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

B1 The name and address of the applicant

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

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. 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.

The VIT2 protein occurs naturally in wheat and across many other plants and fungi. In the proposed lines we have expressed the native wheat *VIT2* gene ectopically in the wheat endosperm using a wheat promoter. Thus, the application mostly pertains the expression of a wheat gene in a different tissue than normally expressed. In addition to the wheat gene and promoter, the plants also contain two selectable markers, *nptI* kanamycin resistance gene for selection of bacteria during the cloning process, and the *Hyg* gene to facilitate the selection of transgenic plants after transformation.

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

The plants will be released on an area of arable land no larger than 75 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 includes 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).

Each year the area planted with the genetically modified plants will be approximately 25 metres squared. In accordance with wheat planting practice, the plot will rotate within the release site each year of the trial. For each year of the field trial we estimate that the release will not exceed 600 transgenic plants.

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 application seeks authority to investigate the effects of over-expressing the wheat *TaVIT2* gene in the endosperm of wheat plants in the field. Specific questions to be examined are:

1. Does the over-expression of *TaVIT2* lead to increased iron accumulation in the endosperm in field grown plants (i.e. as seen in greenhouse/controlled environment conditions)?
2. Are there any effects on phytate accumulation and distribution in field grown plants?
3. Is there any change to the content of other micronutrients in the grain when the transgenic lines are grown in the field (i.e. manganese, zinc)?
4. Is there any effect of over-expressing *TaVIT2* on basic agronomic performance (phenology, yield components, etc)
5. Is there any difference in agronomic performance between the different transgenic lines?
6. Are the effects consistent across years?

B5 The intended dates 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).

B6 The environmental risk assessment.

The proposed transgenic lines have an endosperm-specific expression of the transgenes and as such are indistinguishable from the non-GM equivalent except for the increase iron accumulation in the seeds. This modified composition is found only in the seeds of the GM wheat lines and is absent from all other vegetative tissues (e.g. leaves, roots, stems). There are no known hazards associated with this modification. The gene introduced into the plants proposed for release do not confer characteristics that would increase the competitiveness of plants in unmanaged ecosystems. It is thus highly unlikely that intended or unintended effects of the genetic modification of increased endosperm iron content will result in major changes in invasiveness or persistence of the transgenic wheat.

The donor organism of the gene is hexaploid wheat (*Triticum aestivum*) itself and both inserted sequences (promoter and *TaVIT2* coding sequences) are already present in all modern wheat cultivars. These sequences are not known to be pathogenic or allergenic to humans, and none of the genes under investigation, or 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 occur since the wheat grain harvested from the trial is not intended for general human or animal consumption.

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 cultivars 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 procedures to minimise the spread of seeds or pollen will further reduce the probability of these events occurring. Appropriate physical barriers (fenced growing area and full height netted framework) throughout the growing season) will be employed to prevent access by mammals and birds. 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.

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 genes nor the marker genes would be expected to confer a selective advantage in the field environment under consideration. 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 the transgenic wheat is small and temporary; less than 25 m2 per year and lasting between 5 to 6 months.

Bearing in mind its limited scope, overall risk of harm to human health or the environmental arising from this trial is assessed as very low.

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

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.

Emergency procedures: 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 farm. 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.