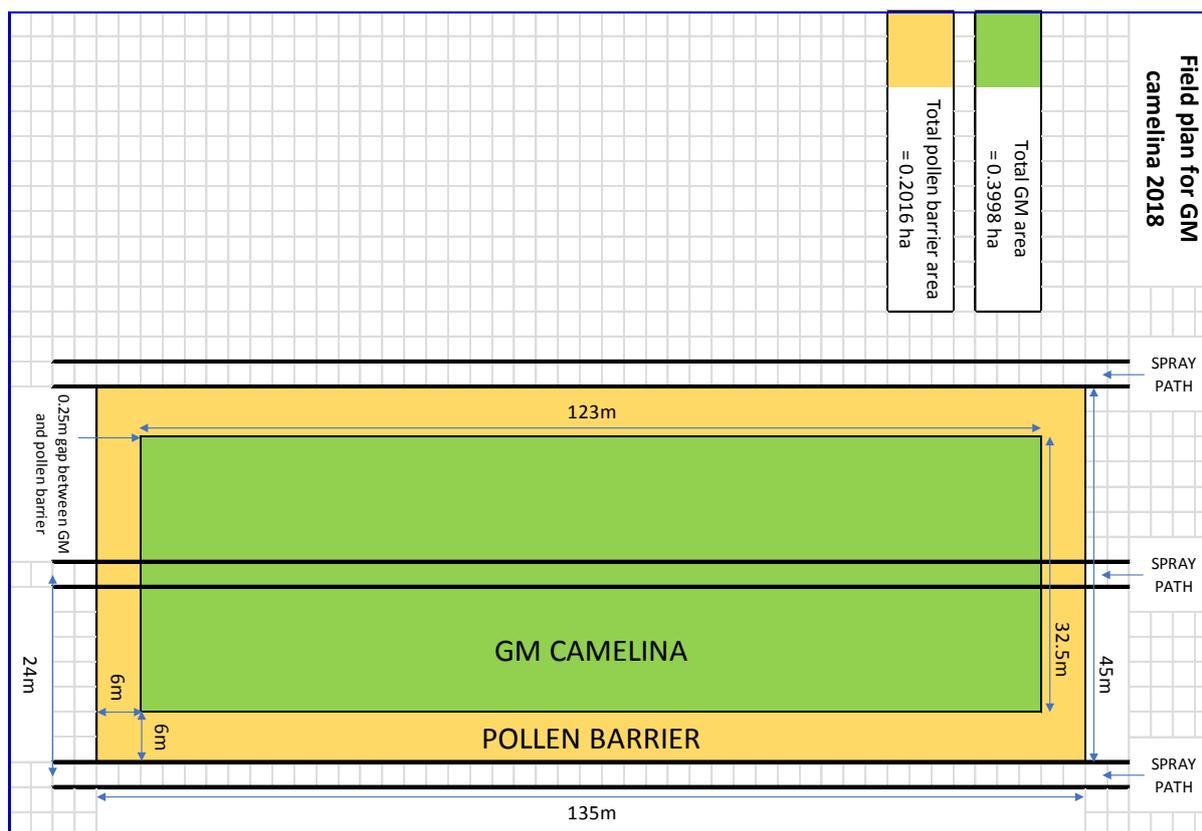


Fig 3. Plot layout for Brooms Barn trial

Sowing density will be to produce a stand density of less than 300 plants per m<sup>2</sup> to accommodate any variation in germination rates.

The outer pollen barrier will be sown with non-GM *C. sativa* of the same variety as the GM plots.

## 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. The drills will be filled on the trial area and will be thoroughly cleaned before leaving the trial area.

To minimise the possibility of seed loss, the plants will be harvested just prior to full maturity. All straw will be chopped and left on site.

Measures (suspending wires across the area to provide bird scaring as well as gas guns and hawk kites) will be used to keep out birds for the rest of the season. 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.

Pollen dispersal be minimised through the placing of wildtype *C. sativa* on the external strip of the experimental plot – this will serve as a pollen-trap for pollen released from the GM *C. sativa*.

### **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.

### **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 on site at Rothamsted Research, all samples taken from the field will be closely monitored and records kept of weights and movements of seeds and straw. All samples removed from the trial site will eventually be destroyed by an approved technique. The remainder of the site will be then be harvested by the plot combine. The grain obtained will be disposed to deep landfill using an approved contractor. The plot combine will be cleaned in an uncultivated region of the fenced area 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.**

The site 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 intend to carry out GM field trials at Rothamsted Research in the near future. 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).
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 Ottawa, Ontario. (2012) The Biology of *Camelina sativa* (L.) Crantz (Camelina). <http://www.inspection.gc.ca/plants/plants-with-novel-traits/applicants/directive-94-08/biology-documents/camelina-sativa-l-eng/1330971423348/1330971509470>

Dalal J, Lopez H, Vasani NB, Hu Z, Swift JE, Yalamanchili R, Dvora M, Lin X, Xie D, Qu R, Sederoff HW. 2015 *Biotechnol Biofuels*. 29;8:175 doi: 10.1186/s13068-015-0357-1

Frisch DA, Harris-Haller LW, Yokubaitis NT, Thomas TL, Hardin SH, Hall TC. (1995) Complete sequence of the binary vector Bin19. *Plant Mol Biol*. 27(2): 405-9.

Haslam RP, Ruiz-Lopez N, Eastmond P, Moloney M, Sayanova O, Napier JA. (2013) The modification of plant oil composition via metabolic engineering--better nutrition by design. *Plant Biotechnol J*. 11(2): 157-68.

Jach G, Binot E, Frings S, Luxa K, Schell J. (2001) Use of red fluorescent protein from *Discosoma* sp. (dsRED) as a reporter for plant gene expression. *Plant J*. 28(4): 483-91.

Julié-Galau S, Bellec Y, Faure JD, Tepfer M. (2013) Evaluation of the potential for interspecific hybridization between *Camelina sativa* and related wild Brassicaceae in anticipation of field trials of GM camelina. *Transgenic Res*. 23(1):67-74.

Koncz C, Schell J. (1986) The promoter of TL-DNA gene 5 controls the tissue-specific expression of chimaeric genes carried by a novel type of *Agrobacterium* binary vector. *Mol Gen Genet* 204: 383-396.

Lu C, Kang J. (2008) Generation of transgenic plants of a potential oilseed crop *Camelina sativa* by *Agrobacterium*-mediated transformation. *Plant Cell Rep*. 27(2):273-8.

Morineau C, Bellec Y, Tellier F, Gissot L, Kelemen Z, Nogué F, Faure JD. (2107) Selective gene dosage by CRISPR-Cas9 genome editing in hexaploid *Camelina sativa*. *Plant Biotechnol J*. 15(6):729-739. doi: 10.1111/pbi.12671.

Ruiz-López N, Haslam RP, Venegas-Calderón M, Li T, Bauer J, Napier JA, Sayanova O. (2012) Enhancing the accumulation of omega-3 long chain polyunsaturated fatty acids in transgenic *Arabidopsis thaliana* via iterative metabolic engineering and genetic crossing. *Transgenic Res*. 21(6):1233-43.

Ruiz-Lopez N, Haslam RP, Napier JA, Sayanova O. (2014) Successful high-level accumulation of fish oil omega-3 long-chain polyunsaturated fatty acids in a transgenic oilseed crop. *Plant J*. 77: 298-308

Ruiz-Lopez N, Broughton R, Usher S, Salas JJ, Haslam RP, Napier JA, Beaudoin F. (2017)

Tailoring the composition of novel wax esters in the seeds of transgenic *Camelina sativa* through systematic metabolic engineering. *Plant Biotechnol J*. 15(7):837-849.

Sambrook J, Fritsch EF, Maniatis T (1989). *Molecular Cloning: A Laboratory Manual* (Second Edition). New York: Cold Spring Harbor Laboratory press.

Sayanova O, Ruiz-Lopez N, Haslam RP, Napier JA. (2012) The role of  $\Delta 6$ -desaturase acyl-carrier specificity in the efficient synthesis of long-chain polyunsaturated fatty acids in transgenic plants. *Plant Biotechnol J.* 10(2):195-206.

Séguin-Swartz G, Nettleton JA, Sauder C, Warwick S I, Gugel, RK (2013). Hybridization between *Camelina sativa* (L.) Crantz (false flax) and North American *Camelina* species. *Plant Breeding* 132: 390-396.