Department for Environment, Food and Rural Affairs

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

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

Part I General information

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

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

The Rothamsted Farms team will carry out the trial and have extensive experience conducting GM field trials:
Head of Farms
Field Trials Manager
Farm Manager
Farm Staff

2. The title of the project.

Investigating altered agronomic performance of wheat through the expression of plant regulators of photosynthesis

Part II 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	N/A

(e) cultivar/breeding line Cadenza

(f) common name Common wheat / bread 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; and

The generation time is 20-25 weeks. For Cadenza (when sown as a winter-wheat type), one season is normally from September/October to August /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* and *Elytrigia* (formerly *Agropyron*) are present in the UK but there are no reports of wheat x *Agropyron* 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) & 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% (eg. 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.

N/A.

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 (Homoptera: Aphididae) species, the bird cherry-oat aphid, Rhopalosiphum padi, the grain aphid, Sitobion avenae, and the rose grain aphid, Metopolophium dirhodum, the orange wheat blossom midge, Sitodiplosis mosellana (Diptera: Cecidomyiidae) and 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 III Information relating to the genetic modification

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

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

11. The nature and source of the vector used.

The gene of interest sequence (described below) was cloned into vector pRLF12R1R2-SCV which is based on a super clean vector described in (Firek et al., 1993) and which contains the nptII (neomycin phosphotransferase II) gene under control of the Subterranean clover stunt virus Sc4 promoter (Schunmann et al., 2003). Derivatives of this vector are widely used for plant transformation, enabling the insertion of so-called t-DNA sequence fragment into a plant genome mediated by Agrobacterium tumefaciens.

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

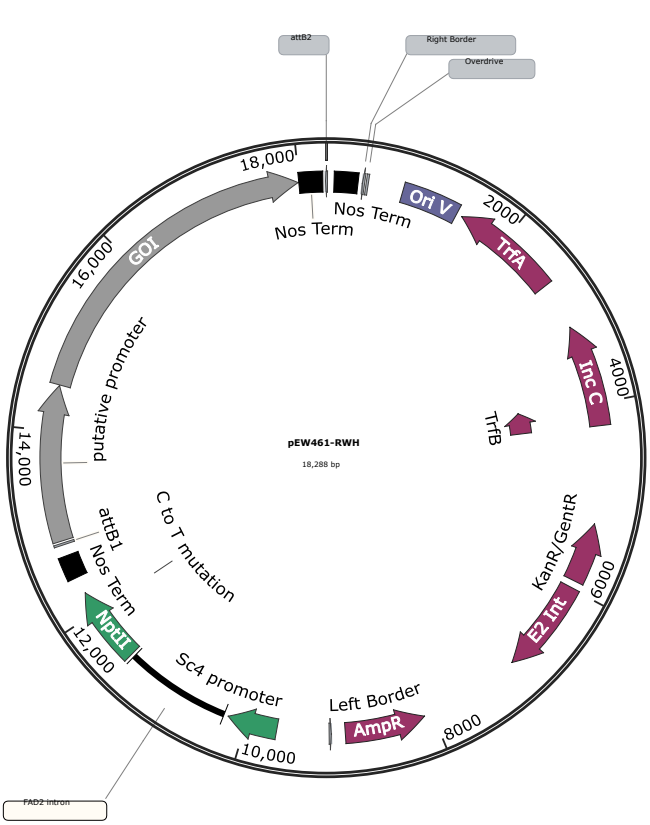
The gene of interest is derived from the wheat photoreceptor PHYTOCHROME B (PHYB) from *Triticum aestivum*. This version of PHYB is 99.8% identical to the native version, differing by two amino acid substitutions that are also found in the natural diversity of this gene. The PHYB gene was placed downstream of the Zoysia japonica PHOSPHOENOLPYRUVATE CARBOXYKINASE (PCK) promoter (Nomura et al., 2005). The intended function of this fragment is to boost PHYB activity in specific vegetative tissues of the plant, resulting in enhanced photosynthesis. The PCK promoter and PHYB fragment were both synthesized de novo and inserted into the plasmid described above. The t-DNA of the plasmid also contained the nptII kanamycin resistance gene downstream of the Sc4 promoter for selection of transformed plants, and the right and left t-DNA borders. Details on donor organisms for these gene fragments are given below.

pRLF12R1R2-SCV / PHYB

Element	Size (bp)	Donor Organism	Description and Intended Function
Left Border	23	*Agrobacterium tumefaciens*	T-DNA left border
Right Border	23	*Agrobacterium tumefaciens*	T-DNA right border
Sc4 promoter	532	*Subterranean clover stunt virus*	Promoter sequence from *Subterranean clover stunt virus*
FAD2 intron	1135	*Arabidopsis thaliana*	First intron of the *AtFAD2* gene. Known to enhance promoter efficiency in plants.
NptII	813	*Escherichia coli*	Bacterial selection gene conferring resistance to Kanamycin and other antibiotics
Nos Term	253	*Agrobacterium tumefaciens*	Nopaline synthase terminator
attB1, attB2	21, 21	-	Sequence elements for Gateway (recombination) cloning
Putative promoter (PCK)	1656	*Zoysia japonica*	Promoter sequence of the *Zoysia japonica* PCK gene
GOI (gene-of-interest: PHYB)	3501	*Triticum aestivum*	Coding sequence for the wheat *PHYB* gene to enhance leaf photosynthesis.

Map of pRLF12R1R2-SCV /

PHYB



Part IV Information relating to the genetically modified plant

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

One of the determinants of crop yield is the photosynthetic rate per unit leaf area. PHYB is a known photoreceptor and positive regulator of photosynthetic development in plants (Legris et al., 2016). Under controlled environment conditions, transgenic lines overexpressing PHYB under control of the *Zoysia japonica* PCK promoter have been shown to have enhanced leaf-level photosynthetic carbon assimilation, increased vegetative biomass, and an increase in both number and total mass of seeds per plants (unpublished data). This application seeks authority to investigate the effects of up-regulating the levels of PHYB in wheat plants in the field.

The plasmid used also contains the pSc4::nptII::tNOS cassette, which confers resistance to kanamycin but this was used only to select transgenic plants in the laboratory and this trait will not be utilized in proposed field trials.

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.

We propose to grow eight separate transgenic lines in the field trial, each transformed with the same gene construct. In addition, for each transgenic line we also proposed to grow the eight corresponding segregating wild type lines (null segregants). The grain is the so-called T2 generation of the transformation events. Plants were transformed using Agrobacterium tumefaciens, therefore, all transformation events will result in a nuclear localization for the transgenes.

Apart from the expected phenotype of PHYB expression and enhanced photosynthesis and productivity in controlled environments (unpublished data), these plants are indistinguishable from untransformed controls. No other changes to the plant morphology or development are apparent.

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.

All plants expressing the transgene are morphologically indistinguishable from untransformed controls. The inheritance of the transgene over two generations follows normal rules of Mendelian genetics. The insert appears to be stably integrated into the genome.

Part IVA Information on specific areas of risk

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

It is expected that the PHYB-overexpressing 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. Enclosing the whole site will be a deer-proof fence (with lockable double gates) to prevent the entry of rabbits and other large mammals including unauthorised humans.

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,

N/A.

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,

PHYB occurs naturally in all plants and is a key photoreceptor involved in light-signalling during plant development. There appears to be no published toxicity or allergenicity data for PHYB 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 differ from conventional Cadenza plants in that their leaves create additional quantities of a wheat protein that is already abundant throughout the entire wheat plant. This has led to higher seed biomass in controlled environments. The plants also contain a widely used selectable marker (nptII) although that will not be utilised during these field trials. Neither 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.

PCR using primers specific for this PHYB sequence (which lacks introns, compared to the native version already present in the genome) and nptII genes.

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

None.

Part V Information relating to the site of release

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

The area for the proposed field trial, including control and spacing between GM plots, will cover up to 500 m^2. It will be sited within the fenced area used for previous GM experiments in the farm at Rothamsted Research, Harpenden, UK.

It will comprise approximately 32 3m x 1m plots (~100 m^2) planted with the PHYB-overexpressing transgenic lines described plus 32 3m x 1m plots of controls where the transgene has been segregated out of the genome (an additional ~100 m^2). Each plot will be separated from each other by at least 0.5m and from the edge of the trial. The outer edge of the trial has a barrier of non-GM wheat to function as a pollen barrier. No cereals or grass species will be cultivated or allowed to grow for a further 20m from the outer edge of the site. Enclosing the whole site will be a deer-proof fence (with lockable double gates) to prevent the entry of rabbits and other large mammals including unauthorised humans.

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

The release site is an agricultural area forming part of an 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.

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 in the UK are in the

genera *Elymus* and *Elytrigia* (formerly *Agropyron*) although there are no reports of cross-hybridisation between wheat and these genera. The two most common inland species are *Elytrigia repens* (common couch = *Agropyron repens*) and *Elymus caninus* (bearded couch = *Agropyron caninum*). Other related species, such as *Elytrigia juncea* (Sand couch = *Agropyron junceum*), *Elytrigia atherica* (Sea couch = *Agropyron pycnanthum*) and hybrids are largely confined to coastal habitats.

E. repens is common on the Rothamsted estate whereas *E. caninus* is less common and is confined to woods and hedgerows. *E. repens* propagates primarily by vegetative reproduction (rhizomes), rather than by sexual reproduction, and in any case, no reports of wheat x *Elytrigia* or *Elymus* spontaneous hybrids have been reported. *E. repens* will be controlled along with other weeds in and around the trial site using standard farm practices. No wheat or other cereals, 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.

There are no protected areas near the trial site.

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 investigate the effect of increased expression of a photosynthetic regulator on agronomic endpoints of wheat. The results would inform future efforts to develop high yielded wheat seed products.

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

We would commence trials Spring 2023, with additional spring and winter planting over the following five years, finishing with all plants harvested and removed Autumn 2027.

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

Seeds will be drilled using conventional plot-scale farm equipment.

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. 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 plants will be sown in 3m x 1m blocks. Each block will be drilled at approximately 300 seeds / square metre. The control plots will be sown with seeds that are descended from GM plants where the transgene has been segregated out of the genome, of the same variety as the GM plots.

Up to four rows will be drilled, each row containing up to 16 plots (8 GM experimental plots containing the gene of interest, 8 containing suitable azygous segregating controls). Within row spacings of at least 0.5m, between row spacing of at least 1m. The outer edge of the trial has a barrier of non-GM wheat to function as a pollen barrier.

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

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

(2) In sub-paragraph (1) “plant species” means-

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

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 in the UK are in the genera *Elymus* and *Elytrigia* (formerly Agropyron). The two most common inland species are *Elytrigia repens* (common couch = *Agropyron repens*) and *Elymus caninus* (bearded couch = *Agropyron caninum*). Other related species, such as

Elytrigia juncea (Sand couch = *Agropyron junceum*), *Elytrigia atherica* (Sea couch = *Agropyron pycnanthum*) and hybrids are largely confined to coastal habitats.

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The drills will be filled on the trial area and will be thoroughly cleaned before leaving the trial area. kanamycin will not be used in the trial. The grain obtained will be stored in appropriate seed storage facilities in our containment level 2 laboratory. All straw will be chopped and left on site. 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 grain 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 grain and straw. All small 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 in our containment level 2 laboratory. The combine will be cleaned within the GM Compound prior to leaving the site so that all traces of gm plant material will remain in the trial area. The tractor will be driven onto a plastic sheet so that all the material that is removed can be collected in the sheet, bagged securely, and labelled, then disposed of in accordance with the hazardous waste disposal SOP. 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 and after the termination of the trial. 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 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 field trial centre has a good working relationship with the local police who will be informed and have experience of previous and current GM field trials at Rothamsted Research.

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 provided by GENEWIZ UK https://www.genewiz.com/en-GB/
2. Standard molecular biology reagents and methods were used following (Sambrook J et al., 1989)
3. Wheat transformation was performed at the National Institute of Botany (NIAB: https://www.niab.com/) using *A. tumefasciens*–mediated techniques as previously described (Ishida et al., 2015).
4. Transgene copy number and zygosity testing was provided via quantitative (Taqman) PCR and performed by NIAB.
5. All additional analyses were performed by the wild bioscience team.