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 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.

Wild Bioscience is the applicant. The people involved with this project are as follows.

Wild Bioscience Ltd
115K Olympic Avenue
Milton Park
Oxfordshire
OX14 4SA

Job title:

- CEO
- CTO
- Team lead (plant care)
- Team lead (validation)

The Rothamsted Farms team, the John Innes Centre (JIC) Experimental Research (Church Farm) team, and the National Institute of Agricultural Botany (NIAB) Farms team will be contracted to carry out part of the trial and have extensive experience conducting GM field trials. The responsible people will be:

Name and title	Address	Qualifications and experience
Field Experimentation Manager	JIC Field Station, Church Farm, Bawburgh, Norwich NR9 3PY	30+ years of experience in agricultural field trials with 6 years direct experience growing GM trials at JIC.
Field Operations Manager	JIC Field Station, Church Farm, Bawburgh, Norwich NR9 3PY	BASIS Foundation certificate. 5 years' experience at JIC with involvement in GM trials.
Field Team-Machinery and Equipment Specialist	JIC Field Station, Church Farm, Bawburgh, Norwich NR9 3PY	15 years' experience at JIC with involvement in GM trials for 7 years.
Field Team-Seed and Data Processing Specialist	JIC Field Station, Church Farm, Bawburgh, Norwich NR9 3PY	Licenced drone pilot, 10 years' experience at JIC as a Research Assistant, joined field team in November 2024. PA1 and PA6 qualified for application of plant protection products using handheld equipment. 1 year of experience with GM trials.
Field Team	JIC Field Station, Church Farm, Bawburgh, Norwich NR9 3PY	Licenced drone pilot, Joined field team in November 2024. PA1 and PA6 qualified for application of plant protection products using handheld equipment. 1 year of experience with GM trials including monitoring.
Field Team	JIC Field Station, Church Farm, Bawburgh, Norwich NR9 3PY	Joined field team in April 2025. PA1 and PA6 qualified for application of plant protection products using handheld equipment. 1 year of experience with GM trials.
Field Trials Manager	Rothamsted Research, West Common, Harpenden, AL5 2JQ	3 years Field Trials experience and management, including GM monitoring and trial execution.
Farm Manager	Rothamsted Research, West Common, Harpenden, AL5 2JQ	30 years + experience working in agronomy and long-term experiments.
NIAB Trials Coordinator	NIAB, Park Farm Campus, Villa Road, Histon, Cambridge CB24 9AT	BSc (Hons) Agriculture with Agronomy, BASIS, FACTS. 20 years in agricultural field trials
Senior Trials Manager	NIAB, Park Farm Campus, Villa Road, Histon, Cambridge CB24 9AT	City & Guilds NVQ in Agriculture, BASIS Foundation. 38 years in agricultural field trials

Additional responsible people will be highlighted prior to each sowing if not already noted in the above list.

2. The title of the project.

Investigating altered agronomic performance of wheat through the modulation of plant regulators of photosynthesis using gene-edited winter wheat.

Part 2: Information relating to the parental or recipient plant

3. The full name of the plant

(a) family name	Poaceae
(b) genus	Triticum
(c) species	aestivum
(d) subspecies	Not applicable
(e) cultivar/breeding line	Cadenza
(f) common name	Common wheat, bread wheat, winter wheat, spring wheat

4. Information concerning

(a) the reproduction of the plant:

(i) the mode or modes of reproduction

Reproduction is sexual leading to formation of seeds. Wheat is approximately 99% autogamous under natural field conditions; with self-fertilization normally occurring before flowers open. Wheat pollen grains are relatively heavy and any that are released from the flower remain viable for between a few minutes and a few hours. Warm, dry, windy conditions may increase cross-pollination rates on a variety-to-variety basis (see also 6 below).

(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

The generation time is 20-25 weeks. For Cadenza (when sown as a winter-wheat type), one season is normally from September or October to August or September.

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

Wheat is naturally self-pollinating but under experimental conditions wheat can be crossed with various wild grasses. Of these, only the genera *Elymus* are present in the UK but there are no reports of wheat x *Elymus* spontaneous hybrids. Wheat can also be forced using laboratory techniques to cross to rye, triticale and a limited number of other cereals.

5. Information concerning the survivability of the plant:

(a) its ability to form structures for survival or dormancy,
(b) any specific factors affecting survivability.

5 a) and b) Wheat is an annual species and survives from year to year only via seed production. In normal farming practice, mature seeds may fall from the plant prior to or at the time of harvest and not be collected. If not managed, these seeds may over-winter in the soil and germinate the following spring as 'volunteers'. Cadenza is a UK milling variety, which is photoperiod-sensitive (ppd-D1) but has a negligible vernalising requirement and relatively high levels of frost tolerance which means it can be sown either as a spring or winter type with good frost-tolerance under typical UK winter conditions (Whaley et al 2004).

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
(b) any specific factors affecting dissemination.

Pollen can be disseminated by the wind. Such dissemination is limited by the relatively large size and weight of wheat pollen. The risk of cross-pollination is also reduced by its short period of viability. Reports quantifying the rate of cross pollination state that out-crossing rates are usually less than 1% (for example Hucl 1996). Under certain growing conditions individual genotypes may have out-crossing rates of up to 4-5% (Griffin 1987; Martin 1990). Seed is usually retained by the plant 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 in Europe.

Wheat is grown in temperate zones worldwide, mainly in Europe, North America and Asia.

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.

Wheat plants have a range of pests and fungal pathogens. The main insect pests in the UK are:

- Three aphid (Hemiptera: *Sternorrhyncha*) species
    - The bird cherry-oat aphid, *Rhopalosiphum* padi
    - The grain aphid, *Sitobion avenae*
    - The rose grain aphid, *Metopolophium dirhodum*
- Orange wheat blossom midge, *Sitodiplosis mosellana* (Diptera: Cecidomyiidae)
- Wheat bulb fly Delia coarctata (Diptera: Anthomyiidae).

Wheat also interacts with beneficial insects, for example Aphidius rhopalosiphi (Hymenoptera: Aphidiinae) which attack aphid pests.

Wheat is not toxic and a major world bulk commodity food but may cause gastro-intestinal intolerance, coeliac disease and/or ‘bakers’ asthma’ in susceptible individuals.

Plants and seeds arising from this trial will not enter the food or feed chains.

Part 3: Information relating to the genetic modification

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

Transgenic wheat plants were produced using standard *Agrobacterium tumefaciens*-mediated transformation protocols described in (Ishida et al., 2015). The constructs were introduced into immature embryos of *T. aestivum* cv. Cadenza by Agrobacterium-mediated inoculation. Whole plants were regenerated and selected from immature embryos induced in tissue culture.

Targeted genome editing in *Triticum aestivum* cv. Cadenza was performed using the CRISPR–Cas9 system. For this, a plasmid vector for inducing gene-targeted mutations via the CRISPR/Cas9 system was introduced into the plant's genome. Single guide RNAs (sgRNAs) were designed based on the available annotated wheat reference genome to target regions of the Wild Solar 003 and Wild Solar 005 gene loci in the A, B and D sub genomes. Guide sequences were selected to minimise potential off-target activity.

Wild Solar 003 gene

For targeting the three homeolog copies of the gene of interest within each sub genome (A, B and D), two sgRNA sequences were selected for an area of homology in the promoter region of the gene of interest for the three sub genomes. These sequence edits are designed to enahance translation of this target gene. A combination of two guides was delivered to the plant (plasmid pWB0354: sgWB00016 + sgWB00018; plasmid pWB0357: sgWB00017 + sgWB00018 - Table 1).

Wild Solar 005 gene

For targeting the three homeolog copies of the gene of interest within each sub genome (A, B and D), two sgRNA sequences were selected for an area of homology in the CDS (Coding Sequence) region of the gene of interest for the three sub genomes. These sequence edits are designed to disrupt expression of the target gene. Each guide was delivered independently to the plant (plasmid pWB0360: sgWB0021; plasmid pWB0361: sgWB0020 - Table 1).

Table 1 - Level 2 binary plasmids used for plant transformation

Gene of interest	Vector plasmid name	Guide RNAs
Wild Solar 003	pWB0354	sgWB0016, sgWB0018
Wild Solar 003	pWB0357	sgWB0017, sgWB0018
Wild Solar 005	pWB0360	sgWB0021
Wild Solar 005	pWB0361	sgWB0020

Please note that Wild Solar 003 and Wild Solar 005 involve entirely separate genetic modifications, distinct from those described in our previous release (22/R55/01: Wild Solar 001).

11. The nature and source of the vector used

All vectors used are binary plasmids whose backbone (i.e. the regions outside of the T-DNA, derives like-for-like from the well-used plant transformation vector pCambia2301). The T-DNA of these binary vector plasmids all contain the plant selection resistance gene for hygromycin (*hptII*), the Cas9 and one or two sgRNAs, specific for the gene of interest.

The individual constructs contained within the T-DNA of the binary vector are: **pOsAct1::HptII::2xtNos**; **pZmUbi1::SpCas9(TaCO)::tZmUbi** (Wild Solar 003) or **pZmUbi1::SpCas9(TaCO)::tNos** (Wild Solar 005); and **pTaU3::sgWB0016_P1::sgWB0018_P2** or **pTaU3::sgWB0017_P1::sgWB0018_P2** (for Wild Solar 003) and **pTaU3::sgWB0020_P1** or **pTaU3::sgWB0021_P1** (Wild Solar 005).

For the sgRNA cassette, a tRNA-based system was used, which enables the expression of multiple sgRNAs from a single promoter as a single polycistronic transcript. Endogenous tRNA-processing enzymes cleave the transcript to release individual, functional sgRNAs.

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

Wild Solar 003

Schematic maps and details of the genetic elements present in the Level 2 binary plasmids used to generate the Wild Solar 003 gene-edited lines included in this release are provided in Figures 1 and 2 and Table 2.

Plasmids pWB0354 and pWB0357 were used to target the Wild Solar 003 trait. These plasmids differ only in the sgRNAs they contain: pWB0354 includes sgRNAs sgWB0016 and sgWB0018, whereas pWB0357 includes sgRNAs sgWB0017 and sgWB0018 (see Table 1).

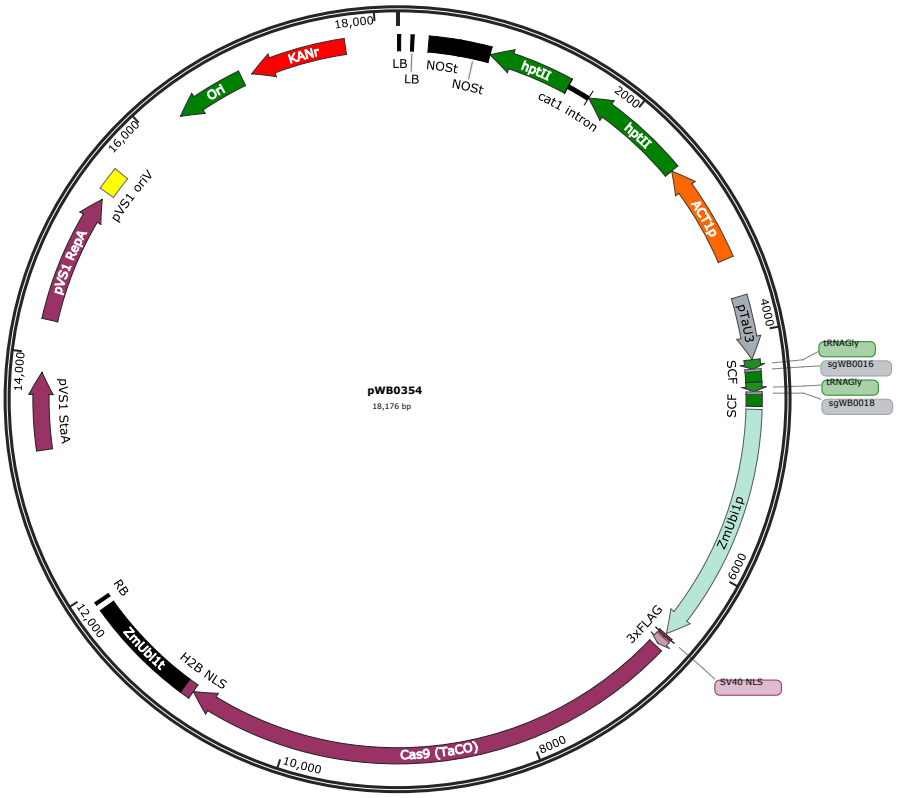

Figure 1 - Level 2 binary plasmid pWB0354. This plasmid contains two sgRNAs (sgWB0016 and sgWB0018) specific to the **Wild Solar 003 trait**. The information about the fragments can be found in Table 2.

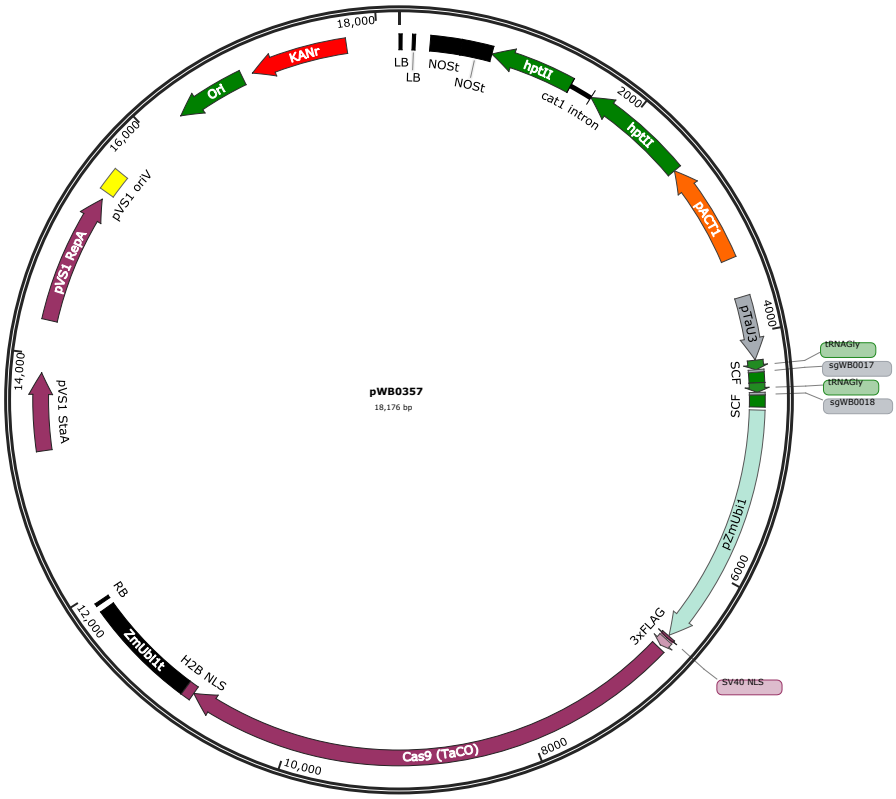

Figure 2 - Level 2 binary vector pWB0357. This plasmid contains two sgRNAs (sgWB0017 and sgWB0018) specific to the **Wild Solar 003 trait**. The information about the fragments can be found in Table 2.

Table 2 - Genetic elements present within the region intended for insertion (the T-DNA) in the Level 2 binary plasmids pWB0354 and pWB0357.

Element	Donor organism	Description, size, and intended function
2 X LB	*Agrobacterium tumefaciens*	2 X T-DNA Left border (2 X 25bp)
2 X NOSt	*Agrobacterium tumefaciens*	2 X Nopaline synthase terminators (2 X 253bp)

hptII with intron	*Escherichia coli* and *Ricinus communis*	Hygromycin phosphotransferase (or *hph*) selection gene containing the CAT1 intron from *Ricinus communis* catalase-1 gene (1778bp)
pACT1	*Oryza sativa*	Rice Actin1 promoter (843bp)
pTaU3	*Triticum aestivum*	U3 promoter sequence (520bp)
tRNAGly	*Oryza sativa*	tRNA Glycine, glycine transfer RNA (tRNA) (to process and express multiple guide RNAs simultaneously) (2 X 77bp)
sgWB0016 sgWB0017 sgWB0018	*Triticum aestivum*	sgRNAs (21bp each)
SCF	*Streptococcus pyogenes.*	sgRNA scaffold for the CRISPR/Cas9 System (86bp + 98bp)
pZmUbi1	*Zea mays*	Maize Ubiquitin1 promoter (1992bp)
Cas9 (TaCO) with NLS signal peptides	*Streptococcus pyogenes*	Endonuclease gene for the CRISPR/Cas9 system, generates RNA-guided double strand breaks in DNA (4104bp). Codon-optimized for wheat. To ensure proper nuclear localization, the SV40 nuclear localization signal (NLS) from simian virus 40 large T antigen (21bp) and a triple FLAG epitope tag (with an enterokinase cleavage site) (66bp) were fused to the N-terminus. An additional nuclear localization signal derived from the core histone protein H2B (78bp) was fused to the C-terminus.
ZmUbi1t	*Zea mays*	Maize Ubiquitin1 terminator (910bp)
RB	*Agrobacterium tumefaciens*	T-DNA Right Border (25bp)

Wild Solar 005

Schematic maps and details of the genetic elements present in the Level 2 binary plasmids used to generate the Wild Solar 005 gene-edited lines included in this release are provided in Figures 3 and 4 and Table 3.

Plasmids pWB0360 and pWB0361 were used to target the Wild Solar 005 trait. These plasmids differ only in the sgRNAs they contain: pWB0360 includes sgRNA sgWB0021, whereas pWB0361 includes sgRNA sgWB0020 (see Table 1).

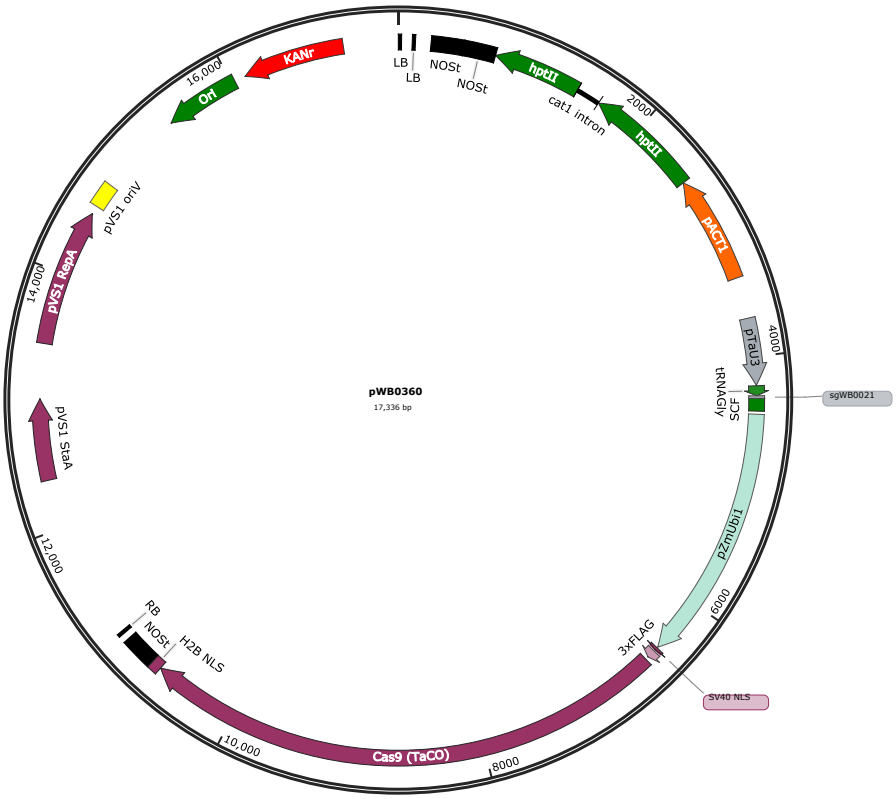

Figure 3 - Level 2 binary plasmid pWB0360. This plasmid contains one sgRNA (sgWB0021) specific to the **Wild Solar 005 trait**. The information about the fragments can be found in Table 2.

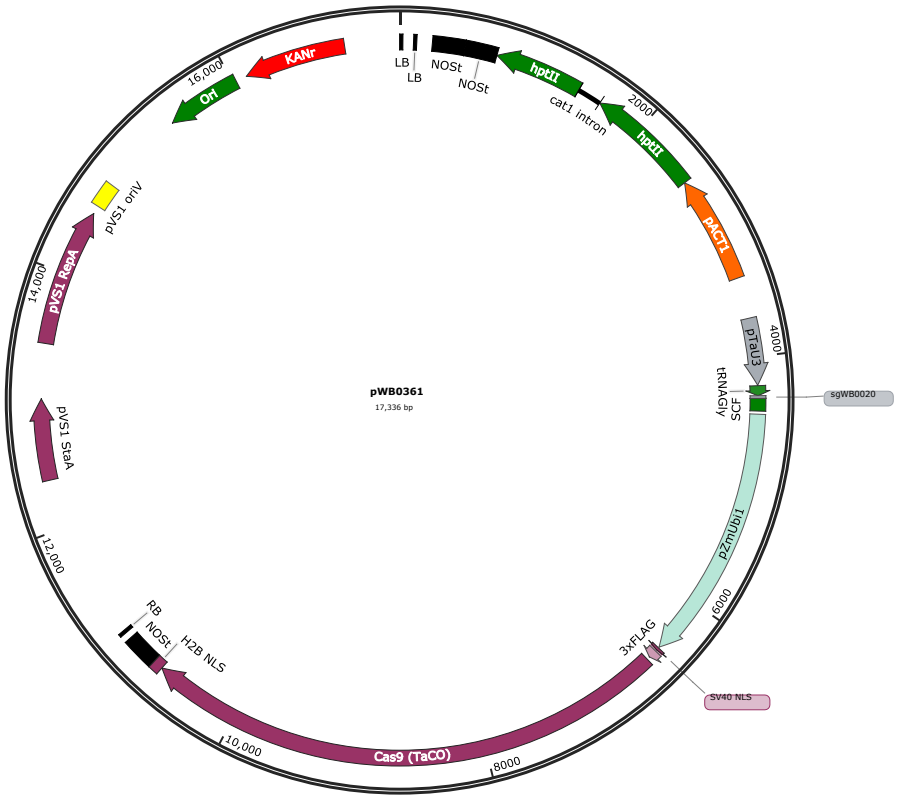

Figure 4 - Level 2 binary plasmid pWB0361. This plasmid contains one sgRNA (sgWB0020) specific to the **Wild Solar 005 trait**. The information about the fragments can be found in Table 2.

Table 3 - Genetic elements present within the region intended for insertion (the T-DNA) in the Level 2 binary plasmids pWB0360 and pWB0361

Element	Donor organism	Description, size, and intended function
2 X LB	*Agrobacterium tumefaciens*	2 X T-DNA Left border (2 X 25bp)
3 X NOSt	*Agrobacterium tumefaciens*	3 X Nopaline synthase terminators (3 X 253bp)

hptII with intron	*Escherichia coli* and *Ricinus communis*	Hygromycin phosphotransferase (or *hph*) selection gene containing the CAT1 intron from *Ricinus communis* catalase-1 gene (1778bp)
pACT1	*Oryza sativa*	Rice Actin1 promoter (843bp)
pTaU3	*Triticum aestivum*	U3 promoter sequence (520bp)
tRNAGly	*Oryza sativa*	tRNA Glycine, glycine transfer RNA (tRNA) (to process and express multiple guide RNAs simultaneously) (77bp)
sgWB0020 sgWB0021	*Triticum aestivum*	sgRNAs (21bp each)
SCF	*Streptococcus pyogenes.*	sgRNA scaffold for the CRISPR/Cas9 System (98bp)
pZmUbi1	*Zea mays*	Maize Ubiquitin1 promoter (1992bp)
Cas9 (TaCO) with NLS signal peptides	*Streptococcus pyogenes*	Endonuclease gene for the CRISPR/Cas9 system, generates RNA-guided double strand breaks in DNA (4104bp). Codon-optimized for wheat. To ensure proper nuclear localization, the SV40 nuclear localization signal (NLS) from simian virus 40 large T antigen (21bp) and a triple FLAG epitope tag (with an enterokinase cleavage site) (66bp) were fused to the N-terminus. An additional nuclear localization signal derived from the core histone protein H2B (78bp) was fused to the C-terminus.
RB	*Agrobacterium tumefaciens*	T-DNA Right Border (25bp)

Part 4: 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

Wild Solar 003 is described as a positive regulator of photosynthesis. The sequence edits are meant to **improve the translation efficiency** of this target gene, which is expected to enhance photosynthetic efficiency and agronomic performance (*i.e*., increase yield).

Wild Solar 005 was identified from a bioinformatics screen looking for negative regulators of photosynthesis. This target gene is therefore considered a putative negative regulator of photosynthesis, and its modification is intended to enhance photosynthetic efficiency and agronomic performance.

This cassette was segregated from the progeny by advancing edited plants through successive generations, enabling segregation of the T-DNA region, including the Cas9 expression cassette and the *hptII* selectable marker. However, residual T-DNA-derived sequences can still remain detectable in the genome, predominantly as non-functional fragments that do not constitute intact expression cassettes; as a result, these lines can still fall within the regulatory definition of genetically modified (GM) plants.

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.

The genetically modified wheat lines were generated using *Agrobacterium tumefaciens*-mediated transformation. The size of the transgenic cassette inserted into the plant genome of the genetically modified plants is approximately 11,081bp for Wild Solar 003 and 11,921bp for Wild Solar 005. The cassette spans from the LB to the RB sequence (T-DNA Left- and Right-Border, respectively) and contains all the elements necessary for the CRISPR/Cas9 system to generate edits in the gene target, as well as the hygromycin selection marker, as depicted in the plasmid figures and tables, detailed in section 12.

Digital PCR (dPCR) was applied to detect the endogenous reference gene (TaGamyB) and the selectable marker gene (*hptII*, hygromycin resistance) to identify and calculate the number of T-DNA (containing the Cas9 machinery and resistance marker gene) copies inserted into the plant's genome. Plants carrying the edits of interest were advanced through successive generations to **identify individuals homozygous for the edits** and **lacking the *hptII* marker gene (copy number = 0)**.

The plant lines intended for this multisite trial are listed in Table 4 using their original T1 identifiers for traceability. Plants used for field release will be derived from subsequent generations advanced from these T1 lines. In later generations, an additional suffix will be appended to the original identifier to denote the specific generation and individual plants used (for example sdWB01334-XXX-XXX).
Prior to field release, the parental plants of each line will be molecularly characterised to confirm the presence and zygosity of the intended edits. The expected genetic modifications for each trait are described in the sections below.

Table 4 - Plant line IDs at T1 generation

Plant Line ID	Genotype	Trait
sdWB01334	Edited line	Wild Solar 003
sdWB01407	Edited line	Wild Solar 003
sdWB01348	Edited line	Wild Solar 003
sdWB01341	Edited line	Wild Solar 003
sdWB01350	Edited line	Wild Solar 003
sdWB01405	Edited line	Wild Solar 003
sdWB01694	Edited line	Wild Solar 005
sdWB01695	Edited line	Wild Solar 005
sdWB01696	Edited line	Wild Solar 005
sdWB01698	Edited line	Wild Solar 005
sdWB01628	Edited line	Wild Solar 005
sdWB01642	Edited line	Wild Solar 005
sdWB01643	Edited line	Wild Solar 005
sdWB01552	Edited line	Wild Solar 005
sdWB00526	Wild Type (WT)	Not applicable

Wild Solar 003
Targeted mutations were introduced into the Wild Solar 003 loci located on the A, B and/or D sub genomes of wheat. sgRNAs were delivered as pairs with the aim of generating fragment deletions between guide target sites within the promoter region

of the gene of interest. Where fragment deletions did not occur, other insertions and/or deletions within the promoter region may be present.

The precise nature and size of the deletions will be determined by PCR using gene-specific primers flanking the designed gRNA target sites, followed by amplicon sequencing to assess zygosity.

Wild Solar 005
Targeted mutations were introduced into the Wild Solar 005 loci located on the A, B and/or D sub genomes of wheat. The edits consist of insertions and/or deletions within the CDS (Exon 1), resulting in frameshift mutations and predicted loss of gene function.

The precise nature and size of the deletions will be determined by PCR using gene-specific primers flanking the designed gRNA target sites, followed by amplicon sequencing to assess zygosity.

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.

b). Conclusions on the molecular characterisation 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.
Molecular characterisation will confirm that the wheat lines selected for field release will contain the intended targeted edits in the Wild Solar 003 and 005 loci. All lines selected for field release will be copy number = 0 for the *hptII*, hygromycin resistance marker gene.

Part 4A: 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

It is expected that the transgenic lines will not differ from conventional wheat in their capacity to self or cross pollinate via sexual reproduction (see parts 4 and 6). A low rate (approximately 1%) of cross pollination with closely adjacent wheat plants within the trial is anticipated.

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

arising

None.

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

Not applicable.

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.

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.

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

None.

22. Any toxic, allergenic or other harmful effects on human health arising from the genetic modification

There appears to be no published toxicity or allergenicity data for Wild Solar 003 and Wild Solar 005 at the levels expected to be generated by these plants and because they will not enter the food or feed chains, we consider the potential toxic or harmful effects to be negligible.

23. Conclusions on the specific areas of risk

The genetically modified plants of Wild Solar 003 and Wild Solar 005 differ from conventional Cadenza plants in that they may contain additional quantities of an already existing wheat protein (Wild Solar 003) or have a wheat protein being knocked out (Wild Solar 005). None of these changes affect the ability of the genetically modified plants to transfer DNA to microorganisms, interact with other species, or interactions with the abiotic environment. Neither do they elicit changes in agricultural practices or present novel harmful effects on human health arising from genetic modification.

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

dPCR was applied to detect the endogenous reference gene (TaGamyB) and the

selectable marker gene (*hptII*, hygromycin resistance) to identify and calculate the number of T-DNA (containing the Cas9 machinery and resistance marker gene) copies inserted into the plant's genome.

Genetic modifications introduced by CRISPR/Cas9 will be detected and characterised using conventional PCR followed by sequencing, either Sanger sequencing or next-generation amplicon sequencing, or both. Primers were designed to flank the CRISPR target region, enabling the identification of insertions and deletions.

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

Not applicable.

Part 5 Information relating to the site of release

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

We propose to carry out trials across the three locations that were used in the consent **22/R55/01**. The locations we propose are Rothamsted Research GM field trial site, Harpenden, Hertfordshire, the NIAB Park Farm trial site in Histon, Cambridge and the John Innes Centre (JIC) trial site at Sparrow's Hill, Norwich. Grid references will be provided prior to sowing for each location.

The maximum total proposed area across all trial sites and all traits will be no more than 10,000m^2 per calendar year. This area will consist of all GM cropped area, excluding any Wild Type plots, empty space between plots and Pollen barrier.

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

All the release sites are located in the East of England and are agricultural areas of

the experimental farms. In particular, the farms at Bawburgh, and Histon, pertain geographically to counties to the area denominated as East Anglia.

In general, the ecosystem of East Anglia hosts species of lowland grass and heath to semi-natural woodland, coastal marshes and freshwater reed beds. East Anglia has one third of the country's stock of the most productive Grade 1 and 2 soils, much of this from the area spreading from The Wash through The Fens into the southern parts of Suffolk. The water source in this region is the large chalk aquifer that stretches from the Northeast to the Southwest of the region.

On the other hand, Harpenden includes habitats such as acidic grasslands, woodlands, and the human-made Southdown Ponds. The acidic grasslands occur on soils with a pH of 5.5 or less and support an array of specialist species, a range of ground dwelling and burrowing insects. The Harpenden grassland hosts species such as grazing animals, bumblebees, green-winged orchids, harebells, and slender St John's wort. The Harpenden woodland is of secondary origin; the cessation of grazing has allowed tree seeds to germinate and grow on previous meadow areas to produce the largely oak-dominated woodland that occurs today. The Ponds are inhabited by waterfowl, amphibians and nesting birds.

Specifically, the areas of the release sites, according to the MAGIC interactive map system from Defra, include the habitats:

- JIC Church Farm, Bawburgh (NR9 3PY): acid, calcareous, neutral grassland; bare ground, and dwarf shrub heath.
- NIAB Park Farm, Histon (CB24 9AT): broadleaved, mixed and yew woodland; acid, calcareous, neutral grassland, and built-up areas and gardens.
- Rothamsted West Common, Harpenden (AL5 2JQ): acid, calcareous, neutral grassland; broadleaved, mixed and yew Woodland; and built-up areas and gardens.

In relation to climate, the temperature of the East of England shows both seasonal and diurnal variations; with a mean annual temperature of 9.5-10.5°C, the maximum temperatures are 6-8°C during winter and 20-23°C during summer. Through most of the region there are about 30 rain days (rainfall greater than 1 mm) in winter and less than 25 days in summer. Eastern England is one of the most sheltered parts of the UK to wind - the windiest areas are closer to the storms from the Atlantic.

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

Wheat is a self-pollinating crop with very low rates of cross-pollination with other wheat plants. The only wild relatives of wheat commonly found across the UK are in the genera *Elymus,* and there are no reports of cross-hybridisation between wheat and species of these genera. The two most common inland species are the common couch grass (*Elymus repens*, formerly *Elytrigia/Agropyron repens*) and the bearded couch grass (*Elymus caninus*, formerly *Agropyron caninum*). Other related species, such as the sand couch (*Elytrigia juncea*, formerly *Agropyron junceum*), sea couch (*Elytrigia atherica*, formerly *Agropyron pycnanthum*) and hybrids are largely confined to coastal habitats.

According to the records of the National Biodiversity Network, in a 5 km radius of the respective postcodes of the trial sites, the incidence of the two most common grasses is as follows:

The common couch grass is prevalent in the areas around the JIC Church Farm

(Bawburgh, NR9 3PY) and the Rothamsted West Common (Harpenden, AL5 2JQ) trial sites, but it less frequent around the NIAB Park Farm (Histon, CB24 9AT). In general, the bearded couch is less common than the couch grass. The bearded couch is frequent around the West Common site. This bearded couch grass has minimal number of records or no records at all around the Park Farm and the Church Farm, respectively.

The common couch grass propagates primarily by vegetative reproduction through rhizomes rather than by sexual reproduction – common couch is self-sterile and, as each spreading colony is usually a single clone, seeds are not often produced In the case of the bearded couch, a previous study using seeds from wild populations of this grass and of bread wheat (collected in the immediate vicinity of the bearded couch) in England, did not find any spontaneous hybridizations; this study concluded that introgression of bread wheat traits into the bearded couch population were improbable, disregarding the fact that these populations tend to grow in the same vicinity (Guadagnuolo, *et al.*, 2001).

In summary, the lack of reports of spontaneous hybrids between wheat and common couch or wheat and bearded couch, alleviates concerns in relation to potential cross-pollination events with GM pollen. Nevertheless, in all the trial sites, these grasses will be controlled along with other weeds in and around the trial site using standard farm practices.

No wheat or sexually compatible cereals and weeds, including *E. repens* will be cultivated or allowed to grow within 20m from the trial.

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

The proximity of the release sites to protected areas, denoted as SSSI (Site of Special Scientific Interest) conservation designations, are:

- Bawburgh (NR9 3PY): to the northeast, by ~5.4 km, there is the Sweetbriar Road Meadows SSSI.
- Histon (CB24 9AT): to the south, by ~1.5 km, there is the Histon Road SSSI; and to its southwest there are the Traveller's Rest Pit and the Madingley Wood SSSIs, by ~2.7 km and ~4.6 km, respectively.
- Harpenden (AL5 2JQ): to the northwest, by ~9 km, there is the Sherrardspark Wood SSSI.

Despite the proximity of the release sites to the above SSSIs, we consider that this multisite trial represents a minimal risk to any officially recognised biotopes to the protected areas because: (i) the trial sites are located in England within a sheltered area to wind (decreasing the chances of pollen dissemination), and (ii) the SSSIs are

not in the immediate vicinity if the trial sites (all the trial sites are >1 km distant to the closest SSSI).

Part 6 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.

The purpose of this release is to conduct a research field trial to evaluate the agronomic performance of gene-edited wheat lines in which wheat regulators of photosynthesis have been modified. The study aims to generate data under field conditions to assess the effects of these edits on plant growth and yield-related characteristics.

Phenotypic observations under glasshouse conditions showed consistent growth, development, flowering time, and fertility among individuals within each line and when compared to non-GM controls, except for the expected phenotype associated with the edits.

The plants generated in this study are intended for research purposes only. The results will inform future research and breeding strategies, including the potential development of transgene-free edited lines for crop improvement. No commercial use is proposed as part of this release. Material from this trial will not enter the food or feed chain.

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

We aim to commence trials in the Autumn (September/October) of 2026, with additional spring and autumn sowings over the following 7 years, with all plants harvested and removed by Autumn 2033.

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

Seeds will be drilled using conventional plot-scale farm equipment or by hand sowing where sowing is restricted by equipment size or capabilities.

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

The site will be prepared according to standard agronomic practices for wheat cultivation. The release will be monitored regularly during all stages of development and harvested at maturity.

Seeds from the GM and control plots will be conditioned, threshed and stored in

appropriate GM seed stores. Plant residue may be left on the trial area to decompose naturally. All other material 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 material will be in secure containers.

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

GM and control plots will be drilled at standard agronomic seed rates, dependant on the sowing date and quality of the seed. Typical spring sowing density ranges from 350-400 seeds/ m^2 while autumn wheat sowing density ranges from 300-350 seeds/ m^2. This is to achieve a target plant population of 250-300 plants per m^2.

Part 7 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

(a) See section 28 for information on wild relatives that are present in the area, noting that spontaneous crosses between these species and wheat have not been observed.

(b) Wheat is a self-pollinating crop with very low rates of cross-pollination with other wheat plants. Wheat can be forced, using laboratory techniques, to cross with rye, triticale and a limited number of other cereals, but spontaneous crossing in the field is extremely rare if it occurs at all. Nevertheless, the outer edge of the trial has a 3 m wide strip of non-GM wheat to function as a pollen barrier.

No wheat or sexually compatible cereals or weeds, including E. repens will be cultivated or allowed to grow within 20m from the trial.

Following the example of Consent 24/R57/01 and Variation of **Consent 22/R55/01** (22/05/2024), other GM crops that are the subject of separate consents or notifications may be grown simultaneously within the trial sites as long as a wheat pollen barrier of at least 3 metres width surrounding the GMOs, is sown on the same day as the GMOs, at the same sowing density as the GMOs, with a variety of similar growing characteristics (height, flowering time) within the perimeter of the plot.

The drills will be filled on the trial area and will be thoroughly cleaned before leaving the trial area. The grain obtained will be stored in appropriate seed storage facilities in a secure GM containment level 1 facility. All remaining plant material will be chopped and left on site to decompose naturally.

At drilling all care will be taken to ensure that no seed remains on the surface. Bird scaring devices including gas guns and hawk kites will be used to keep out birds during the growing season.

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 two cropping years.

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

At harvest, a sample of the plots will be collected with a plot combine to obtain yield measurements. The plant material sampled will be analysed on site at each trial site. All samples taken from the field will be closely monitored and records kept of weights and movements of grain and straw. All plant material samples removed from the trial site will eventually be destroyed by an approved technique. The remainder of the site will be harvested by either a commercial combine or the plot combine. The grain obtained will be stored in appropriate seed storage facilities on the site of the trial or in Wild Bioscience's containment level 2 laboratory.

The clean down of the combine will use a combination of compressed air as well as vacuuming with the vacuum being emptied into a bag before leaving the trial site. Because this is done on the trial area, any grain falling to the ground will be treated as a volunteer. Any waste collected will then disposed of in accordance with the hazardous waste disposal SOP. The trial area will remain in stubble for 1 year to enable monitoring of volunteers and a broad-spectrum herbicide such as glyphosate will be applied as required. After a year of stubble and volunteer control, a non-sexually compatible crop can be grown with the provision that any remaining wheat volunteers are able to be manually removed and/or chemically destroyed.

38. A description of monitoring plans and techniques.

The trial sites will be monitored regularly (on a weekly basis) for volunteers and weeds during the growing period and for two years after the termination of the trial. The soil will be treated by lightly tilling down to 5 cm depth to encourage volunteers; and when detected, these volunteer plants will be recorded before being destroyed

prior to flowering, either by hand-pulling or by application of herbicides.

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 wheat plots) 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 release site will be fenced to protect against animal damage and entry by unauthorised persons. Human access to the trial sites will be restricted to only those personnel who have been informed of the limitations and conditions of the consent. The release sites will be securely fenced. A sign will be posted indicating entry by unauthorised persons is prohibited.

The Rothamsted Research, JIC and NIAB trials teams have experience of previous GM field trials and the relevant management procedures at those sites. In addition, the Rothamsted Research has a movement-activated camera security system, and the trials team has a good working relationship with the local police, who will be informed of the trial and have experience of previous and current GM field trials at Rothamsted Research. GM inspectorate will have access to the trial sites on request.

Part 8: 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
DNA synthesis was provided by GenScript Biotech Corporation and Integrated DNA Technologies (IDT).

2. Molecular biology methods
Standard molecular biology reagents and methods were used as described in Sambrook et al. (1989).

3. Wheat transformation
Wheat transformation was performed at Wild Bioscience using *Agrobacterium tumefaciens*-mediated techniques as previously described (Ishida et al., 2015).

4. Molecular characterisation and genotyping
Probe-based digital PCR (dPCR) was performed in a multiplex format to detect the hygromycin resistance gene (hptII) alongside an endogenous reference gene, enabling copy number determination of the hygromycin target.

Screening for CRISPR/Cas9-induced genome edits was conducted using PCR followed by Sanger sequencing and/or next-generation amplicon sequencing of subgenome-specific amplicons generated with gene-specific primers for each homoeolog.

Primer design, genomic DNA isolation, and genotyping analyses were carried out at Wild Bioscience using established molecular biology protocols.

5. References

Griffin, W.B. (1987). Out-crossing in New Zealand wheats measured by occurrence of purple grain. *N.Z Journal of Agricultural Research* 30: 287-290.

Guadagnuolo, R., Savova-Bianchi, D., Keller-Senften, J. and Felber, F. (2001). Search for evidence of introgression of wheat (*Triticum aestivum* L.) traits into sea barley (*Hordeum marinum s.str.* Huds.) and bearded wheatgrass (*Elymus caninus*

Hucl, P. (1996). Out-crossing rates for 10 Canadian spring wheat cultivars. *Canadian Journal of Plant Science* 76: 423-427.

Ishida Y, Tsunashima M, Hiei Y, Komari T. Wheat (Triticum aestivum L.) transformation using immature embryos. Methods Mol Biol. 2015;1223:189-98. doi: 10.1007/978-1-4939-1695-5_15. PMID: 25300841

Martin, T.J. (1990). Out-crossing in twelve hard red winter wheat cultivars. *Crop Science* 30: 59-62.

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

Whaley, J.M., Kirby, E.J.M., Spink, J.H., Foulkes, M.j., Sparkes, D.L. (2004). Frost damage to winter wheat in the UK: the effect of plant population density. *European Journal of Agronomy* 21: 105-115.