Advisory Committee on Releases to the Environment

Advice on an application for deliberate release of a GMO for research and development purposes

Applicant: John Innes Centre (JIC)

Application: To release genetically modified wheat lines that have been biofortified with higher iron levels in their grain.

Reference: 21/R52/01

Date: February 2022

Advice of the Advisory Committee on Releases to the Environment to the Secretary of State under section 124 of the Environmental Protection Act 1990

The Advisory Committee on Releases to the Environment (ACRE) is satisfied that all appropriate measures have been taken to avoid adverse effects to human health and the environment from the proposed release. ACRE sees no reason for the release not to proceed according to the following advice.

To minimise the likelihood that GM wheat from this trial will enter the human food or animal feed chains, the applicant should:

1. Ensure that the 20m surrounding the trial site is planted with a non-cereal crop and that cereal volunteers are controlled (prior to flowering) in this area during the trial.

2. Plant a wheat pollen barrier of 2m width, to flower at the same time as the GM wheat as an additional precautionary measure.

3. Control *Elytrigia repens* (Couch Grass) using a glyphosate herbicide and hand weeding, if necessary, within the trial site and the surrounding 20m, before flowering and for the duration of the trial.

4. Ensure that any GM or non-GM wheat plant material remaining in the area of release at the end of the trial is disposed of appropriately.

5. Ensure that following harvest, the area of release is lightly tilled twice (once after harvest and again in the following spring) to a depth of 5cm to stimulate germination of any wheat plant volunteers. The release areas should be left fallow and monitored for wheat plant volunteers for 2 years following harvest.

6. Record the number of wheat plant volunteers that germinate before destroying them with an application of glyphosate herbicide or hand pulling them prior to flowering.

7. Ensure that suitable measures (such as those described in the John Innes Centre's application) are put in place to keep large birds out of the trial area and that the efficacy of these measures are kept under review.

8. Ensure that machinery used on the site is cleaned thoroughly onsite, including between using it with GM and non-GM material, and that clothing and equipment such as vehicles used by personnel on the site are also cleaned thoroughly before leaving the site.

Comment

ACRE considered the risks to human health and the environment posed by the proposed release of wheat that has been genetically modified to enhance micronutrient accumulation in its grain by over-expressing in the same plant both the wheat vacuolar iron transporter 2 (*TaVIT2*) gene in the endosperm, and the rice nicotianamine synthase 2 (*OsNAS2*) gene.

The aim of this research is to investigate the effect of this over-expression in the field. Glasshouse studies found that the effect of these two genes was additive, but the limits on the number of plants that were grown under such containment prevented mineral bioavailability from being tested.

Furthermore, under these conditions the three transgenic lines intended for release were indistinguishable from untransformed controls. By contrast previous, separate trials of plants transformed with one or other of these two genes revealed either a decrease in grain size (for *TaVIT2* in UK trials) or a small, but consistent reduction in plant height (for *OsNAS2* trialled in Australia).

Key characteristics of this field trial with respect to its environmental risk assessment are:

1. It will be on a small scale. This application is to release approximately 60 seeds per m^2 over an area of 50m^2 comprised of three GM wheat lines and one control line making for a maximum of 2,000 GM plants being grown each year. The proposed release will be conducted within the GM field trial site at JIC Church Farm site, Bawburgh, Norfolk. The applicant has proposed that the release will take place at different locations within their GM trial site over three years. The trial will be planted first in the Spring of 2022 and then sequentially until the final harvest in Autumn of 2024.
2. The GM wheat and non-GM wheat grown in this trial will not be put into the human food chain or fed to livestock.

The applicant intends to trial 3 genetically modified lines; all contain the gene construct TaVIT2-OsNAS2 T-DNA. The GM lines may also contain the antibiotic resistance genes encoding neomycin phosphotransferase I (*npt1*) and hygromycin phosphotransferase (*hyg*). NPT1 confers resistance to aminoglycoside antibiotics such as kanamycin and neomycin.

These genes are used in the development of GM plants to facilitate the selection of bacteria and plants (respectively) that have been transformed successfully.

Molecular Characterisation

ACRE noted that the plants for this trial were a genetically modified US spring wheat variety cv. Fielder. It was modified using *A. tumefaciens* mediated transformation to incorporate two genes, as a single T-DNA construct, into a nuclear location. These two genes are the wheat vacuolar iron transporter 2 gene (*TaVIT2*) and the rice (*Oryza sativa, Os)* nicotianamine synthase gene (Os*NAS*).

Within the inserted T-DNA cassette these two genes are, respectively, under the control of a wheat endosperm-specific promoter (*HMWG*) and the maize Ubiquitin 1 promoter (*UBI*1; for ubiquitous expression in the plant). JIC plan to release three transformed lines containing the gene construct TaVIT2-OsNAS2 T-DNA: Line B4 which contains 2 copies of the construct per haploid genome, B12 containing 6 copies, and B14 containing 3 copies.

Some of the public representations criticised the JIC's molecular characterisation of the GM lines for not including information on unintended effects on the genome, including the role this may play in altered phenotype of the resultant plants. These data are not required in applications for small trial releases of GM plants unless they are needed to inform the risk assessment.

ACRE considered whether this information would provide useful data on the biological and agronomic characteristics of these plants compared to gathering data from the field. As part of this, ACRE were reminded of their previous discussions on what intrinsic characteristics of wheat these (or other) alterations would need to change in order for them to confer an environmental risk, for example, to make wheat a problem weed[1].

[1] Chepil W.S. (1946) Germination of Weed Seeds I. Longevity, Periodicity of Germination, and Vitality of Seeds in Cultivated Soil. Scientific Agriculture 26: 307-346.
Anderson, R. L. and G. Soper. 2003. Review of volunteer wheat (Triticum aestivum) seedling emergence and seed longevity in soil. Weed Technol 17:620–626.

It is inevitable that there will be differences between plant lines. This is the case for conventional plant breeding as much as it is for GM. Attempting to interpret these differences is challenging and not constructive unless there is an indication of what hazard to look for.

Under controlled conditions, the GM plants are indistinguishable from untransformed plants. An objective of the trial is to determine whether this is the case under field conditions. Monitoring of GM plants is a standard requirement in any consent that is issued for a GM field trial.

ACRE considered further the implications of the inserted gene cassette including that of the presence of two selectable marker genes, and on the basis that the nptI marker gene is outside the T DNA right and left borders, the JIC's statement that it was present in transformed plants was correct.

The structure of the genes and their associated promoters in the T-DNA cassette was standard for experiments of this type and therefore ACRE did not conclude that there were any further molecular aspects to be concerned about.

Members noted that the insertion site was not characterised in molecular detail, but given the phenotypic analysis, and that this will be a small-scale trial where plants will not enter into the human food chain, ACRE concluded that in the case of this particular trial, additional data on molecular characterisation would not be helpful in addressing risk-based questions.

The environmental risk assessment

ACRE concluded in its advice on a previous trial involving one of the inserted genes (*TAVIT2*) that it was very unlikely that increased levels of iron in the endosperm of GM wheat seeds would alter the characteristics or biology of the plants in a way that would transform them into a problem agriculture weed or increase their ability to invade and persist in habitats outside of arable conditions.

Based on results from that trial, and also from the Australian trial of the second gene (*OsNAS*), ACRE were content that this conclusion was equally applicable to this new trial.

If the JIC were to apply for wide scale cultivation of these GM plants in the future, data from small-scale field trials on the comparative agronomic and phenotypic characteristics are likely to be required.

Wheat is naturally self-pollinating but under experimental conditions can be crossed with various wild grasses. The application discusses sexual compatibility with wild relatives present at the trial site. *Elytrigia repens* (common couch) is the only one of these common on the JIC Church farm site and it is proposed to control it and other grasses and weeds in and around the larger GM trial site either by applying herbicides or hand pulling.

No cereals or grass species will be allowed to grow within 20m of the trial area itself. It should be noted that the applicant reports that no spontaneous hybrids between wheat x *Elytrigia* have been found.

There is within the application an assessment of the likelihood of horizontal transfer of the gene cassette and specifically of the antibiotic resistance genes, along with consideration given to recombination with soil bacteria. Furthermore, a large proportion of public representations reflected concern that growing plants containing antibiotic resistant marker genes would compromise the use of associated antibiotics in human and veterinary medicine.

ACRE has discussed the use of resistance marker genes in GM plants on a number of occasions and taken into consideration the statement from the European Medicines Agency (EMA) on the importance of preserving the therapeutic relevance of the antibiotics.

ACRE emphasised that both the nptI and hyg genes are present at high frequency in agricultural soils[2]. (Although the nptI gene is, for reasons discussed under molecular characterisation, unlikely to be present in the transformed plants).

Antibiotic resistant bacteria occur naturally in the environment, but many are a result of contamination with human and animal excreta in sewage, slurry and manure. Antibiotic resistance in humans and other animals has resulted from the strong selective pressure associated with the substantial use of industrially made antibiotics in human and veterinary medicine and as food supplements for farm animals.

ACRE gave the following advice on plant to bacterial gene transfer in a previous field trial application:

Even though the scientific consensus is that selection pressure on bacteria containing antibiotic resistance genes is the driver of antibiotic resistance gene frequency in the environment, ACRE discussed the potential for bacteria in the environment to be transformed with antibiotic resistance genes from the gene edited wheat plants. Studies of horizontal gene transfer from plants to bacteria suggest that this phenomenon is extremely rare (refer to the review by Keese, 2008[3]).

ACRE noted that even if a recombination event were to occur between DNA from a plant and a bacterial genome, in order for the gene to be expressed, it would need to be combined as a fully functional transcription unit in the bacterium, which is unlikely.

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[2] Walsh F, Duffy B (2013) The Culturable Soil Antibiotic Resistome: A Community of Multi-Drug Resistant Bacteria. PLoS ONE 8: e65567.
[3] Keese P. (2008). Risks from GMOs due to horizontal gene transfer. Env Biosafety Research. 7(3): 123 – 149

If it were to occur, it would most likely result from a homologous recombination event at a site in the bacterial genome where a version of antibiotic resistance gene already exists.

A number of public representations referred to a paper about the relatively high levels of a synthetic antibiotic resistance genes detected in Chinese rivers, which the authors (Chen et al. 2012[4]) attributed to improper disposal of laboratory waste.

By way of contrast, LaPara et al. (2015)[5] did not detect any of these genes in wastewater effluent or river water samples from the upper Mississippi River in the USA. The authors attribute this to stringent regulations on destroying laboratory waste containing recombinant DNA being followed.

The UK's Genetically Modified Organisms (Contained Use) Regulations 2014, apply to the use of plasmids with antibiotic resistance genes under laboratory conditions and address the management of waste.

There were also public representations recommending that heavy metal toxicity studies should be carried out. This is not generally necessary for small-scale trials where material will not enter the food or feed chains unless there is a plausible hypothesis whereby such limited exposure to the plant material could cause harm to humans and other animals. The consideration of any altered toxicity if this crop were to be used in food production is therefore beyond the remit of ACRE.

Managing the trial site

ACRE has considered the potential risks of this trial to human health and the environment in the context of it being a small-scale trial from which no material will enter the food or feed chains, the committee considered, in detail, management plans to minimise the persistence of GM material at the trial site and the dispersal of GM material from the site.

ACRE recognised that, although the proposed trial was larger than a previous wheat trial in terms of area, a 2m pollen barrier and a 20m isolation distance was adequate to minimise the probability of out-crossing to an acceptable degree.

The relatively small scale of the trial is reflected by the fact that GM plants are being planted by hand as seedlings into the release site and that harvesting will also be undertaken by hand. A wheat pollen barrier will be in place that is designed to flower at

[4] Chen, J.; Jin, M.; Qiu, Z.-G.; Guo, C.; Chen, Z.-L.; Shen, Z.-Q. Wang, X.-W.; Li, J.-W. A survey of drug resistance bla genes originating from synthetic plasmid vectors in six Chinese rivers. Environ. Sci. Technol. 46: 13448−13454.

[5] LaPara, T.M., Madson, M., Borchardt,S., Lang, K. S and Johnson T. J (2015). Multiple Discharges of Treated Municipal Wastewater Have a Small Effect on the Quantities of Numerous Antibiotic Resistance Determinants in the Upper Mississippi River. Environ. Sci. Technol. 49: 11509−11515.

approximately the same time as the GMO crop, ACRE acknowledge that this is not an exact science.

However, the trial is further contained by a surrounding 20m isolation distance and the probability of crossing with wild species particularly *Elymus* and *Elytrigia* is very low indeed.

The GM plants are susceptible to a wide range of herbicides and therefore, if necessary, it will be straightforward to kill off the GM plants. ACRE considered that the post-harvest processing protocol was robust, and that the described trial management procedures reflected the level of experience that the JIC have in handling GM trials.

Gene flow

Wheat is a self-pollinating crop with very low rates of cross-pollination with other wheat plants. This is because fertilisation often occurs before the florets open, which makes out-crossing unlikely, in addition, wheat pollen is relatively heavy and tends to travel shorter distances than pollen from other grass species that are wind-pollinated.

Studies have detected cross-pollination rates of 1 to 2 per cent between wheat plants in close proximity, but this rapidly decreases with the distance between plants.

There are several relevant studies involving GM wheat field trials, most recently those of Foetzki et al. (2012)[6] and Miroshnichenko et al. (2016)[7].

The area for the proposed trial will comprise 250m^2 total area per year (comprised of up to 50m^2 GM and c.200m^2 non-GM pollen barrier and gaps) and will be sown at a density of 60 seeds per m^2.

There will be 1m separation between plots and a wheat pollen barrier of 2m width entirely surrounding the trial plots, with a further 20m surrounding that, in which no cereals or grass species will be left to grow. ACRE noted that the separation distance required to prevent hybridisation between different wheat varieties when certified seed is produced for marketing purposes is 2 metres.

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[6] Foetzki A., Diaz Quijano C., Moullet O., Fammartino A., Kneubuehler Y. and Mascher F. (2012). Surveying of 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(2), 115–122.

[7] Miroshnichenko D., Pushin A and Dolgov S (2016). Assessment of the pollen-mediated transgene flow from the plants of herbicide resistant wheat to conventional wheat (*Triticum aestivum* L.). Euphytica 209:71–84.

The application proposes to sow a 2-metre-wide wheat pollen barrier (comprising the same variety as the GM wheat) around the trial. ACRE recommended a 2-metre-wide pollen barrier in its advice on previous GM wheat trials as this is an additional precautionary measure to the 20-metre separation distance.

But, recognising the similarly small size of this trial, ACRE sees no reason to extend this to ensure an acceptable probability of no unacceptable gene flow. To maintain the separation distance, ACRE advises that the 20m surrounding the trial site is planted with a non-cereal crop and that cereal volunteers are controlled (prior to flowering) in this area during the trial and for two years afterwards.

ACRE members considered that in terms of the pollen barrier, the key was timing to make sure both the experimental crops and the pollen barrier crop were at the same stage of development.

That can be difficult if one is looking at experimental seed that does not have all the characteristics and stability of a commercial variety. The committee concluded that, in their view, if synchronisation proves difficult, then the 20m separation distance would be an acceptable risk mitigation.

The applicant plans to move the specific site around within the bounds of the JIC Church Farm GM field trial site to allow post trial monitoring and to avoid the effects of take-all disease on the plants. Therefore, the location of the specific growing site will require careful consideration to ensure that the 20m isolation distance remains within the bounds of the Church Farm site as a whole.

Wheat plant volunteers

The trial will receive standard farm practice as regard to herbicides, fungicides, nitrogen, sulphur and other fertilisers. The site will be monitored regularly (at least once a week) both during and for 2 years after the trial. For the Post- trial monitoring period, the trial area will remain in stubble to enable monitoring of volunteers.

The applicant does mention shallow cultivation being carried out to encourage volunteers but does not specify when this will be done. ACRE advice on previous trials has been lightly till twice (once after harvest and again in the following spring) to a depth of 5cm to stimulate germination of any wheat plant volunteers. The persistence of such volunteers from winter wheat in cultivated soil has been studied for a long time and is well-characterised. [8],[9]

[8] Chepil W.S. (1946) Germination of Weed Seeds I. Longevity, Periodicity of Germination, and Vitality of Seeds in Cultivated Soil. Scientific Agriculture 26: 307-346.

[9] Anderson, R. L. and G. Soper. 2003. Review of volunteer wheat (Triticum aestivum) seedling emergence and seed longevity in soil. Weed Technol 17:620–626.

In common with previous GM wheat field trial applications, public representations have raised the issue of volunteers found by the US Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) in three states some years after trials had been carried out.

There had been more than 100 large-scale trials conducted across 16 states, and although USDA's investigations were inconclusive, it was as previously discussed by ACRE, most probably due to the persistence of volunteers that GM seed had mixed with other seed and in this way become spread to other fields.

There are several relevant publications, of which the most detailed are 2 specifically designed to consider longevity of spring wheat in the seed bank in the context of GM (Kristi et al. 2007 and Ryan et al. 2009).

These studies conclude that survival of buried seed beyond the next spring is extremely rare and longer-term persistence in a field is most likely to occur from seed produced from volunteers that escape detection in the following season and then set seed. This conclusion is supported by the more recent study by Kalinina et al. in 2015[10].

This trial proposed by the JIC is on a very small scale and has a number of measures, including post trial monitoring, to ensure that any volunteers are detected and removed.

Seed movement

ACRE were content with the applicant's outline of how the release will be monitored regularly during all stages of development and harvested at maturity. Some seeds from the GM and control plots will be conditioned, threshed and stored in appropriate GM seed stores. A sample of plants may be hand-harvested, conditioned and threshed to supply seeds for research purposes.

All such small samples removed from the trial site will be stored in containment prior to use and will eventually be autoclaved before disposal. The remainder of the site will be harvested by the plot combine.

[10] Olena Kalinina, Simon L. Zeller, Bernhard Schmid (2015). Persistence of seeds, seedlings and plants, performance of transgenic wheat in weed communities in the field and effects on fallow weed diversity. Perspectives in Plant Ecology, Evolution and Systematics 17: 421–433.

Grain that is not required for analysis or to provide seed for future trials and all other material, including that from the pollen barrier rows, will be disposed of by incineration, autoclaving, or deep burial at a local authority-approved landfill site using an approved contractor, while any material remaining after analysis will be autoclaved before disposal.

Transportation of waste materials will be in secure containers. All straw will be chopped and left on site. The combine will be cleaned prior to leaving the site so that all traces of plant material from the trial will remain in the trial area. All transport of material will be logged.

Other items arising from public representations

Some 80 public representations were received, where these covered areas within the remit of ACRE they were addressed within its assessment, as summarised above. Many of the representations concerned areas beyond the remit of ACRE or these topics are not relevant to the environmental risk assessment when considering a small-scale field trial.

Some of these comments would be of relevance if the application had been for commercial-scale cultivation or food and feed use.

Others were more political in nature and are outside of ACRE's remit, for example, they questioned whether GM techniques should be used in the development of plant varieties and more specifically whether it is necessary to develop wheat with enhanced micronutrient accumulation using this technology.