**Department for Environment, Food and Rural Affairs**

# Application for consent to release a GMO

## Part A2: Data or results from any previous releases of the GMO

**Give information on data or results from any previous releases of this GMO by you either inside or outside the European Community [especially the results of monitoring and the effectiveness of any risk management procedures].**

The GMOs that are subject to this application have not been released before.

## Part A3: Details of previous applications for release

**Give details of any previous applications to release the GMO made to the Secretary of State under the 2002 Regulations or to another member state under the Deliberate Release Directive 2001/18/EC.**

There have been no previous applications to release these GMOs. However, Rothamsted Research and the John Innes Centre have received previous consents to release GM wheat, most recently: 22/R55/01 and 21/R52/01, respectively.

## Part A4: Risk assessment and a statement on risk evaluation

### Summary

#### Environmental risks

It is not expected that the *sp1* gene-edited GM plant lines intended for this multisite trial will differ from conventional wheat in their capacity to invade the ecosystem, to transfer genetic material via cross-pollination or to have an impactful interaction with other organisms. Moreover, the transgene cassette is fully integrated into the plant genome; and the risk of non-sexual, horizontal gene transfer to other species is extremely low.

The transgenic plants have not been modified to display a selective advantage/disadvantage over conventional wheat. Our observations on the *sp1* lines growing in our greenhouses show that these lines complete their full life cycle in a similar way that the control lines. The stay-green trait of the *sp1* plants is expected to improve crop yield and only parameters related to yield will be evaluated to confirm

preliminary assessments in our greenhouses. The release of these *sp1* gene-edited GMOs will be monitored regularly during all stages of development and harvested at maturity by fully authorised and experienced staff. Seeds from the GM and control plots will be conditioned, threshed and stored in appropriate GM seed stores.

To minimise the dissemination of pollen, the experimental plots containing the GM lines will be surrounded by a 2 m pollen barrier and surrounding this whole trial area no cereals or grasses, with the exception of other GM trials, will be allowed to grow for 20 m (or 20 m from a GM plot at the JIC Church Farm, as indicated in Part A1, Section 26). Additionally, management procedures will be in place to minimise the prevalence of volunteers or access from animals and/or unauthorised people. These preventive measures will be in place in all four trial sites.

Previous advice from ACRE for the release of GMs that contain similar transgenic elements (*i.e.*, the Cas9 along with the sgRNA, and the selective marker) did not conclude that this created concerns with regards to risk assessing any environmental impact (Application Ref: 21/R08/01).

Therefore, the overall risk of harm to the environment arising from this trial is assessed as extremely low.

**Human health risks**

The transgenic elements (*i.e.*, plant-specific promoters and coding sequences) are not known to be pathogenic or allergenic to humans, and none of these DNA sequences are expected to result in the synthesis of products that are harmful to humans because of the improbability of gene-expression in a non-plant cell environment. Moreover, the sgRNAs are designed to target a gene that localises in the chloroplasts of plants, and importantly, no off-target sequences are predicted for this pair of sgRNAs. The fact that no further gene-edits were found in the progeny of each selected line despite the presence of the transgenic cassette, strongly suggests that these components are no longer expressed or are not active in the generations of plants intended for this multisite trial.

On the other hand, the hygromycin-resistance marker is not used in human clinical medicine, and it is not restricted to be used in GM plants for field experimentation, according to the EFSA. More importantly, because these *sp1* gene-edited GM plants will not enter the food or feed chains, any potential toxic, allergenic or harmful effect on human health is negligible.

In summary, the overall risk of harm to human health arising from this trial is assessed as extremely low.

## Risk assessment

Conclusions on the potential environmental impact from the release or the placing on the market of GMOs.

**i. Likelihood of the genetically modified higher plant (GMHP) becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats.**

Although the *sp1* gene-edited GM lines are resistance to hygromycin, plants remain sensitive to all other herbicides. The elements present in the transgenic cassette are not anticipated to confer any advantage in relation to persistence in agricultural habitats or invasiveness in natural habitats and no emergent hazard is predicted. Our observations of the *sp1* lines growing in our greenhouses show that these lines are indistinguishable to non-GM control lines in their general morphology, and in the completion of the full life cycle; also, similarly to non-GM wheat, the GM lines do not persist by vegetative reproduction. Other traits such as the capacity to self-pollinate and to set seeds do not appear to differ from non-GM controls either.

The management procedures of this multisite trial are aimed to minimise the dissemination of pollen or seeds outside the trial areas. In summary, the likelihood of the GMHP to become more persistent or invasive in natural habitats is extremely low.

**ii. Any selective advantage or disadvantage conferred to the GMHP.**

It is not expected that the *sp1* plant lines to be released in this multisite trial will display any selective advantage/disadvantage compared to non-GM wheat.

The stay-green trait for which they have been intentionally gene-edited, has been reported to improve tolerance to abiotic and biotic stresses (such as drought, salinity, or foliar fungal diseases) besides yield (Danful et al., 2019; Kamal, et al., 2019). Therefore, the only potential advantage of the GM plants would be that the plants could cope better against abiotic and/or biotic stresses but because of the genetic edition rather than the presence of the transgenic elements. At the moment of the application for consent to release, this potential ability to better tolerate abiotic/biotic stresses in the GMHP *sp1* gene-edited lines has not been tested yet; also, this assessment will not be part of this multisite trial.

**iii. Potential for gene transfer to the same or other sexually compatible plant species under conditions of planting the GMHP and any selective advantage or disadvantage conferred to those plant species.**

The frequency of pollen mediated gene flow (PMGF) in wheat is low even for plants in close proximity and decreases rapidly with distance to the pollen source;

some studies have concluded that, at a distance of 10 m from the pollen source, PMGF events were reported under 0.5% for spring and winter wheat, even for farm-scale fields (see Foetzki *et al.*, 2012); thus, only a low rate of cross-pollination with closely adjacent wheat plants within the trial plots could potentially occur.

The wild relatives of wheat *Elytrigia repens* (common couch) and *Elymus caninus* (bearded couch) are reported around the trial sites, being the common couch grass the more prevalent. However, the lack of reports of spontaneous hybrids between wheat and common couch or wheat and bearded couch, alleviates concerns in relation to potential cross-pollination events with GM pollen. Nevertheless, in all the trial sites, these grasses will be controlled along with other weeds in and around the trial site using standard farm practices. No wheat, cereals or grasses (including common and bearded couch) other than those cultivated as part of this application or another GM trial, will be cultivated or allowed to grow within the trial site and the surrounding 20 m from the trials (or 20 m from a GM plot at the JIC Church Farm, as indicated in Part A1, Section 26). All the above measure will minimise the potential for gene transfer around the trial sites.

In summary, there is very limited potential for gene transfer among the GMHP and other sexually compatible plant species in this multisite trial.

**iv. Potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GMHP and target organisms, such as predators, parasitoids and pathogens (if applicable).**

Not applicable.

**v. Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms, (also taking into account organisms which interact with target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens.**

It is not expected any immediate and/or delayed impact on other organisms resulting from the release of these *sp1* gene-edited GM lines.

The stay-green trait (see Section ii above) could improve tolerance to abiotic and biotic stresses (including fungal pathogens) without affecting the completion of the full life cycle of the plants; a potential impact of this trait is the improved tolerance to pathogens. The *sp1* lines are not expected to become persistent (see Section i above), and relevant protective measures will be in each site to minimise cross-pollination, spread of seeds or establishment of volunteers.

**vi. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMHP and persons working**

**with, coming into direct contact with, or in the vicinity of the GMHP release(s).**

It is not expected any immediate and/or delayed effect on human health as a result of this multisite trial. The transgenic elements are not known to be pathogenic or allergenic to humans, and none of these DNA sequences are expected to result in the synthesis of products that are toxic or harmful to humans; also, the sgRNAs are designed to target a gene that localises in the chloroplasts of plants, and importantly, no off-target sequences are predicted for this pair of sgRNAs. In addition, the transgene-expression is unlikely in a non-plant cell environment.

Because the modified plants will not enter the food or feed chains, the possibility of any undesired effect on human health is negligible.

**vii. Possible immediate and/or delayed effects on animal health and consequences for the food/feed chain resulting from consumption of the GMO and any products derived from it if it is intended to be used as animal feed.**

It is not expected any immediate and/or delayed effect on animal health as a consequence of this multisite trial. The transgenic elements are not known to be pathogenic or allergenic to animals, and none of these DNA sequences are expected to result in the synthesis of products that are harmful to animals or the environment; the sgRNAs are designed to target a gene that localises in the chloroplasts of plants, and importantly, no off-target sequences are predicted for this pair of sgRNAs; in addition, the transgene-expression is unlikely in a non-plant cell environment.

The release of the *sp1* gene-edited GM lines will have the purpose to assess potential improvements in crop yield. The *sp1* lines will not enter the food or feed chains. The seeds to be collected will be stored in designated containers and will be used for studies of yield-related traits; these seeds will not enter the food or feed chains. Because of the above, no effects on animal health, no consumption and consequences for the food/feed chain are expected to occur.

In addition, all the trial sites will have protective measures to minimise dissemination of pollen and/or seeds, and access of animals or unauthorised people.

**viii. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).**

No immediate and/or delayed effect on any biogeochemical process is expected from the release of these GMO and their potential minimal interactions with other organisms.

**ix. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs.**

No environmental effect is expected from the release of these GMHP. The cultivation, management and harvesting practices at the multisite trials are standard procedures and are performed by fully trained staff. Any effect is expected to be comparable to that of non-GM wheat cv Fielder under conventional agricultural practice.

| | **Step1:** ***Potential hazards which may be caused by the characteristics of the novel plant*** | **Step 2:** ***Evaluation of how each hazard could be realised in the receiving environments*** | **Step 3:** ***Evaluation of 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 of harm caused by the release for each hazard*** |
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| **a** | **Increased invasiveness in natural habitats or persistence in agricultural habitats.** | Increased invasiveness may arise from intended or unintended effects of the genetic modification that resulted in wheat plants with a more weed-like 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 | 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 become established | Harvested seeds will be transported from the site in sealed containers. Machinery will be cleaned thoroughly prior to removal from the site. The 2 m barrier zone will minimise the spread of pollen, as advised by ACRE on previous GM wheat field trials (Ref: 19/R52/02, 21/R52/01). Surrounding this whole trial area no | Overall risk is very low. |

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| | | | 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.<br><br>The magnitude of harm if the hazard was realised is considered to be very small. | elsewhere. This is very unlikely as wheat pollen is relatively heavy so does not travel far, and it has a short half-life. Cereals and grasses, **with the exception of other GM trials,** will not be allowed to grow within 20 m of the trial site (or 20 m from a GM plot at the JIC Church Farm as indicated in Part A1, Section 26), and spontaneous crossing between wheat and its closest wild relatives in the UK has not been observed. Seed removal from the site will be rigorously managed. The chances of modified wheat plants | cereals or grasses, with the exception of other GM trials, will be allowed to grow for 20 m (or 20 m from a GM plot at the JIC Church Farm as indicated in Part A1, Section 26). Appropriate physical barriers and/or deterrents will be employed to minimise access by large mammals and birds. | |

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| | | | | establishing themselves outside the trial site are considered to be negligible. | | |
| **e** | **Selective advantage or disadvantage conferred to sexually compatible plant species** | Selective advantage (*i.e.*, stay-green phenotype for improving yield) or disadvantage may result from intended or 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 the trial site. | The basal ability for commercial cereal crop cultivars to survive in uncultivated environments is very low. We anticipate that the stay-green phenotype will not provide any selective advantage compared to other factors determining a plant's ability to survive in unmanaged ecosystems. | However, this hazard would be realised only if GM pollen were to spread from the trial site and become established; but pollen of wheat is relatively heavy so does not travel long distances, and it has a short half-life. Cereals and grasses, with the exception of other GM trials, will not be allowed to grow within 20 m of the trial site (or 20 m from a GM plot at the JIC Church Farm, as above), | There will be a 2 m pollen barrier surrounding the trial site and there is a large buffer zone that minimises any risk. Surrounding this whole trial area no cereals or grasses, with the exception of other GM trials, will be allowed to grow for 20 m (or 20 m from a GM plot at the JIC Church Farm, as above). Appropriate physical barriers and/or deterrents will be employed to minimise access by | Overall risk is very low. |

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| | | | | and spontaneous crossing between wheat and its closest wild relatives in the UK has not been observed. Therefore, the likelihood of this hazard resulting in environmental harm is extremely low. | large mammals and birds. | |
| **f** | **Potential environmental impact due to interactions between the novel plant and target organisms** | This hazard could not be realised because there are no target organisms. | Not applicable. | Not applicable. | Not applicable. | No risk. |
| **g** | **Potential environmental impact due to interactions between the novel plant and non-target organisms** | Changes in the plant interactions with non-target organisms could result from the intended (*i.e.*, stay-green phenotype with a consequent improved tolerance to biotic stress) or | Wheat plants have a range of pests and fungal pathogens. Wheat also interacts with beneficial insects that attack aphid pests. It is extremely unlikely that the genetic modification will | Impact of this hazard on the environment would only occur if seeds or pollen from the GM plants were to spread from the trial site and become established. The measures described above that minimise | There will be a 2 m pollen barrier surrounding the trial site and there is a large buffer zone that minimises any risk. Surrounding this whole trial area no cereals or grasses, with the | Overall risk is very low. |

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| | | unintended effects of the genetic modification. This could have an environmental impact if changes in interactions with non-target organisms resulted in the plants being better able to thrive in uncultivated environments or to persist in agricultural habitats. | affect these non-target organisms in any way.<br>Wheat plants can become infected by several fungal pathogens in the UK, e.g. spot blotch causes major yield loses to wheat crops. The novel stay-green phenotype of these plants may confer improved tolerance to fungal disease. | the spread of GM pollen will make the likelihood of this hazard to be considered extremely low. | exception of other GM trials, will be allowed to grow for 20 m (or 20 m from a GM plot at the JIC Church Farm, as above). Appropriate physical barriers and/or deterrents will be employed to minimise access by mammals and birds. | |
| **h** | **Potential effect on human or animal health due to the introduced genes** | By contact or ingestion of GM plant material. | The transgenic sequences (i.e., *Cas9*, *hptII* and sgRNAs) are not expected to result in the synthesis of products that are harmful to human or animal health. The null expression of *sp1* in these GM plants is only | People operating machinery at the farms and scientists and/or farm staff in the trial site will come into physical contact with the plants. However, it is extremely unlikely that they will ingest any plant material. Other small animals | 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 of animals. | Overall risk is very low. |

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| | | | expected to be plant-specific, by delaying the senescence of the plant and potentially improving yield grain with no expected toxic or allergenic effects. | (mice, insects or invertebrates may come into contact and/or ingest plant material). | | |
| **i** | **Potential effects on biogeochemical processes (changes in soil decomposition of organic material)** | No detrimental effect on the soil is expected from the introduced genes. | Soil decomposition or any other biogeochemical process is not expected to be affected any differently due to the cultivation of GM wheat plants compared to wild-type cv Fielder. | Any effect is expected to be comparable to that of non-GM wheat cv Fielder under conventional agricultural practice. | Conventional agricultural practice for these type of GM trials will be followed strictly, no modifications are expected. | Overall risk is very low. |
| **j** | **Possible environmental impact due to changes in cultivation practice** | The cultivation practices at the multisite trials are standard procedures and are likely to have a negligible environmental impact. | The magnitude of any effects arising from changes in cultivation practice will be negligible. | The frequency that this hazard may be realised is low. Changes on cultivation practices are unlikely to occur during the two years of this trial. | Conventional agricultural practice for these type of GM trials will be followed strictly, no modifications are expected. | Overall risk is very low. |

## 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.**

Detailed information of gene names and genetic modifications/editions are sensitive. The application of CHLORAD as a technology for crop improvement is covered by a patent application (no. WO2019/171081 A1).

## 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.

## References

Danful, R., Kassim, Y.B., Puozaa, D.K., Oteng-Frimpong, R., Rasheed, M.A., Wireko-Kena, A., and Akromah, R. (2019). Genetics of Stay-Green Trait and Its Association with Leaf Spot Tolerance and Pod Yield in Groundnut. *International Journal of Agronomy* 2019:1

Foetzki, A., Diaz Quijano, C., Moullet, O., Fammartino, A., Kneubuehler, Y., Mascher, F., Sautter, C. and Bigler, F. (2012). Surveying the pollen-mediated crop-to crop gene flow from a wheat field trial as a biosafety measure. *GM Crops and Food: Biotechnology in Agriculture and the Food Chain* 3: 115-122

Kamal, N.M., Gorafi, Y.S.A., Abdelrahman, M., Abdellatef, E., and Tsujimoto, H. (2019). Stay-Green Trait: A Prospective Approach for Yield Potential, and Drought and Heat Stress Adaptation in Globally Important Cereals. *International Journal of Molecular Sciences* 20:5837