Department for Environment, Food and Rural Affairs

Application for consent to release a GMO – Higher plants

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

Part 1: General information

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

Applicant:

Project Leader, John Innes Centre, BSc in Agronomy, PhD in Genetics; over 16 years of experience with field trials in wheat and over 16 years of experience in wheat genetics and genomics.

Scientists:

Project Leader, John Innes Centre: Scientist who has led the research on the *VIT2* gene and the transgenic high-iron wheat lines. MSc in Molecular Biology (Wageningen, The Netherlands) and D.Phil. in Biological Sciences (Oxford, UK). Over 20 years of experience in Plant Biotechnology.

Field Experimentation Manager, John Innes Centre: Responsible for cultivation of the wheat lines. BSc in Environmental Biology, with 29 years' experience in cereal breeding, general agriculture and field experimentation. BASIS and FACTS qualified.

Research Assistant, John Innes Centre: Scientist who will be responsible for the day to day management and phenotyping of the field experiment. BSc in Environmental Science with 20 years of experience in the management and coordination of wheat field trials.

John Innes Centre
Norwich Research Park
Norwich
NR4 7UH
UK

2. The title of the project.

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	Not applicable
(e) cultivar/breeding line	Fielder
(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 fruits (caryopsis) which are traditionally referred to as seeds or grains. 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 cultivar to cultivar basis (see 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 20-24 weeks. For Fielder (spring-wheat type), one season is normally from March/April to August in the UK.

(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.

(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'. Fielder is a US spring wheat which has no vernalisation requirement

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, South 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.

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 (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.

The wheat grain harvested from the trial is not intended for general human or animal consumption.

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 the standard protocol by *Agrobacterium*-mediated transformation described in Rey et al 2018. The constructs were introduced to *T. aestivum* cv. Fielder 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 sequence of interest (*TaVIT2-D*; TraesCS5D02G209900) was cloned into vector pRRes14_RR.301 containing the promoter sequence for the endosperm-specific wheat high molecular weight glutenin subunit 1Dx5 (Lamacchia et al 2001). This fragment was then cloned into vector pBract202 (Smedley and Harwood, 2015) which is based on the pGREEN backbone (Hellens et al 2000).

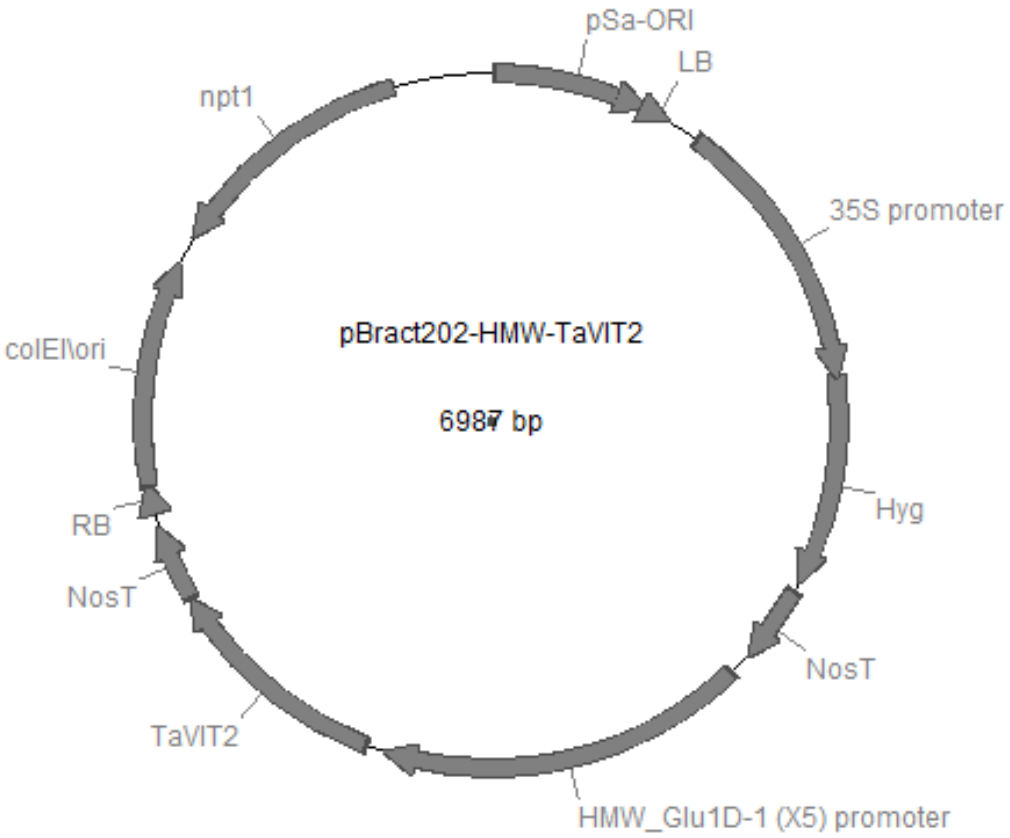
12. The size, intended function and name of the donor organism or organisms of each constituent fragment of the region intended for insertion.

A sequence encoding wheat Vacuolar Iron Transporter *TaVIT2-D* (TraesCS5D02G209900) was synthesised by Genscript and cloned into vector pRRes14_RR.301 containing the promoter sequence for the endosperm-specific wheat high molecular weight glutenin subunit 1Dx5 (nucleotides -1,187 to -3 with respect to the ATG start codon of the *GLU-1D-1* gene). The promoter-gene fragment

was then cloned into vector pBract202 containing a hygromycin resistance gene and left and right border elements for insertion into the plant genome (Smedley and Harwood, 2015). The intended function of expressing *TaVIT2-D* in the endosperm was to increase iron levels in this tissue (confirmed in Connorton et al 2017). The plasmid also contained the *nptI* kanamycin resistance gene for selection of bacteria, and the *Hyg* gene for selection of plants (under control of the cauliflower mosaic virus 35S promoter). See part VIII section 41 for more details on the methods.

pBRACT202-HMWG-TaVIT2

Element	Size (bp)	Donor organism	Description and intended function
colE1\ori	724	E. coli	Origin of replication for plasmid replication in *E. coli*
pSa-Ori	484	Agrobacterium tumefaciens	Origin of replication for plasmid replication in *Agrobacterium*
nptI	812	E. coli	Bacterial selection gene conferring resistance to kanamycin
RB	25	Agrobacterium tumefaciens	T-DNA right border
LB	24	Agrobacterium tumefaciens	T-DNA left border
35S promoter	897	Cauliflower mosaic virus (CaMV)	Promoter sequence from CaMV
Hyg	672	E. coli and Ricinus communis	Plant selectable marker gene encoding hygromycin phosphotransferase gene, including CAT-1 intron from *Ricinus communis* catalase-1 gene.
NosT	244	Agrobacterium tumefaciens	Nopaline synthase terminator
HMW_Glu1D-1(x5) promoter	1,185	Triticum aestivum	Promoter sequence for high molecular weight glutenin subunit 1Dx5
TaVIT2	738	Triticum aestivum	Coding sequence for Vacuolar Iron Transporter *TaVIT2* D genome copy



Part IV: Information relating the genetically modified plant

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

Increasing the intrinsic nutritional quality of crops, known as biofortification, is viewed as a sustainable approach to alleviate micronutrient deficiencies. In particular, iron deficiency anaemia is a major global health issue, but the iron content of staple crops such as wheat has been difficult to improve using conventional breeding. We have shown that the wheat *VACUOLAR IRON TRANSPORTER 2* gene (*TaVIT2*) functions as an iron transporter in wheat (Connorton et al 2017). Overexpression of *TaVIT2* under the control of a wheat endosperm-specific promoter increases iron in white flour fractions by greater than 2-fold, in controlled environment grown plants. The antinutrient phytate was not increased and the iron in the white flour fraction was bioavailable in vitro, suggesting that food products made from the biofortified flour could contribute to improved iron nutrition. The single-gene approach did not affect plant growth as defined by several phenotypic measurements including plant height, tillers per plant, grain number per plant nor grain weight in controlled environment grown plants (Connorton et al 2017).

This application seeks authority to investigate the effects on enhancing micronutrient accumulation in the grain by over-expressing the wheat *TaVIT2* gene in the endosperm of wheat plants in the field.

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 three separate lines, each transformed by the same gene construct in the field trial. Line 4-5 contains 9 copies of the pBract202-HMW-TaVIT2 plasmid per haploid genome, line 4-6 contains 8 copies and line 4-7 contains 11 copies, all determined by quantitative (Taqman) PCR performed on genomic DNA by iDNA Genetics (Norwich, UK). Plants were transformed using *A. tumefaciens*; therefore, all transformation events will result in a nuclear location for the transgenes. Segregation analysis using PCR of genomic DNA indicates that in all lines, all the gene insertions are carried in the chromosomal DNA and are stably inherited. The three lines proposed 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 increased iron content in the endosperm (checked by Perls' staining and confirmed by ICP-OES analysis) these plants are indistinguishable from untransformed controls, when grown in glasshouses or in controlled environment rooms. No other changes to the plant morphology or development are apparent (Connorton et al 2017).

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 *TaVIT2* and the hygromycin resistance genes are under the transcriptional control of the wheat *High Molecular Weight Glutenin-D1* (*HMW*) gene promoter (Lamacchia et al 2001) and the cauliflower mosaic virus promoter 35S (+ intron), respectively.

The wheat *HMW* promoter is specific to the endosperm of developing wheat grains and expression is not observed in any other vegetative tissue during the life cycle of

the plant. The 35S promoter is known to give broadly constitutive expression in wheat and therefore expressed in all tissue-types of the plant to varying degrees.

16. Information on how the genetically modified plant differs from the parental or recipient plant in the following respects -

(a) mode or modes and/or the rate of reproduction,

(b) dissemination,

(c) survivability.

Except for the ectopic expression of the wheat *TaVIT2* which is present in all wheat cultivars, all aspects of the phenotypes of events 4-5, 4-6 and 4-7 including morphology, pollination and seed-set appear to be identical to non-transgenic control wheat plants.

No difference in seed set, seed size or germination was observed. No difference in fertility was observed. Vegetative performance of the transgenic plants was unaltered. We would expect dissemination of pollen to be the same as for non-transgenic wheat plants given the very localised expression of the HMW promoter. We do not expect any changes in survivability of these plants in unmanaged systems.

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.

None known or expected. It is expected that the events will not differ from conventional wheat in their capacity to self or cross pollinate via sexual reproduction. A low rate (approximately 1%) of cross pollination with closely adjacent wheat plants within the trial can be anticipated. No cereals or grass species will be cultivated or allowed to grow for a further 20 metre from the outer edge of the trial area (see sections below). Enclosing the whole site will be a 2.4 metre high chain-link fence to prevent the entry of rabbits and other large mammals including unauthorised humans. In addition, rabbit-proof fence and netting will be used to further mitigate the risk of spread of transgenic materials by animals or birds.

The plasmid used possesses two antibiotic resistance genes (*nptI* and *Hyg*) 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 appropriate antibiotics are present). The *neomycin phosphotransferase I* (*nptI*) gene is under the control of a bacterial promoter to facilitate the maintenance and replication of plasmid vectors containing the genes of interest in dividing bacterial cells before they are used to transform plant cells. Their expression in plant cells is very unlikely and the gene is already widely present in the environment. The *hygromycin phosphotransferase* (*Hyg*) gene is under the control of a constitutive 35S promoter to facilitate the selection of transgenic plants after transformation. According to EFSA (EFSA 2009) genes conferring resistance to hygromycin are included in the first antibiotic resistance marker genes (ARMG) group. They state that, "with regard to safety there is no rationale for inhibiting or restricting the use of genes in this category, either for field experimentation or for the purpose of placing on the market." It is worth mentioning that *nptII* (which is closely related to *npt1*) is also classified alongside the *Hyg* gene in the EFSA guidelines.

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

The VIT2 protein occurs naturally in wheat and across many other plants and fungi. In this transgenic line we have expressed the native wheat *VIT2* gene ectopically in the wheat endosperm (using a native wheat promoter). There appears to be no published toxicity or allergenicity data for TaVIT2 but at the levels expected to be generated by these plants and because they will not enter the general food or feed chains, we consider the potential toxic or harmful effects to be negligible. The resistance genes in the construct have been described above (section 18).

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.

The modified plants and grains are not intended to be used as animal feed. Thus, there will be no risk of the genetically modified material entering the 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.

There are no known or predicted interactions associated with this trait and non-target organisms.

23. The potential interactions with the abiotic environment.

There are no known or predicted interactions associated with this trait and abiotic stress.

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

PCR primers for specific detection of the introduced T-DNAs are available and details are given in PART VIII (Section 41).

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

None. These plants have not been previously released.

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.

In each of the three years, the plants will be released on an area of arable land no larger than 25 metres squared located at the John Innes Centre (JIC, Ordnance Survey map grid reference TG 179 075). It will be situated within the fenced area used for GM experiments at the John Innes Centre which include genetically modified potatoes (under consents 16/R29/01 and 17/R29/01) and will correspond to the land that was previously sown with GM potatoes under consent 10/R29/01 (2010 – 2012). In accordance with wheat planting practice, the 25 m2 plot will rotate within the release site each year of the trial, therefore a total of 75 m2 will be used for the release across the three years.

In each year. the release will comprise ten 1 x 1.45 metre plots (1.45 m2) planted with two rows each of the three transgenic events and one non-transgenic control (eight 1 m rows per plot separated by 18 cm). Each 1.45 m2 plot will be separated by a 0.5 metre gap to the neighbouring plot. This covers an area of 9.25 x 2.5 metres = 23.125 m2. No cereals or grass species will be cultivated or allowed to grow for 20 metre from the outer edge of release area.

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

The release site (Ordnance Survey map grid reference TG 179 075) is arable land located at the John Innes Centre (JIC); some areas are bordered by deciduous hedges or trees. Flora in the immediate vicinity will be unknown until decisions on other local (non-GM) field trials are made each year but will likely be limited to

cereals (wheat/barley) and peas. Except for the experimental plots themselves, no wheat crop will be grown within the accepted separation distance of 20 metres from the release site. The fauna is typical of agricultural land in the East of England.

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 grass = *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 in the JIC site whereas *E. caninus* is uncommon in Norfolk (National Biodiversity Network). *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 20 meters from the trial.

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

There are no officially recognised biotopes, protected areas or Sites of Special Scientific Interest (SSSIs) within 4 km of the release site. The closest SSSI to the release site is Sweet Briar Road Meadows which is ~4 km away and is a series of unimproved wet meadows. The fact that wheat is not a wind-pollinated crop and is not visited frequently by pollinators means that there is minimal risk to any officially recognised biotopes or protected areas listed by Natural England.

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.

The purpose is to investigate the effects of the ectopic over-expression of the wheat *TaVIT2* gene in the endosperm of wheat grains and determine its effect on micronutrient accumulation and agronomic performance in the field.

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

If consent is granted, this year's field trial will start in Spring/Summer 2019. The plants will be transplanted in April and harvested in August/September. The exact timing of harvesting of the trial will depend upon weather conditions at the time. The trial will then proceed for two more growing seasons (2020 and 2021).

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

Seedlings of transgenic lines will be grown under controlled glasshouse conditions and will be planted in the field by hand following the randomised complete block design shown in section 34.

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

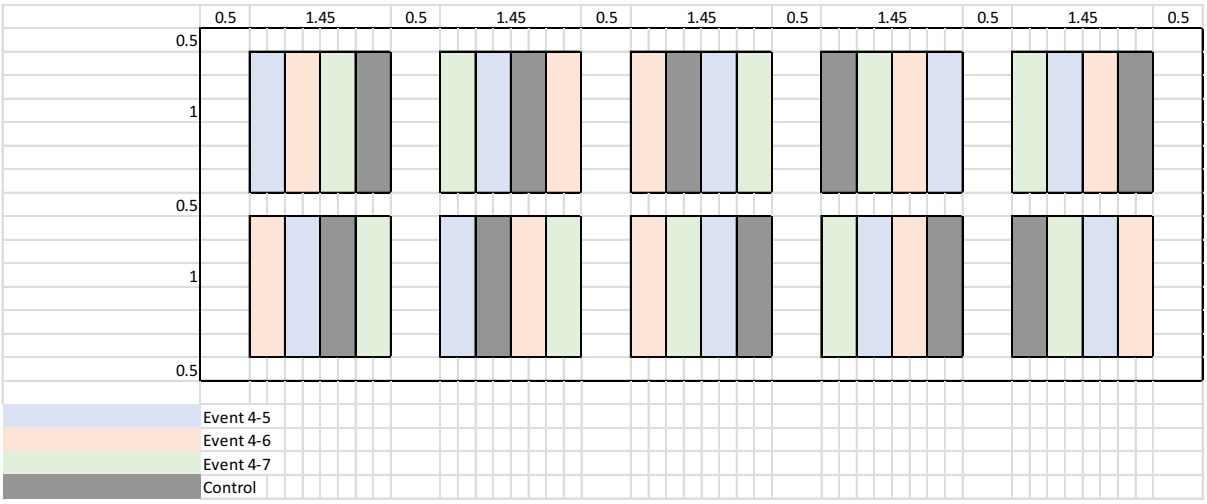
The ground will be prepared by staff from the John Innes Centre (JIC) Field Experimentation team who carry out field work on the Norwich Research Park (NRP) site, according to normal agricultural practices for spring wheat cultivation. Ground preparations will consist of existing plants being sprayed with herbicide to clear the ground. Compost will be applied if necessary and the ground will be prepared for planting using shallow mechanical cultivation

Harvest will occur August/September depending on weather conditions at the time (if the plants senesce prior to this then harvesting will be brought forward). Ears (spike/inflorescences) of transgenic and control plants will be hand harvested, conditioned, and manually threshed in a separate designated area, with seeds being stored in appropriate GM seed stores.

The plot will be monitored for volunteer plants immediately following harvest. This will include a shallow light tillage (minimum depth 5 cm) to encourage volunteers in autumn. If volunteers are present we will apply systemic herbicide (glyphosate) to ensure their destruction. The area will be left fallow over winter. In spring another shallow light tillage will be performed and glyphosate applied in case any volunteers are present. The area will be monitored for volunteers in this growing season (2019-2020) and the following season (2020-2021) during which time it will remain uncropped with wheat. Any volunteers detected in this two-year post-harvest period will be recorded and then destroyed by application of glyphosate or by autoclaving before ear emergence.

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

We will transplant 10 plants/m and the experiment will consist of sixty 1 metre rows of transgenic lines (three independent events x 10 replications/blocks x 2 rows/replication). Therefore, we estimate that the release will not exceed 600 transgenic plants.



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*) although there are no reports of cross-hybridisation between wheat and these genera. The two most common inland species are *Elytrigia repens* (common couch grass = *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 in the JIC site whereas *E. caninus* is uncommon in Norfolk (National Biodiversity Network). *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 20 meters 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).

To avoid dispersal of seed while sowing, we will hand-transplant seedling plants grown in controlled environment conditions. Ears of all transgenic lines and controls for all ten replications will be hand-harvested, conditioned and threshed for

phenotypic assessment and research purposes. Any remaining grain will be disposed by autoclaving and disposing in agreement with JIC standard operating procedures for transgenic material. All straw will be chopped and left on site.

Pollen will be allowed to be produced as we require grain but it's short period of viability and the separation distance of the transgenic plants from other wheat crops (at least 20 metres) will minimise the risk of cross-pollination. Full height framework and netting will protect the planting from birds and mammals throughout the growing season (from transplant to harvest).

We have excluded the use of a pollen barrier given that its inclusion would more than quadruple the area under the release; a 2 m pollen barrier surrounding the release area would translate to a total area of 106.9 m2 compared to the 23.1 m2 of transgenic material. We are prepared to include a 2 m pollen barrier if requested (e.g the designated area is large enough, etc) but we propose that the separation distance of 20 m should minimise dispersal of any modified material.

Prior to planting, seedlings will be transported from JIC controlled environment rooms to the release site and the plants will not be mixed with either other plants or with equipment used for working on other agricultural land. Any equipment used during the growing season, including for planting and harvesting of transgenic material, will be thoroughly cleaned after use and before it is allowed to leave the release site.

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

Harvest will occur August/September depending on weather conditions at the time (if the plants senesce prior to this then harvesting will be brought forward). Ears (spike/inflorescences) of transgenic and control plants will be hand harvested, conditioned, and manually threshed in a separate designated area with seeds being stored in appropriate GM seed stores.

The plot will be monitored for volunteer plants immediately following harvest. This will include a shallow light tillage (minimum depth 5 cm) to encourage volunteers in autumn. The area will be left fallow over winter and in spring another shallow light tillage will be performed and glyphosate applied in case any volunteers are present. The area will be monitored for volunteers in this growing season (2019-2020) and the following season (2020-2021) during which time it will remain uncropped with wheat. Any volunteers detected in this two-year post-harvest period will be recorded and then destroyed by application of glyphosate or by autoclaving before ear emergence.

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

Ears (spike/inflorescences) of transgenic and control plants will be hand harvested, conditioned, and manually threshed in a separate designated area. Seeds will be stored in appropriate GM seed stores.

38. A description of monitoring plans and techniques.

The purpose of the monitoring plan is to enable early detection of any unintended effects related to the release of the transgenic wheat plants.

The release site will be visited by trained laboratory personnel who are working on the project at no less than weekly intervals. Visits will usually occur more frequently and records will be kept of each visit. Any unexpected occurrences that could potentially result in adverse environmental effects or the possibility of adverse effects on human health will be notified to the national inspectorate immediately. Should the need arise to terminate the release at any point the emergency plans detailed below will be followed.

Post-trial the release site will remain fallow to enable easy identification of volunteers. The site will be inspected fortnightly between harvest and September and any volunteers identified will be immediately destroyed either by application of a systemic herbicide or by hand pulling plants and digging out the root systems. These will then be autoclaved within JIC. If volunteers are found at the end of the 2-year period, Defra recommendations will be followed for the management of the release site.

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 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 using our 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 field personnel.

40. Methods and procedures to protect the site.

The release site will be fenced to protect against animal damage and entry by unauthorised persons. We will include additional measures for birds and rabbits by enclosing the planting in framework and netting throughout the growing season. The site will also be monitored by remote security cameras visible from the John Innes Centre (JIC) reception which is manned throughout the day by JIC reception staff and by security guards out of normal working hours.

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.

From Connorton et al 2017, “The *TaVIT2* gene was amplified using primers TaVIT2-NcoIF (AGACCATGGTGAAGCCTGTGCAG) and TaVIT2-SpeIR (TGAACTAGTCAGATGGCCTGCACG) and cloned into vector pRRes14_RR.301 containing the promoter sequence comprising nucleotides -1,187 to -3 with respect to the ATG start codon of the *GLU-1D-1* gene, which encodes the high-molecular-weight glutenin subunit 1Dx5 (Lamacchia et al 2001). The promoter-gene fragment was then cloned into vector pBract202 containing a hygromycin resistance gene and left border and right border elements for insertion into the plant genome (Smedley and Harwood, 2015). The construct was checked by DNA sequencing. Transformation into wheat (cv Fielder) was performed by the BRACT platform at the John Innes Centre using Agrobacterium tumefaciens-mediated techniques as described previously (Wu et al 2003; Harwood et al 2009). Transgene insertion and copy number in T_0 plants were assessed by iDNA Genetics using quantitative PCR with a Taqman probe. For the T_1 generation, the presence of the hygromycin resistance gene was analyzed by PCR with primers Hyg-F (ATGAAAAAGCCTGAACTCACC) and Hyg-R (CTATTCCTTTGCCCTCGGA).”

References

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