APPLICATION FOR CONSENT TO RELEASE A GMO – HIGHER PLANTS

PART A1: INFORMATION REQUIRED UNDER SCHEDULE 1 OF THE GENETICALY 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.

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

2. The title of the project.

Investigating altered agronomic performance of wheat

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,

(ii) any specific factors affecting reproduction,

(iii) generation time; and

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

ai) 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).

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

aiii) The generation time is 20-25 weeks. For Cadenza (sown as a spring-wheat type), one season is normally from March/April to August /September.

b) 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.

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 the United Kingdom, 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 by microprojectile bombardment Sparks & Jones (2014).

The gene of interest was precipitated onto gold particles along with a vector containing a *bar* gene selectable marker cassette and co-bombarded into scutella of immature zygotic 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 was carried on a binary vector pBract302 (www.bract.org) to give pBract302/SBPase. This was co-transformed with pAHC20 containing the *bar* gene under the control of the Ubiquitin1 promoter sequence (Christensen and Quail 1996). Both were prepared in *E. coli* Invitrogen DH5α sub-cloning-efficiency competent cells (Genotype F- φ80lacZΔM15 Δ(lacZYA-argF)U169 recA1 endA1 hsdR17(rk-, mk+) phoAsupE44 thi-1 gyrA96 relA1 λ-). Plasmids were purified using a Qiagen plasmid purification Midi kit.

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

A sequence encoding the enzyme sedoheptulose-1,7-biphosphatase (SBPase) was cloned from the model grass *Brachypodium distachyon* and inserted into a pBract302 vector between the rice tungro bacilliform virus promoter (RTBVP) and 35S terminator. The intended function of over-expressing SBPase is to increase regeneration of ribulose 1,5 bisphosphate (RuBP), the substrate for RuBisCO. The plasmid also contained the *nptI* kanamycin resistance gene for selection of bacteria and the *bar* gene for phosphinothricin resistance under control of the maize ubiquitin1 promoter + intron for plant selection. The pBract302 plasmid carries right and left T-DNA border

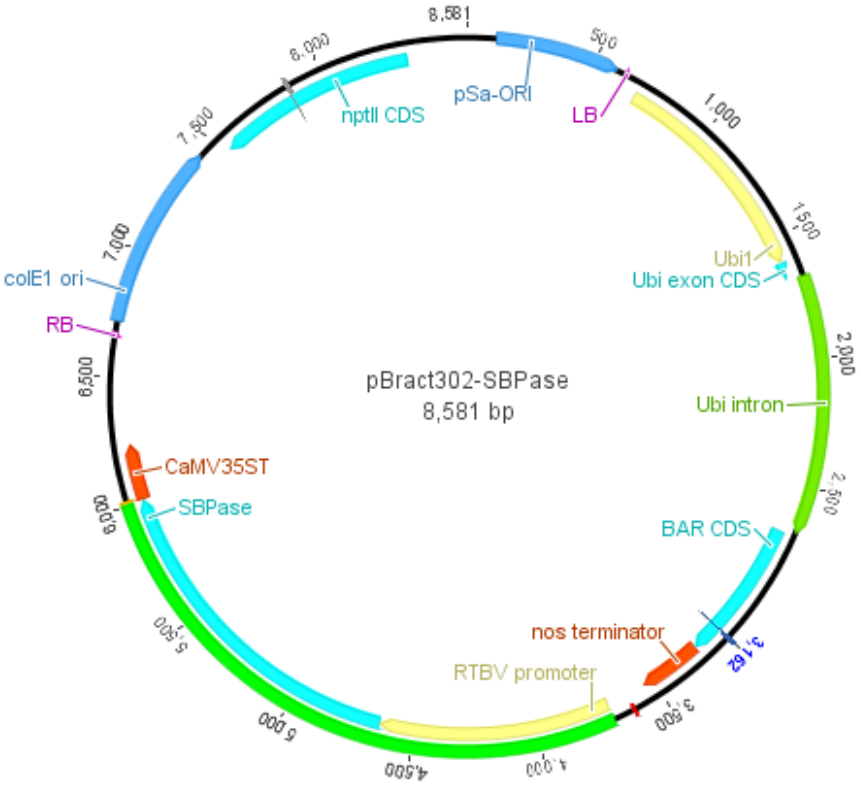
sequences, origins of replication and bacterial selectable marker genes necessary for maintenance in *E.coli* and *Agrobacterium*. A plasmid possessing a *bar* cassette under the control of the maize ubiquitin1 promoter + intron for plant selection was also co-bombarded.

pBract302/SBPase

Element	Size	Donor Organism	Description and Intended Function
ColE1	724bp	*E.coli*	Origin of replication for plasmid replication in *E. coli*
pSa-Ori	484bp	*Agrobacterium tumefaciens*	Origin of replication for plasmid replication in *Agrobacterium*
nptI (aph(3')-Ia)	812bp	*E.coli*	Bacterial selection gene conferring resistance to Kanamycin and other antibiotics
RB	25bp	*Agrobacterium tumefaciens*	T-DNA Right border
LB	24bp	*Agrobacterium tumefaciens*	T-DNA Left border
RTBVP	939bp	Rice tungro bacilliform virus	Promoter sequence from rice tungro bacilliform virus
SBPase	1367bp	Brachypodium distachyon	Coding sequence for expression of sedoheptulose-1,7-biphosphatase
CaMV35ST	233bp	Cauliflower mosaic virus (CaMV)	Terminator of the 35S viral transcript
Ubi+intron	1991bp	*Zea mays*	Maize ubiquitin 1 promoter + first intron driving constitutive expression in wheat
Bar coding sequence	581bp	*Streptomyces hygroscopicus*	Plant selectable marker gene encoding phosphinothricin acetyltransferase conferring resistance to herbicides with active ingredient glufosinate ammonium. (N.B. *Bar* gene inefficient/inoperative in this plasmid)
nos T	257bp	*Agrobacterium tumefaciens*	Nopaline synthase terminator

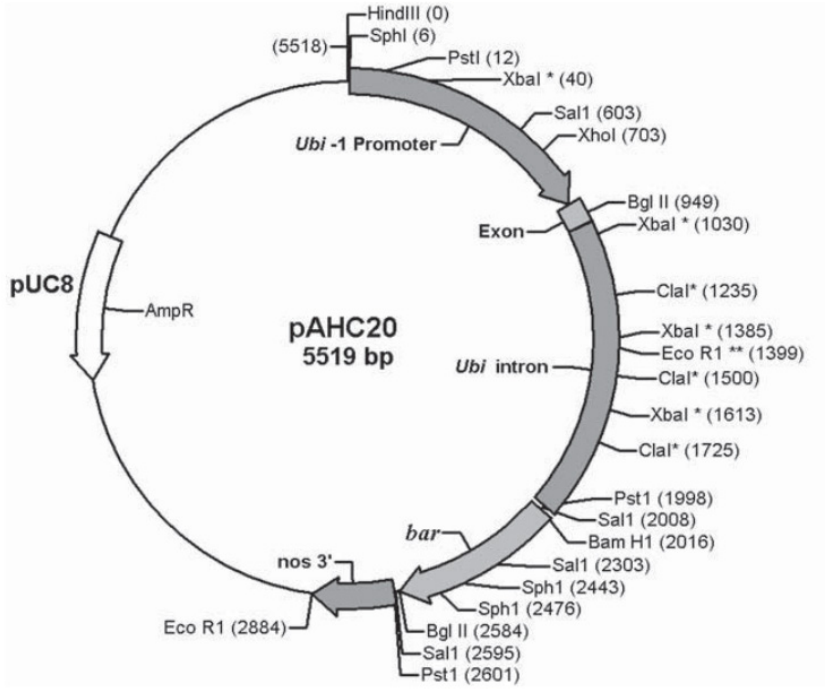
pAHC20UbiBar

Element	Size	Donor Organism	Description and Intended Function
ColE1	619bp	*E.coli*	Origin of replication for plasmid replication in *E. coli*
bla (beta-lactamase)	930bp	*E.coli*	Bacterial selection gene conferring resistance to ampicillin
Ubi+intron	1992bp	*Zea mays*	Maize ubiquitin 1 promoter + first intron driving constitutive expression in wheat
Bar coding sequence	541bp	*Streptomyces hygroscopicus*	Plant selectable marker gene encoding phosphinothricin acetyltransferase conferring resistance to herbicides with active ingredient glufosinate ammonium.
nos T	250bp	*Agrobacterium tumefaciens*	Nopaline synthase terminator for gene of interest



Map of pBract302/SBPase

Map of pAHC20



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. SBPase is a key enzyme in the Calvin cycle and is pivotal in the regeneration of Ribulose-1,5-bisphosphate (RuBP) and the dephosphorylation of sedoheptulose - 1,7-biphosphate (SBP) to sedoheptulose-7-phospate (S7P) (Poolman et al., 2000; Zhu et al., 2007; Raines, et al., 2011). It is also vital for maintaining the balance between carbon leaving the Calvin cycle and that necessary for RuBP regeneration (Raines, 2003). Transgenic lines with increased SBPase protein levels and activity grown under greenhouse conditions showed several significant differences compared to controls including; enhanced maximum carboxylation efficiency of Rubisco (Vc,max) and regeneration of RuBP via photosynthetic electron transport (Jmax), increased vegetative biomass, and an increase in both number and total mass of seeds per plant (Raines / Parry, *pers comm*). This application seeks authority to investigate the effects of up-regulating the levels of SBPase in wheat plants in the field.

The plasmids used also contain the Ubi1::bar::nos cassette which confers resistance to herbicides with active ingredient glufosinate ammonium but this was used only to select transgenic plants and this trait will not be utilised 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 include two GM events of the same gene constructs in the field trial. Event Sox44 contains two copies of the pBract302/SBPase plasmid per haploid genome, as determined by quantitative (Taqman) PCR performed on genomic DNA by iDNA Genetics (Norwich, UK) using regions of the coding sequence as primers/probe. Event Sox23 contains 6 copies per haploid genome of plasmid pBract302/SBPase. Segregation analysis using PCR of genomic DNA indicates that in both lines, all the gene insertions are carried in the chromosomal DNA and stably inherited as a single genetic locus. Both events selected for field trial are homozygous.

We have not analysed the position or the structure of the insertion nor sequenced the flanking genomic DNA. Apart from the expected phenotype of enhanced SBPase expression and, under glasshouse growth conditions, increased total biomass and dry seed yield (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) 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 SBPase and *bar* genes are under the transcriptional control of the rice tungro bacilliform virus promoter and maize Ubi1 promoter + intron respectively. Both are known to give broadly constitutive expression in wheat. However, it is known that the RTBV promoter is not active in pollen or root tissue of wheat.

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.

Except for the enhanced expression of SBPase which is present in all wheat varieties and de-novo expression of the *bar* gene, all aspects of the phenotype of events Sox23 and Sox44 including morphology, pollination and seed-set appear to be identical to non-transgenic control wheat plants. We would expect dissemination of pollen and seeds to be the same as for non-transgenic wheat plants. The survivability of these plants in unmanaged systems may be affected by their enhanced photosynthesis. In addition, these plants possess the ability to tolerate glufosinate-based herbicides which would increase their survivability in environments where these herbicides were the only ones used.

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

We have not specifically investigated genetic or phenotypic stability of these lines but all plants expressing the transgene are morphologically indistinguishable from untransformed controls. The inheritance of the transgene over three generations follows normal rules of Mendelian genetics.

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

It is expected that the events Sox23 and Sox44 would 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. A 3m wheat pollen barrier will completely surround the outer separator strip (see Figure in Section 34). Enclosing the whole site will be a 2.4m high chain-link fence (with lockable double gates) to prevent the entry of rabbits and other large mammals including unauthorised humans.

Both the plasmids used possess a bacterial origin of replication and antibiotic resistance and we have assumed that these are integrated into the plant genomic DNA along with the genes of interest. These elements may increase the rates of horizontal gene transfer and establishment in soil bacteria because they provide a theoretical mechanism for homologous recombination and selection (if aminoglycoside antibiotics are present). However, we estimate the rate of horizontal gene transfer is low and, if it were to occur, these genetic elements are already present in bacteria and in soil microbes in particular.

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

SBPase occurs naturally in all plants and is a key enzyme of the Calvin cycle. There appears to be no published toxicity or allergenicity data for SBPase but 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.

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.

None of the plant material from the field trial will enter the human food- or animal feed-chain.

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

Not applicable. There are no target organisms.

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

None.

23. The potential interactions with the abiotic environment.

None.

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

PCR using primers specific for SBPase and *bar* genes.

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

None.

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.

The area for the proposed field trial, including controls and spacing between GM plots will cover 13.5m x 18m. It will be sited within the fenced area used for previous GM experiments in the farm at Rothamsted Research, Harpenden, UK and at grid reference TL121131. It will comprise eight 1.8 x 6m plots (86.4 m2) planted with events Sox23 or Sox44 plus four 1.8m x 6m plots of non-transgenic controls. Each plot will be separated from each by 0.5m and from the edge of the trial by a wheat pollen barrier of at least 3m which will completely surround the outer perimeter of the trial (see Figure in Section 34). No cereals or grass species will be cultivated or allowed to grow for a further 20m from the outer edge of the pollen barrier. Enclosing the whole site will be a 2.4m high chain-link 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 SBPase on agronomic endpoints of wheat.

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

Spring/ summer 2017 and 2018. The plants will be sown in March/April 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.

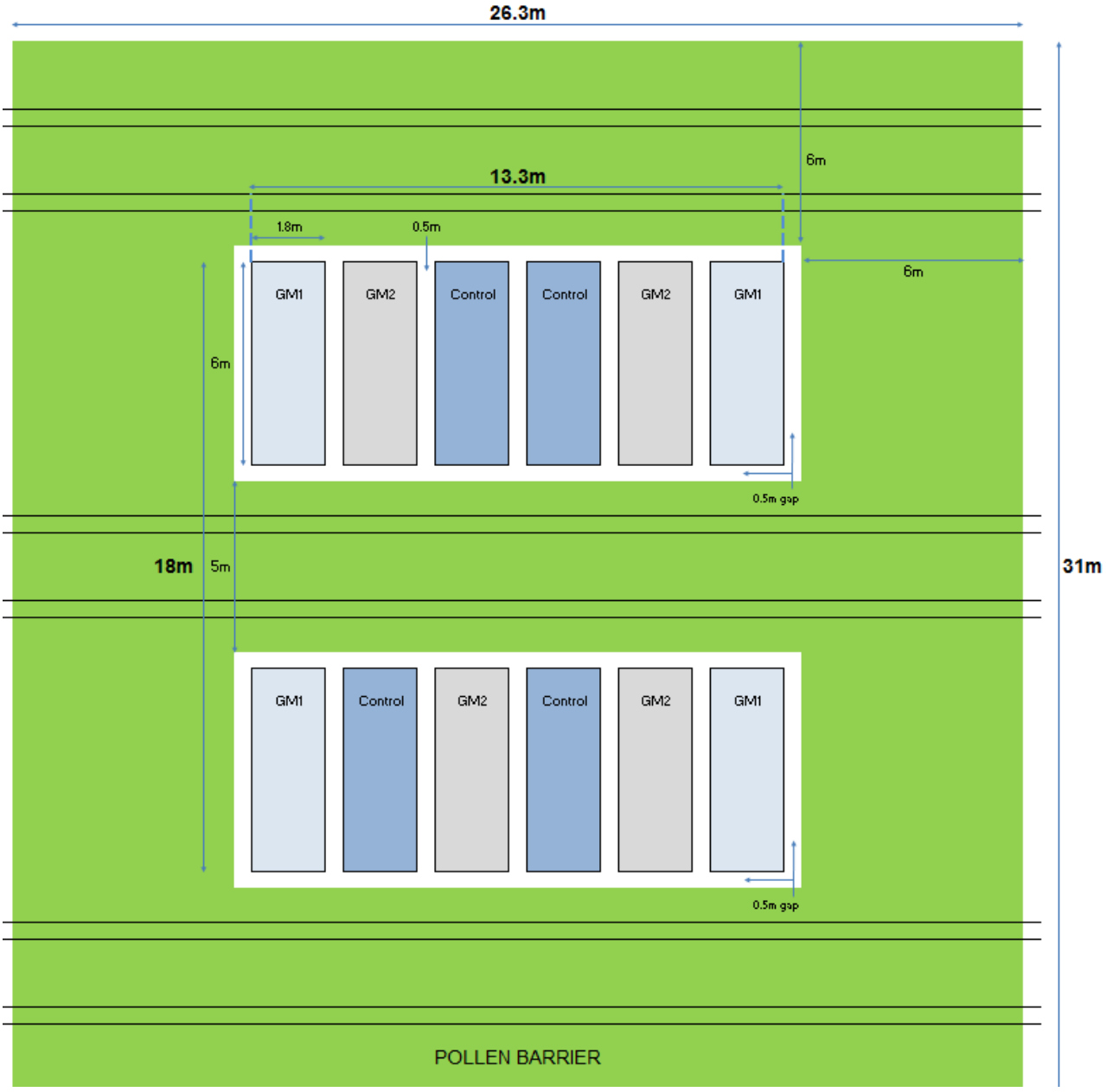
33. The method for preparing and managing the release site, prior to, during and after the release, including cultivation practices and harvesting methods.

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

Some seeds from the GM and control plots will be conditioned, threshed and stored in appropriate GM seed stores. All other material, including that from the pollen barrier rows 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. GM and control plants will be sown in four blocks. Each block will contain 2 GM plus 1 control plot each of 1.8m x 6m. The control plots and surrounding guard row will be sown with non-GM wheat of the same variety as the GM plots. Planting density will be approximately 300 seeds per m^2.



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.

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.

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. The outer edge of the trial has a 3m barrier of non-GM wheat to function as a pollen barrier (see Figure in Section 34). No wheat or other cereals, including *E. repens* will be cultivated or allowed to grow within 20m from the trial.

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

The outer edge of the trial has a 3m barrier of non-GM wheat 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. Glufosinate-based herbicide will not be used in the trial. A sample of plants will be hand-harvested, conditioned and threshed to supply seeds for future trials or other research purposes. The remaining grain obtained will be disposed of in deep landfill using an approved contractor. 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 cropping year.

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 disposed of in deep landfill using an approved contractor. All straw will be chopped and left on site. The combine will be cleaned in the empty half 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 (Mar-Aug/Sept) 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 wheat plots, and pollen barrier rows) 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.

We have 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. The trial will be contained within the approximately 3ha trial site which protected by a 2.4m high chain-link fence (with lockable double gates) and has a movement-activated camera security system.

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 GenScript Inc. USA http://www.genscript.com/index.html

2. Standard molecular biology reagents and methods were used following Sambrook et al., (1989).

3. Wheat transformation was performed using biolistics as described in Sparks and Jones, (2014)

4. Transgene copy number and zygosity testing was provided via Taqman PCR by iDNA Genetics Norwich UK. http://www.idnagenetics.com/

References

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PART A2: DATA OR RESULTS FROM ANY PREVIOUS RELEASES OF THE GMO

Events containing these genes have not previously been released.

PART A3: DETAILS OF PREVIOUS APPLICATIONS FOR RELEASE

Rothamsted Research has received previous consents to release GM wheat:

97/R8/3, 01/R8/4, 11/R8/01, 14/R8/01

PART A4: RISK ASSESSMENT AND A STATEMENT ON RISK EVALUATION

Summary

Observations on the general plant morphology of glasshouse-grown plants, timing of flowering, fertility, seed shape and germination show that the two GM wheat events Sox23 and Sox44 are indistinguishable from their non-GM equivalents except for the expected phenotype of enhanced SBPase expression and, under glasshouse growth conditions, increased total biomass and dry seed yield (unpublished data). No other changes to the plant morphology or development are apparent. The gene donor organisms (*Brachypodium distachyon* and *Streptomyces hygroscopicus*) are not known to be pathogenic or allergenic and neither the gene under investigation, nor the selectable marker genes are expected to result in the synthesis of products that are harmful to humans, other organisms or the environment. Any unknown hazards arising from the expression and ingestion of foreign proteins will not be realised because the wheat plants will not be consumed by humans.

The probability of seeds escaping from the trial site or the transfer of inserted characteristics to sexually-compatible species outside the trial area is estimated as very low. Commercial wheat varieties do not establish easily or thrive in uncultivated environments and are naturally self-pollinating with out-crossing being a rare event. Wheat seeds are relatively large and not normally dispersed by wind. Management measures including netting when the wheat is in ear and the use of gas guns and hawk kites will be

employed to mitigate the risk of seed removal by birds. Management procedures to minimise the spread of seeds or pollen will further reduce the probability of these events occurring. There will be no cereals grown for 20 metres from the boundary of the experimental plots and no sexually-compatible wild relatives of wheat exist in the vicinity. If out-crossing to plants outside the trial area where to somehow occur, selection pressure to maintain the genes in the environment would exist only where glufosinate-based herbicides were applied. Even if the up-regulation of SBPase resulted in significantly enhanced photosynthesis, the chances of successful establishment of these wheat plants in unmanaged ecosystems is extremely low.

The risk of non-sexual, horizontal gene transfer to other species is extremely low. In the event of horizontal gene transfer to bacteria, neither the trait gene nor the selectable marker genes would be expected to confer a selective advantage in the field environment under consideration. The plasmid backbone sequences, *nptI* gene, origins of replication, border sequences etc. come originally from *E coli* and *Agrobacterium tumefaciens*, two common gut and soil bacteria respectively and these sequences are already widespread in the soil metagenome. Although this makes potential homologous recombination events more likely, we estimate the likelihood of horizontal gene transfer as low and the consequences, were it to occur, as negligible. The area proposed to be planted with GMOs is small; eight 1.8m x 6m plots (total area 86.4m^2) and temporary (lasting between 5 and 6 months).

Although the above-ground plant material will be cleared from the site, the *nptI* gene contained in the plant root DNA will decompose into the soil. The transgene is fully integrated into the plant DNA and the copy number is low thus the *nptI* gene represents a very small proportion (much less than one millionth) of the total DNA in any one cell of our transformed wheat plants. This excess of competing DNA will significantly dilute the rate of any *nptI* natural bacterial transformation. In addition, enzymatic degradation of free plant DNA in the soil and the low level of spontaneous bacterial competence to take up free DNA will significantly reduce the incidence of natural transformation. Although the transfer of functional gene units from plants to soil bacteria is accepted to be extremely low under natural conditions (Schluëter et al 1995, Nielsen et al 1997, EFSA, 2009), it cannot be completely discounted that some bacteria may successfully take up the *nptI* gene. However, there will be no antibiotics applied to the soil to provide additional selection pressure for the gene to persist in the environment. The source of the *nptI* gene is the gut bacterium *E. coli* carrying a plasmid containing the transposable element (Tn 903). R plasmids possessing resistance to aminoglycoside antibiotics are already naturally found in the soil and other environments. The *nptI* gene encodes the enzyme Aminoglycoside 3'-phosphotransferase which confers resistance to kanamycin and related aminoglycoside antibiotics. Although these antibiotics still have some clinical applications, alternatives are readily available. Taken together, and bearing in mind the limited scope of this trial, the risk of generating of any additional antibiotic resistance within the soil microbial community or risks to human health or the environment if this were to occur as a result of the proposed trial is considered to be extremely low.

The overall risk of harm to human health or the environmental arising from this trial is assessed as *very low*.

Detailed evaluation of hazards, magnitude of exposure and management strategies to minimise risk.

We adopted a classic six-step process of risk assessment. Systematic identification of all potential hazards arising from this field trial; evaluation of hazard-realisation in the specific field-trial environment; potential for harm; frequency of exposure; mitigation of risk by appropriate management and finally, an estimate of the overall risk.

Step1: ***Potential hazards which may be caused by the characteristics of the novel plant***	**Step 2:** ***Evaluation of how above hazards could be realised in the receiving environments***	**Step 3:** ***Evaluation the magnitude of harm caused by each hazard if realised***	**Step 4:** ***Estimation of how likely/often each hazard will be realised as harm***	**Step 5:** ***Modification of management strategies to obtain lowest possible risks from the deliberate release***	**Step 6:** ***Overall estimate of risk caused by the release***
Increased invasiveness in natural habitats or persistence in agricultural habitats due to inserted trait.	Increased invasiveness may arise from intended or unintended effects of the genetic modification that resulted in wheat plants with a more 'weedy' habit that are better able to establish and thrive in uncultivated environments or to persist in agricultural habitats.	Wheat is an annual species that requires active management to out-compete weedier plants. Left unmanaged, wheat does not establish and survive in nature and thus has a low base line of invasiveness and persistence. Even if intended or unintended effects of the genetic modification resulted in major changes in invasiveness or persistence, it is considered that this would not result in significant environmental harm for agricultural or unmanaged ecosystems. Wheat is a benign plant that can be easily managed by cultivation or herbicides. The magnitude of harm if the hazard was realised is considered to be very small.	It is highly unlikely that intended or unintended effects of the genetic modification will result in major changes in invasiveness or persistence. If it were to occur, this hazard would be realised only if seeds or pollen possessing genes encoding these traits were to spread from the trial site and successfully become established elsewhere. This is very unlikely as wheat pollen is relatively heavy so does not travel far, it has a short half-life and there are no sexually compatible species for out-crossing for at least 20m from the trial site. Seed removal from the site will be rigorously managed (see step 5). The chances of modified wheat plants establishing themselves outside the trial site are negligible.	Harvested seeds will be transported from the site in sealed containers. Machinery will be cleaned thoroughly prior to removal from the site. There is a large buffer zone to minimize the spread of pollen. Surrounding the trial site is a 20 metre area in which no cereals will be grown so it will be easy to see any cereal plants in the surrounding area. Appropriate physical barriers and/or deterrents will be employed to minimise access by large mammals and birds. Glufosinate herbicides will not be used on the trial site.	Overall risk is negligible.
Selective advantage or disadvantage conferred to wheat or other sexually compatible plant species.	Selective advantage or disadvantage may result from the intended traits (improved photosynthetic rates and tolerance to glufosinate	The basal ability for commercial cereal crop varieties to survive in uncultivated environments is very low. We anticipate that the conferred trait of improved photosynthetic rates will provide only minor selective advantage compared to other factors determining a plant's ability to survive in unmanaged ecosystems. The genetic modification resulting in increased	This hazard would be realised only if seeds or pollen possessing genes encoding these traits were to spread from the trial site and successfully become established in environments were the appropriate selection pressures were present. This is very unlikely as wheat pollen is	Harvested seeds will be transported from the site in sealed containers. Machinery will be cleaned thoroughly prior to removal from the site. There is a large buffer zone to minimize the spread of pollen. Surrounding the trial	Overall risk is very low.

	herbicides) or as a result of unintended effects of the genetic modification. These hazards could be realised in the receiving environment via dispersal of GM seeds from trial site to the surrounding environment or via out-crossing to sexually-compatible species outside trial site.	tolerance to glufosinate herbicides has the potential to confer a major selective advantage only where those herbicides are used routinely.	relatively heavy so does not travel long distances, it has a short half-life and there are no sexually compatible species for out-crossing for at least 20m from the trial site. Seed removal from the site will be rigorously managed. The use of glufosinate herbicides in the surrounding agricultural fields may be expected. Overall, the frequency of this hazard resulting in environmental harm is very low.	site is a 20 metre area in which no cereals will be grown so it will be easy to see any cereal plants in the surrounding area. Appropriate physical barriers and/or deterrents will be employed to minimise access by large mammals and birds. Glufosinate herbicides will not be used on the trial site.	
Potential effect on human or animal health due to introduced sedoheptulose -1,7-biphosphatase (SBPase)	By contact or ingestion of GM plant material.	Although there are no robust toxicity data available for SBPase, it is considered that the magnitude of harm caused by contact, inhalation or ingestion of these GM plants is negligible. SBPase is already consumed by humans and other animals when they eat leafy vegetables and other green plant parts. SBPase is a Calvin-cycle enzyme and as such is present in the cells of all photosynthetic plants. In the quantities produced by the GM plants, SBPase is not considered harmful. lower molecular weight compounds.	Some contact between the GM plants and humans or animals is expected. People operating farm machinery and scientists working in the trial site will come into physical contact with the plants. Small mammals such as mice, invertebrates and birds may also come into contact and/or ingest plant material.	No plant material from the trial will enter the food or animal feed chain. Appropriate physical barriers and/or deterrents will be employed to minimise access by large mammals and birds. Machinery will be cleaned before being removed from the trial site	Overall risk is very low.
Potential effect on human or animal health due to introduced phosphinothricin acetyl transferase (PAT)	By contact or ingestion of GM plant material.	The magnitude of harm caused by contact, inhalation or ingestion PAT in these GM plants is extremely low. The source organism for this gene (*Streptomyces hygroscopicus*) is ubiquitous in the soil and there have been no reports of its adverse effects on humans, animals or plants. The product of the *bar* gene, phosphinothricin acetyl transferase (PAT) has been evaluated on numerous occasions by EFSA and found	Some contact between the GM plants and humans or animals is expected. People operating farm machinery and scientists working in the trial site will come into physical contact with the plants. Small mammals, invertebrates and birds may also come into contact and/or ingest plant material.	No plant material from the trial will enter the food or animal feed chain. Appropriate physical barriers and/or deterrents will be employed to minimise access by large mammals and birds. Machinery will be cleaned	Overall risk is very low.

		to raise no safety concerns. For example: "The enzyme phosphinothricin acetyl transferase (PAT) is not likely to present safety problems. Its enzymatic function is specific to a substrate which is not naturally present in humans, namely phosphinothricin, and furthermore, it is degraded and inactivated in simulated gastric fluid containing pepsin at pH 1-1.2. It is therefore unlikely to retain any enzymatic activity in vivo. Furthermore, no sequence homology between the PAT protein and known toxins has been found. The native PAT protein (51% purity) has been tested for acute toxicity in mice and no toxicity has been reported at a dose of 5 g per kg body weight."		before being removed from the trial site	
Potential direct effect on human or animal health due to introduced neomycin phosphotransferase	By contact, inhalation or ingestion of GM plant material.	The magnitude of harm caused by contact, inhalation or ingestion of plant material containing NPTI is extremely low. The source organism for gene encoding this enzyme (*E. coli*) is present in the large intestine of healthy humans and any NPTI ingested is expected to be broken down by digestive enzymes in the stomach and small intestine. Although specific toxicity data on neomycin phosphotransferase I (also known as aminoglycoside 3'-phosphotransferase type 1) could not be found, there are several studies reported in scientific literature of the safety of a functionally related enzyme NPTII. For example, acute oral toxicity of NPTII was studied in mice that had received an oral dose of 100, 1000, or 5000 mg NPTII/kg bodyweight and subsequently monitored for adverse effects over the following seven days. The authors concluded that no treatment-related adverse health	The frequency of exposure is very low. The promoter driving expression of the *nptI* gene is prokaryote-specific so NPTI protein will not be present in the modified plants.	No plant material from the trial will enter the food or animal feed chain.	Overall risk is very low.

		effects had occurred (Fuchs et al., 1993).			
Consideration of the potential risk of the *nptI* gene becoming more prevalent in the soil as a result of the trial	By decomposition of plant root DNA into the soil and natural transformation of competent microbes that subsequently became established in the soil community.	Although the transfer of functional gene units from plants to soil bacteria is accepted to be extremely low under natural conditions (Schluëter et al 1995, Nielsen et al 1997, EFSA, 2009), it cannot be completely discounted that some bacteria may successfully take up the *nptI* gene. However, there will be no antibiotics applied to the soil to provide additional selection pressure for the gene to persist in the environment. The source of the *nptI* gene is the gut bacterium *E. coli* carrying a plasmid containing the transposable element (Tn 903). R plasmids possessing resistance to aminoglycoside antibiotics are already naturally found in the soil and other environments. The *nptI* gene encodes the enzyme Aminoglycoside 3'-phosphotransferase which confers resistance to kanamycin and related aminoglycoside antibiotics. Although these antibiotics still have some clinical applications, alternatives are readily available.	The transgene is fully integrated into the plant DNA and the copy number is low thus the *nptI* gene represents a very small proportion (much less than one millionth) of the total DNA in any one cell of our transformed wheat plants. This excess of competing DNA will significantly dilute the rate of any *nptI* natural bacterial transformation. In addition, enzymatic degradation of free plant DNA in the soil and the low level of spontaneous bacterial competence to take up free DNA will significantly reduce the incidence of natural transformation.	Seeds and other above-ground plant biomass will be harvested and removed from the site. No antibiotics will applied to the soil to provide additional selection pressure for the gene to persist in the environment.	The risk of generating of any additional antibiotic resistance within the soil microbial community is considered to be very low.
Potential effects on human or animal health due to horizontal gene transfer of recombinant DNA	By contact, ingestion or infection with bacteria that had received recombinant DNA via horizontal gene transfer.	The magnitude of harm caused by contact, ingestion or infection with bacteria that had received the recombinant DNA via horizontal gene transfer is low. The SBPase gene is not expected to be expressed in bacteria and would have no safety concern if they were. Horizontal gene transfer of a complete *nptI* fragment could confer functional antibiotic resistance to receiving bacteria. Some aminoglycoside antibiotics including kanamycin are important for clinical treatment, especially for second line treatment for multi-resistant tuberculosis (kanamycin) and in gut	The rate of horizontal gene transfer from genetically modified plants to other species is accepted to be extremely low (EFSA, 2009). However, the presence of plasmid backbone sequence and origins of replication which are derived from *E. coli* and *Agrobacterium tumefaciens*, increase the chances of homologous recombination between plant and microbial DNA in the soil. If recombinant DNA were to move by horizontal transfer to soil	No plant material from the trial will enter the food or animal feed chain. No antibiotics will be applied to the soil to give selective advantage.	Overall risk is very low.

		irrigation in, for example, encephalopathy (neomycin). However, this resistance is already widespread in the environment. The source of the *nptI* gene is the gut bacterium *E. coli* carrying a plasmid containing the transposable element (Tn 903). R plasmids possessing resistance to aminoglycoside antibiotics are already widespread in the soil.	bacteria, it is unlikely to significantly increase the prevalence of resistance to aminoglycoside antibiotics in the environment. The area proposed to be planted with GMOs is small; a total of eight 1.8x6m plots and temporary (lasting between 5 and 6 months).		
Consideration of the risk of horizontal gene transfer into wild-type *Agrobacterium* species in the soil that could infect and transfer DNA to other plant species including risks associated with expression of the genes.	By DNA released from decomposing plant material being taken up into the T-DNA of wild-type *Agrobacterium* and the subsequent expression of functional cassettes in other plants after natural transformation by *Agrobacterium*.	In the very unlikely event that functional *bar* and SBPase cassettes were integrated and expressed in transformed plant cells that subsequently led to production of functional PAT or SBPase, it is theoretically possible that this may enhance the fitness of the transformed cells in these plants but only if the appropriate environmental selection pressures were present.	Horizontal gene transfer between plants and wild-type *Agrobacterium* species, and the subsequent infection of other plant species with recombinant DNA is considered an exceedingly small risk. Although transformation of wild type *Agrobacterium tumefaciens* has been reported in laboratory experiments using pre-inoculated sterile soil and high concentrations of circular Ti plasmid with appropriate antibiotic selection (Demanèche et al 2001), no such demonstration has been reported in the field or with linearised plant DNA with or without selection. Even in optimised laboratory conditions, electroporation or freeze-thaw methods are required to effectively transform *Agrobacterium spp* (Holsters 1978, Mattanovich et al 1989). It is considered highly unlikely that free DNA liberated by degradation of GM wheat roots in the soil would become stabilised in wild-type *Agrobacterium* and capable of autonomous replication. This could theoretically occur if the	This risk will be managed by controlling weeds and not spraying with glufosinate ammonium-based herbicides. Seeds and other above-ground plant biomass will be harvested and removed from the site.	The risk of this is extremely low

			transgene insert liberated by decomposing roots was taken up by wild type *Agrobacterium* either as an intact plasmid or as a DNA fragment and subsequently incorporated into the resident Ti plasmid by for instance, homologous recombination. The former would stabilise only if the host *Agrobacterium* cell shared the same IncR compatibility group as the pSa origin of the transgene vector used in this trial. In the unlikely event that intact *bar* or SBPase cassettes are recombined into the T-DNA region of a virulent *Agrobacterium* Ti plasmid, this homologous recombination event would inevitably result in all or part of the oncogene set on the T-DNA being lost. Thus, even if this modified *Agrobacterium* successfully infected and transferred its T-DNA to wounded plant tissue, it is highly unlikely that a crown gall or hairy root phenotype would form. Whether disease symptoms were evident or not, the plant cells transformed by this wild-type *Agrobacterium* cell would be vegetative not germ-line so no vertical gene transfer of this recombinant DNA is possible. In addition, the *bar* and SBPase genes are under the transcriptional control of the maize *Ubi*1 promoter thus, only cereals or species compatible with this promoter are likely to show expression of these genes.		

Potential effects on biogeochemical processes (changes in soil decomposition of organic material	Changes in biogeochemical processes may result from unintended changes in the modified plants or from unintended changes in soil microbes due to horizontal transfer of DNA.	The magnitude of harm is estimated to be extremely low. Biogeochemical processes are not expected to be affected by the cultivation of the genetically modified plants.	The frequency of changes to biogeochemical processes is considered to be very low. The area proposed to be planted with GMOs is small; a total of eight 1.8x6m plots and temporary (lasting between 5 and 6 months).	None.	It is very unlikely that changes in biogeochemical processes would occur.
Possible environmental impact due to changes in cultivation practice	This modification may result in higher yields.	The magnitude of any changes due to changes in cultivation practice will be negligible.	The frequency that this hazard may be realised is low. The number of plants with higher SBPase expression is small (eight 1.8x6m plots) and will be sown for only one growing season (between 5 and 6 months).	None.	Overall risk negligible.

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PART A5: ASSESSMENT OF COMMERCIAL OR CONFIDENTIALITY OF INFORMATION CONTAINED IN THIS APPLICATION.
Identify clearly any information that is considered to be commercially confidential. A clear justification for keeping information confidential must be given.

This is publically funded research and has no associated commercial confidentiality considerations.

PART A6: STATEMENT ON WHETHER DETAILED INFORMATION ON THE DESCRIPTION OF THE GMO AND THE PURPOSE OF RELEASE HAS BEEN PUBLISHED
Make a clear statement on whether a detailed description of the GMO and the purpose of the release have been published, and the bibliographic reference for any information so published. This is intended to assist with the protection of the applicant's intellectual property rights, which may be affected by the prior publication of certain detailed information, e.g. by its inclusion on the public register.

A description of the GMO and the purpose of the release have not yet been published.