

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

B1 The name and address of the applicant

The application is made on behalf of Crop Science Centre, University of Cambridge/ NIAB at the following address:

Crop Science Centre
Department of Plant Sciences
University of Cambridge
93 Laurence Weaver Road
Cambridge, CB3 0LE, UK

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

The genetically modified plants to be studied in the field trial are spring barley (*Hordeum vulgare*) cv. Golden Promise in which one of the genes involved in the perception and colonization of arbuscular mycorrhizal fungi (AMF) has been rendered dysfunctional using the CRISPR/Cas9 system or modified by overexpression. The study aims to undertake a field trial to examine the impacts of AMF inoculation on biomass and yield of the symbiosis pathway gene-edited and the overexpression gene-modified spring barley cv. Golden Promise in low and rich phosphate soils. Six of the barley cv. Golden Promise genes, namely *SYMRK*, *CCamK*, *Cyclops*, *RAM1*, *NSP1*, and *NSP2* have been individually edited using the CRISPR-Cas9-mediated gene editing system. Genetic edition in these genes led to abortion or significant reduction of AMF colonization in the laboratory conditions. Moreover, one of the said genes, namely *Hordeum vulgare* NSP2 (*HvNSP2*) and its ortholog from *Medicago truncatula* (*MtNSP2*), were modified through overexpression (OX) which led to the promotion of AMF colonization in the laboratory setting.

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

In each of the five years, the field trials will be conducted on NIAB managed land. In the first year, this will be at the Park Farm site at the National Institute of Agricultural Botany (NIAB), Cambridge, UK, grid reference in the region of TL 42949 61941. In subsequent years site grid references in the region of TL 46991 43918 and TL 38108

64920 may also be used.

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).

This is a research trial to investigate the effect of arbuscular mycorrhizal fungi (AMF) in barley biomass and yield in the field. Due to the extensive use of phosphorus fertilizers in agricultural practices in the UK, the field soils typically contain high phosphate levels, which prevents crop plants from taking advantage of beneficial associations with natural microbial symbionts such as AMF. The gene-edited lines which will be used in this field trial will reveal the degree to which crop plants such as barley can benefit from naturally occurring mycorrhizal associations in the field. In these gene-edited plants, key genes such as *SYMRK*, *CCamK*, *Cyclops*, *RAM1*, *NSP1*, and *NSP2* are impaired. These genes are involved in the perception and colonisation by AM fungi. Therefore, such mutants are not able to fully take advantage of AMF colonization. Their impaired yield and growth in the gene-edited lines in comparison to wild-type plants will inform us of the advantages delivered naturally by AMF. Moreover, the overexpression of genetically modified lines (*OxHvNSP2* and *OxMtNSP2*) by their abilities to override phosphate suppression of mycorrhizal colonisation can take advantage of the association with AMF even in the current highly phosphorus-rich soils in the UK. In short, the field evaluation of transgene-derived traits which alter plant association with AMF will be carried out, with the predominant focus being on enhancement to AMF association in the presence and absence of phosphorus fertilizers.

B5 The intended dates of the release.

The trial will run for five seasons, from spring 2022 to summer 2026. The plants will be sown in mid-February/March and harvested in September each year. The exact timing of drilling and harvesting of the trial will depend upon weather conditions at the time.

B6 The environmental risk assessment.

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.

This is a research trial to investigate the effect of arbuscular mycorrhizal fungi (AMF) in barley biomass and yield in the field. The field evaluation of transgene-derived traits which alter plant association with AMF will be carried out, with the predominant focus being on enhancement to AMF association in the presence and absence of phosphorus fertilizers. The purpose of the monitoring plan is to enable early detection of any unintended effects related to the release of the transgenic barley plants. The release site will be visited by trained laboratory personnel who are working on the project 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. The outer edge of the trial has a 3-metre-wide strip of non-GM barley to function as a pollen barrier. No barley, other cereals, or grasses will be cultivated or allowed to grow within 20 metres of the trial. 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 non-selective herbicide or by hand pulling plants and digging out the root systems. These will then be autoclaved. If volunteers are found at the end of the 2-year period, DEFRA recommendations will be followed for the management of the release site. In the unlikely event of the integrity of the site being seriously compromised, the trial will be terminated and all plants (including GM, non-GM and control 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, autoclaving or deep burial at a local authority-approved landfill site using an approved contractor. Transportation of waste materials will be in secure containers. Should the release site be subject to vandalism, care will be taken to ensure that all uprooted plant material within and outside of the trial site is identified and destroyed accordingly as described above.