Department for Environment, Food and Rural Affairs

Part B: information about the release application to be included on the public register

B1 The name and address of the applicant

Principal Investigator
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B2 A general description of the genetically modified organisms in relation to which the application is being made

The genetically modified plant lines for this multisite trial are gene-edited wheat plants (cv. Fielder) with null levels of SP1 (*sp1* plants), a key component of the CHLORAD (chloroplast-associated protein degradation) regulatory system of plastid protein import. The production of the edited plants required the introduction of a binary vector carrying a transgenic cassette necessary for the CRISPR/Cas9-mediated edition and for plant selection. The transgenic cassette was not segregated in the gene-edited lines, except for one. This cassette includes: the gene encoding the Cas9 nuclease, two sgRNAs for the target gene, and the plant selection resistance gene for hygromycin, all associated to plant-specific promoters.

The altered levels of SP1 in the gene-edited plants lead to a delayed leaf senescence, causing a functional stay-green phenotype. Functional stay-green is a valuable trait, associated with prolonged photosynthetic activity, and improved crop-yield.

Except for the delayed leaf senescence and the potential to improve yield, the morphology, pollination and seed-set of the gene-edited plants is fully comparable to non-transgenic control wheat plants.

B3 The location at which the genetically modified organisms are proposed to be released

There are four experimental farm locations proposed for this multisite trial: (i) the Rothamsted Research, West Common (Harpenden, Hertfordshire, AL5 2JQ) and (ii) Brooms Barn (Higham, Bury St Edmunds, IP28 6NP); (iii) the JIC Church Farm (Bawburgh Norwich, NR9 3PY), and the (iv) NIAB Park Farm (Histon, Cambridge, CB24 9NZ).

B4 The purpose for which the genetically modified organisms are proposed to be released (including any future use to which they are intended to be put).

The aim is to obtain proof-of-principle data for the use of CHLORAD as a technology

for crop improvement.

Under controlled environmental conditions, the *sp1* wheat plants display a stay-green phenotype associated with an increase in grain yield. We intend to use the grains collected from the multisite field trials to assess if there are significant differences on yield-related traits (e.g., seed weight, size and number, starch content) in the gene-edited plants when compared to the not edited (non-GM) controls growing in the fields. The results would inform future efforts for crop improvement via the development of transgene-free edited lines of the CHLORAD components.

The *sp1* gene-edited lines, GM and non-GM intended for this multisite trial will not be commercialized.

B5 The intended dates of the release.

In all four locations, the starting date will be Spring 2025. The seeds will be sown in March/April and harvested in August/September, finishing with all plants harvested and removed by Autumn 2025.

B6 The environmental risk assessment.

The *sp1* gene-edited lines have been modified to display a stay-green phenotype; besides this trait, they are indistinguishable from the non-GM wheat plants in relation to their morphology, development, and the ability to complete their life cycle to full senescence. It is not expected that the GM lines intended for this multisite trial will differ from conventional wheat in their capacity to inhabit the ecosystem, to transfer genetic material via cross-pollination or to have an impactful interaction with other organisms.

The transgenic cassette is fully integrated into the plant genome; and the risk of non-sexual, horizontal gene transfer to other species is extremely low. Furthermore, all the transgenic elements are under the control of plant-specific promoters, as functional transcription units; thus, in the unlikely event of horizontal gene transfer the expression of the transgenes in soil microorganisms would be improbable. In addition, the hygromycin-resistance marker is not used in human clinical medicine, and it is not restricted for use in GM plants for field experimentation, according to the EFSA. In agreement with the above, previous advice from ACRE for the release of GMs that contain similar transgenic elements (*i.e.*, the Cas9 along with the sgRNA, and a selective marker) did not conclude that this created concerns with regards to risk assessing any environmental impact (Application Ref: 21/R08/01). Any unknown hazards arising from the expression and ingestion of foreign proteins will not be realised because the GM plants will not enter the food or feed chains.

As for any risk associated with the dissemination of pollen or seeds: (i) there will be management procedures to minimise the dissemination of GM-pollen and potential cross-pollination with other plant relatives across all the four trial sites; such as, a 2 m pollen barrier and a surrounding area of 20 from the trial field no cereals or grasses (with the exception of other GM trials) will be allowed to grow for 20 m (or 20

m away from a GM plot in the JIC Church Farm). (ii) The possibility of GM seed disseminating from the trial sites and/or to establish in uncultivated environments is also very low because in all the trial sites there will be measures to reduce access from animals and/or unauthorised people, and to prevent any volunteer to develop. Therefore, the probability of dissemination of pollen for any potential cross-pollination, and/or the dissemination of seed and/or establishment of GM plants in the surrounding ecosystem is very low in all four trial sites.

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

B7 The methods and plans for monitoring the genetically modified organisms and for responding to an emergency.

The release sites will be visited regularly, at least weekly, during the growing season. 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 Defra immediately. If there is need to terminate the release at any point in any of the trial sites, an emergency plan will be followed (see below).

After the termination of the trial, each site will be monitored regularly for volunteers for two years. During this period, they the soil will be treated by lightly tilling to encourage volunteers; and when detected, these events will be recorded and the volunteer plants will be destroyed, either by hand-pulling and autoclaving or by application of herbicides.

Emergency plan: in the unlikely event of the integrity of any of the four trial sites being seriously compromised, the trial site(s) will be terminated and all plants (including GM, non-GM and control wheat plots, and pollen barrier) 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, autoclaving or deep burial at a local authority-approved landfill site using an approved contractor. Transportation of waste materials will be in secure containers. Site security staff and farm managers will be provided with the phone numbers of all key staff and with a standard operating procedure to follow.