

GM Team Department for Environment, Food and Rural Affairs Second Floor Seacole Building, Marsham Street London SW1P 4DF Submitted by email to gm-regulation@defra.gov.uk

29 June 2021, updated 1 July 2021

Dear Madam/Sir

Re: Application from Rothamsted Research to release a genetically modified organism, reference 21/R08/01 as published at https://www.gov.uk/government/publications/genetically-modified-organisms-rothamsted-research-21r0801

GM Freeze, Open Space Cooperative, 41 Old Birley Street, Hulme, Manchester M15 5RF Tel: 0845 217 8992 Email: liz@gmfreeze.org Web: www.gmfreeze.org Twitter: @GMFreeze We are writing on behalf of GM Freeze, Friends of the Earth (England, Wales and Northern Ireland), GeneWatch UK, GMWatch, Beyond GM, EcoNexus, the Soil Association, Organic Farmers & Growers CIC, the Organic Research Centre, the Biodynamic Association, the Land Workers' Alliance, Whole Health Agriculture, The Kindling Trust, Shepton Farms, Real Seeds, Banc Hadau Llambed / Lampeter Seed Library, Hodmedod, Unicorn Grocery, the Real Bread Campaign, GM Free Somerset, GM Free Cymru, Genetic Engineering Network, Agri-Activism UK, Resurgence Dorset, Sustainable Dorset, SE Essex Organic Gardeners, GM Free Dorset and the Springhead Trust to request that the above application to release genetically modified (GM) Wheat is refused.

GM Freeze is the umbrella campaign for a moratorium on GM in food and farming in the UK.

Friends of the Earth (England, Wales and Northern Ireland) exists to create a just world where people and nature thrive. **GeneWatch UK** is a not-for-profit organisation which aims to ensure genetic science and technologies are used in the public interest. **GMWatch** provides the public with the latest news and comment on genetically modified (GMO) foods and crops and their associated pesticides. **Beyond GM** is an initiative educating and engaging the public to raise the level of debate around the issues of GMOs and sustainable food production in the UK. **EcoNexus** analyses and reports on new technologies that have the potential for significant negative impacts on biodiversity and ecosystems.

The Soil Association is the charity that digs deeper to transform the way we eat, farm and care for the natural environment. **Organic Farmers & Growers** were the first UK organic certification body to be approved by the Government and now certify more than half of UK organic land. **The Organic Research Centre (ORC)** is the UK's leading independent organic research organisation. The **Biodynamic Association** promotes biodynamic methods for healthy farming, forestry and gardening for planet, nature and people. The **Land Workers' Alliance** is a grassroots union representing farmers, growers and land-based workers. **Whole Health Agriculture** is a community of farmers, health professionals and citizens who support and promote those who farm for health and vitality.

The Kindling Trust works with communities, farmers, health providers, activists and policymakers to create a fairer more sustainable food system for all. Shepton Farms are organic farmers and fruit growers. Real Seeds provides open pollinated seed appropriate for growers producing vegetables under sustainable low input conditions. Banc Hadau Llambed/Lampeter Seed Library aims to build stocks of locally adapted open-pollinated food seed as a free Lampeter community resource. Hodmedod works with British farmers to offer a range of foods from diverse arable crops to retail, catering and manufacturing customers. Unicorn Grocery in Manchester has pioneered a cooperative approach to sustainable urban food supply. The Real Bread Campaign finds and shares ways to make bread better for us, better for our communities and better for the planet.

GM Free Somerset is a grass roots campaign supported by individuals, groups, local businesses and charities that exist to promote rural sustainability. **GM Free Cymru** is the community pressure group campaigning to keep Wales free of genetically-modified crops. **Genetic Engineering Network** facilitates the exchange of information between groups and campaigners. **Agri-Activism UK** is a network of people who campaign for cleaner, healthier and more sustainable agricultural and food systems. **Resurgence Dorset** is a monthly community group of environmentalists and nature-lovers set up to discuss articles and host talks to raise public awareness of environmental issues. **Sustainable Dorset** is a central hub for sustainable and resilient activity across the county, with the aim of raising awareness and increasing interest and involvement in sustainability. **SE Essex Organic Gardeners** is a local group of Garden Organic, supporting and working with the Soil Association and Pesticide Action Network UK. **GM Free Dorset** is a grass roots campaign promoting rural sustainability across the county of Dorset. **The Springhead Trust** promotes environmental education, sustainability, organic agriculture and local performing arts.

We are of the strong opinion that this open field trial should not go ahead. The research is at an early stage and should be conducted in a contained environment. The applicant's risk assessment is incomplete. The proposed field trial represents a risk and the research is unlikely to provide any public benefit. In summary, our objection covers the following points:

- 1. The research is still at an early stage and should be conducted in a contained environment
 - 1.1. The genetic modifications are not stable, and the Cas9 gene editing apparatus is still active
 - 1.2. The effects of the genetic modifications are not stable
 - 1.3. The applicant has not reported adequately on their recent GM wheat trial
- 2. The applicant's risk assessment is incomplete
 - 2.1. The genetic modification results in the production of a novel protein that has not been tested for safety or allergenicity
 - 2.2. There is no molecular characterisation to check for unintended genetic modifications
 - 2.3. The planned reduction in free asparagine may have unintended consequences
- 3. The proposed field trial represents a risk through escape, contamination and the inclusion of a herbicide tolerance gene
 - 3.1. There is potential for escape and contamination
 - 3.2. The genetically modified plants are tolerant to glufosinate-based herbicides
- 4. The proposed field trial is unlikely to provide any public benefit
 - 4.1. Genetics are unlikely to be an effective means of reducing free asparagine levels
 - 4.2. GM wheat with reduced free asparagine levels will not provide any significant public benefit

1. The research is still at an early stage and should be conducted in a contained environment

1.1. The genetic modifications are not stable, and the Cas9 gene editing apparatus is still active

The field trial application proforma Part A1, paragraph 15 (a) requests information on the genetic stability of the experimental organisms that the applicant wishes to release. The applicant has left this section completely blank despite clear evidence that the modifications made to the GM plants are not genetically stable.

In the immediately previous paragraph (Part A1, paragraph 14) the applicant describes further genetic changes as a key aim of the trial: *another aim is to enable enough plants to be screened to be able to detect some in which the transgenes have been lost altogether.* This suggests that the GM developers wish to use the proposed open field trial for early development research which could, and should, be done under contained conditions.

More concerning still is the clear evidence that the *Cas9* gene inserted to induce DNA injuries in the GM plants is likely to remain active during the proposed field trial. The applicant's own published paper¹ demonstrates that the Cas9 protein has remained active in successive generations (our emphasis):

"Two edited B genome alleles [...] were also present, one showing a deletion of 173 bases between the 2F and 4R positions and the second a deletion of a single base at 4R. Both edits cause frameshifts that bring stop codons into frame, resulting in the genes encoding truncated proteins. Plants 12 and 60 were homozygous for allele B1 while the other plants were biallelic. Neither of these alleles was observed in the T1 generation, **indicating that editing had continued into the T2 generation**, resulting in novel edited alleles appearing."

And

"Continued editing increases the chances of effective edits being generated, but also adds to the difficulties of characterising the mutations that have occurred. **We conclude that several generations may be required to achieve stability**."

While the application states (Part A1, paragraph 15 (b)) (our emphasis):

"... plants 23.60 and 59.26 did still contain a Cas9 gene and this was segregating in the progeny of these plants being grown to produce seed for the field trial (Table 4). It is conceivable that **editing could continue** in plants in which the Cas9 gene is still present.

These points show clear evidence of genetic instability which is a significant biosafety concern. No genetically modified organism should be released until genetic stability has been established across multiple generations in a contained facility. In this instance, the potential for the Cas9 protein to induce further unpredictable DNA mutations after open release and, potentially, in non-target plants should contamination or out-crossing occur, makes it vital that these experimental plants are not grown in an open field.

The applicant's failure to acknowledge or comment on the potential unintended effects of continued Cas9 activity raises concerns about their attitude to field trials. Moving lab-based experiments into an open field significantly increases the risk of escape, contamination and unintended impacts on the environment. Those risks are born not by the applicant but by society as a whole and GMO regulations are the tool that society uses to safeguard against harm. This application is clearly for an ongoing experiment, rather than even a near-final product. As such, safety in the event of an escape cannot be assured, particularly where the genome-editing apparatus remains in the organism.

1.2. The effects of the genetic modifications are not stable

The genetically modified wheat has been developed to produce less free asparagine. However, significant phenotypic instability has been observed with the applicant team's published paper² reporting a rapid drop off in efficacy with higher levels of free asparagine production in second generation plants than in the first.

The team wishing to plant this trial suggests that this reduced impact might be due to the stress of growing in a glasshouse during the hot summer of 2019. We agree that stressors such as drought appear to have significant impacts on the production of free asparagine and explain elsewhere [see 4.1, below] that this calls into question the rationale behind this experiment.

Regardless of efficacy, the instability of the phenotypic impacts of the intended genetic modification needs to be assessed from a safety perspective. The genetically modified plants should not be grown in an open field while there is such doubt about the stability of the intended trait.

1.3. The applicant has not reported adequately on their recent GM wheat trial

Rothamsted Research received consent for a three-year trial of highly experimental GM wheat in 2017 (consent reference 16/R08/02). The applicant engaged in notable public relations activity when consent was received³ but, despite completing the trial in 2019, offers no assessment of the outcome on its own website (including the Rothamsted repository) or in published scientific papers accessible via a Google Scholar search. Having conducted a detailed search of other sources we understand from a report published by UK Research and Innovation⁴ that the GM wheat *"showed no differences to wild type [the non-GM control]"*, ie the GM trait completely failed in the field. The applicant's silence on these results contrasts starkly with the extensive PR exercise staged in support of this current application⁵, raising questions about their commitment to advancing collective knowledge and their repeated focus on open field trials of GM wheat, on which failure is fast becoming the norm.

This trial should not go ahead but, if it does, the Secretary of State should require the applicant to report the outcome in a timely manner and in ways that are accessible to all stakeholders, including the general public.

2. The applicant's risk assessment is incomplete

2.1. The genetic modification results in the production of a novel protein that has not been tested for safety or allergenicity

The application describes (Part A1, paragraph 30) the purpose of the research trial as *"to investigate the effect of knocking out the TaASN2 gene on free asparagine accumulation in wheat grain in the field"*. A DNA 'knock-out' is generally accepted to mean that the gene concerned will no longer be active in protein synthesis, but this is not what has happened in the laboratory stages of this experiment. Rather, the lines for the proposed trial produced novel truncated proteins with no history of safe use. The applicant team's Raffan et al., 2021⁶ paper states (our emphasis):

"Plants of line 23 [...]showed the same two A genome alleles (labelled A1 and A2 in Figure 2) as seen in the T1 generation, both of which **encode truncated proteins**"

"Two edited B genome alleles (B1 and B2 in Figure 2) were also present, ... resulting in the genes **encoding truncated proteins**.

For line 59: The plants were also homozygous for the single edited D genome allele seen in the T1 generation [...]. This allele has a frameshift in the 13th codon and encodes a **truncated protein** of only 76 residues.

The applicant provides no evidence of having assessed these truncated proteins for function; safety or allergenicity to humans; or, indeed, any other impacts at all. Nonetheless, in Part A1, paragraph 22 of the application, which asks about human health risks, the applicant makes the unfounded claim that: *"No toxic, allergenic or harmful effects on human health are envisaged."*

We are concerned about the direct risks of releasing an untested novel protein into the environment, but this omission also raises a wider concern. The applicant's assumption that they can predict the outcome of processes intended to knock out genes or insert new ones demonstrates an arrogant disregard for the complexities of the genome. Rather than "switching off" the target gene they have disrupted its function, possibly in ways detailed by a study published in Nature in October 2019⁷.

2.2. There is no molecular characterisation to check for unintended genetic modifications

Neither the application itself nor the applicant's 2021 paper⁸ consider potential unintended modifications resulting from either the transgenesis or the genome editing process. Also, the limited analysis that has been done only assessed the target region, rather than considering the whole genome.

Unintended genetic modifications raise potential safety implications so must be investigated. The standard risk assessment practice for doing this is to perform molecular characterisations that will identify disruption to the genome. However, the application does not include any characterisation of the flanking regions surrounding any of the genetic inserts. As a result, we have no indication of where they were inserted, how stable those insertions are, or whether they have disrupted endogenous genes.

Similarly, there is no assessment for unintended effects of the genome editing process in the plants that the applicant wishes to grow in an open field, despite indications that unintended outcomes have, indeed, occurred:⁹

"Two edited B genome alleles (B1 and B2 in Figure 2) were also present, one showing a deletion of 173 bases between the 2F and 4R positions and the second a deletion of a single base at 4R. Both edits cause frameshifts that bring stop codons into frame, resulting in the genes encoding truncated proteins. Plants 12 and 60 were homozygous for allele B1 while the other plants were biallelic."

For line 59: The plants were also homozygous for the single edited D genome allele seen in the T1 generation (Table 1), labelled as D4 in Figure 2. This allele has a frameshift in the 13th codon and encodes a truncated protein of only 76 residues.

Similarly, for line 178: "The A genome edited allele (A11 in Figure 2) has a single additional adenine at the 3R position, causing a frame shift in the sequence and the introduction of a stop codon at the 82nd codon."

There is now a wealth of literature¹⁰, published in mainstream academic journals, detailing unintended genetic changes as a result of genome editing. These include off-target effects, unintended on-target effects, interference with gene regulation and effects from intended and unintended insertion of DNA¹¹. In addition, there may be unexpected outcomes from the intended genetic change, for example, if the edited gene has more than one function. Such changes can lead to a wide range of unplanned effects such as the novel protein synthesis seen in this experiment (see 2.1, above) and the changed amino acid profile (see 2.3, below).

Despite this evidence of unintended genetic changes, both as a general observation of the genome editing techniques used and in the laboratory stages of this experiment, the applicant has not adequately reported on the molecular characterisation of either the edited region or more widely. Such characterisation is required to identify any unintended changes including large deletions, insertions, rearrangements or translocations. Unbiased whole omics profiling should also be performed to assess any unintended changes to gene expression, protein and metabolite levels that may impact food and environmental safety.

These plants should not be grown in an open field until any additional unintended effects have been thoroughly investigated and assessed for their safety through reporting of a detailed molecular characterisation.

2.3. The planned reduction in free asparagine may have unintended consequences

The role and accumulation of free asparagine in wheat is not well understood (as members of the team behind this proposed trial acknowledge¹²) but the amino acid is thought to play a vital role in a range of plant processes including development and tolerance of various forms of biotic and abiotic stresses.

Asparagine has a high ratio of nitrogen to carbon compared with other amino acids and many important biological functions in plants have been described for asparagine. These include nitrogen recycling during abiotic and biotic stresses, nitrogen mobilization and transport from source to sink tissues.¹³ Nitrogen recycling, transport and storage are essential processes for seed germination and plant development so there is a strong argument for precaution around any modifications to asparagine levels.

The laboratory stages of this experiment, as detailed in the applicant team's 2021 paper¹⁴, found that reduced levels of free asparagine were associated with poor germination in the edited lines and that "this was overcome by an exogenous application of 0.1 M asparagine in water, applied as a spray over the surface of the compost." The application acknowledges (Part A1, paragraph 16) that "Reduced accumulation of free asparagine in the grain appears to have a negative effect on germination, and there is some evidence of increased seed size in the edited lines" and suggests (Part A1, paragraph 20) that "the seeds may need to be supplied with asparagine in order to germinate at the required rate". While this aspect of the experiments to date may add to our understanding of the role of free asparagine, it raises a number of questions about the validity of the project as a whole. For example, there is no record of the team investigating whether levels of acrylamide production were reduced in any flour made and cooked from plants that had been treated with an external application of asparagine.

In a separate unexpected outcome, the ratio of amino acids in grains of the genome-edited wheat was changed¹⁵: "reduced free asparagine concentration in the edited lines in this study was associated with increases not only in free glutamine but also in free glutamate and aspartate. In other words, the partitioning between free asparagine, glutamine, aspartate and glutamate was altered." The origins of this change need to be identified. It could be an unexpected outcome of the genome editing process or of the intended trait (reduced levels of free asparagine). Whatever the cause of the change, it is important that any implications for human health, wildlife and the wider environment are investigated before the plants are grown in an open field.

The applicant has answered question 21 in Part A1, "Potential interactions with the abiotic environment and the adverse environmental effects arising" with the single word "*None*." This seems an extraordinarily bold statement given the known and observed adverse effects that the modifications have on plant phenotypes. The field trial application should be not approved until the origins and impacts of the unexpected changes have been thoroughly investigated.

3. The proposed field trial represents a risk though escape, contamination and the inclusion of a herbicide tolerance gene

3.1. There is potential for escape and contamination

As we have detailed in previous objections to UK GMO wheat trials¹⁶, wheat has escaped from field trials in the USA on three separate occasions^{17 18}. The discovery of the GM wheat, between eight and 15 years after the conclusion of the GM field trials from which it escaped, prompted some countries to halt purchasing of US wheat¹⁹ and led to market concerns for US farmers and traders. Investigations by APHIS (United States Department of Agriculture – Animal and Plant Health Inspection Service)²⁰ failed to find the route of contamination in any of these cases.

As we stated in our previous objections:

"Together, these incidents present a worrying picture of how easy it is for GM wheat to escape from field trials and remain a GM contamination threat for many years. The uptake and expression of trialled GM traits in other wheat varieties suggests that pollen escaped from the trials. The timelines show that GM wheat trial escapees can remain either undetected or dormant for over 10 years. The impact of these incidents on US wheat trading demonstrates a considerable risk to UK farmers and processors in the event of any escape from the proposed trial."

It would appear that, no matter what precautions are taken, wheat can escape from GMO field trials. This means that we must be certain that any GM wheat grown outside of a contained facility is safe for human and animal consumption, for other interactions with wildlife, and for the wider environment. Where a GM trait alters a biochemical pathway, as it does here, there needs to be a specific and detailed assessment of possible adverse effects of escape. The application for a field trial should be rejected until such an assessment has been undertaken and subjected to independent review.

Outcrossing to a common weed is also a concern with the proposed trial. The applicant notes (Part A1, paragraph 28) that a wild relative of wheat *"Common couch is quite widespread on the Rothamsted estate"* and that it is *"controlled along with other weeds in and around the trial site using standard farm practices"* However, no details are provided as to what these "standard farm practices" involve.

As GM Freeze et al²¹ raised in 2016 and previously in 2013 there are particular concerns that:

"common couch is already an extremely troublesome weed in cereal and other arable crops, as well as in many other crops and gardens, so the application should be refused to remove the chance of outcrossing occurring. A chance crossing between the GM wheat and a couch plant would result in glufosinate ammonium resistance developing in couch as a consequence of the presence of the marker gene.

"Before the UK trials of GM oilseed rape began it was stated that crosspollination between the crop and the common arable weed charlock (Sinapsis arvensis) was impossible under field conditions. Yet during the Farm Scale Evaluations from 2000-2003 such a cross did occur. This demonstrated that rare events do occur under natural conditions."

3.2. The genetically modified plants are tolerant to glufosinate-based herbicides

The application (part A1, paragraph 13) states that "some of the plants still carry the bar gene encoding phosphinothrycin acetyl transferase and will tolerate phosphinothrycin-based herbicides."

It is unclear whether or not the applicant expects this genetic modification to be bred out and discussion in paragraph 15 about their intention to breed out the *Cas9* transgene does not mention the *bar* gene. We must, therefore, assume that the plants grown in the trial will tolerate applications of glufosinate-based (phosphinothrycin-based) herbicides. However, neither the UK nor the European Union currently approves the use of glufosinate-based pesticides due to concerns regarding mammalian toxicity²². If this trait remains in any GM plants eventually commercialised, or is spread through escape and contamination, its presence is likely to encourage the use of a banned pesticide.

In general, herbicide tolerance is not a desirable trait. GM Freeze et al previously highlighted²³ that:

"in the future the presence of the glufosinate ammonium tolerance gene could be used as an agronomic trait in a commercial variety to make it attractive to farmers wishing to control weeds in cereal crops. The results of the UK's Farm Scale Evaluations clearly showed that GM herbicide tolerant Spring and Winter oilseed rape with tolerance to glufosinate ammonium had a significant impact on the flowering plant species in arable fields compared to the current herbicide regime used on conventional crops. This would also have a significant impact on numbers of arable weeds and insects, which form a vital food resource for farmland wildlife and would harm many species. Furthermore, the development of a dependence on glufosinate ammonium for weed control in cereals could lead to the development of resistance in major arable weeds leading to an escalation in herbicide usage and costs, as has happened in Roundup Ready crops in the US and South America."

As in 2013 and 2016, the presence of the glufosinate tolerance marker gene creates unnecessary risk, and the application should be rejected because of it.

4. The proposed field trial is unlikely to provide any public benefit

4.1. Genetics are unlikely to be an effective means of reducing free asparagine levels

Asparagine production levels are significantly influenced by environmental factors and appear to have limited heritability. This is acknowledged by the applicant team in their 2021 paper²⁴ which attributes the reduction in the intended trait effect in the second generation to heat stress (see 1.2, above). The paper also states more generally that "accumulation of free asparagine in wheat grain is responsive to environmental and crop management factors, increasing, for example, in response to sulphur deficiency and pathogen infection". Asparagine production has also been shown to increase in other species in response to stressors such as drought and nutrient deficiency or excess.

Nitrogen fertilizer is known to be an important factor in increasing grain asparagine levels. Nitrogen application in both conventional and organic farming systems is associated with higher levels of free asparagine in wheat grain²⁵ but organic methods have been shown to achieve lower asparagine levels than conventional farming²⁶.

Key members of the applicant team are co-authors of a 2020 paper²⁷ that states that any attempt to reduce asparagine levels through selection and breeding *"will have to be combined with agricultural practices that reduce levels"* and that *"attempts to reduce free asparagine levels in wheat grain, and thereby dietary acrylamide intake, must be balanced against other aspects of wheat cultivation and strategies to improve public health."*

There are significant knowledge gaps and uncertainties around this entire project and, in particular, the potential impacts of reduced asparagine production on the agronomic qualities required to deal with stress. Far broader research is needed before allowing the genetically modified wheat to be grown in an open field with all the associated risks of escape and contamination (see 3.1, above).

4.2. GM wheat with reduced free asparagine levels will not provide any significant public benefit

Leading charity Cancer Research UK states²⁸ that *"Eating burnt food does not cause cancer"* and *"You don't need to avoid acrylamide to have a healthy balanced diet."* Explaining further, they say²⁹ that *"Studies in people have not shown that eating more foods higher in acrylamide increases cancer risk"* and that *"you can reduce your risk by eating a healthy balanced diet"*.

We embrace the precautionary principle so understand that individuals may wish to reduce the level of acrylamide in their diet despite the assurances given by Cancer Research UK. However, we suggest a range of simple steps that will improve the quality of their diet as a whole, rather than further commodification of health worries through the creation of "cancer safe" processed food products no doubt under patent. For example, anyone who wishes to reduce their consumption of acrylamide would be well advised to:

- Avoid eating crisps which are high in fat and salt
- Eat a range of cereals and other starchy foods, rather than allowing wheat to dominate their starch intake
- Cook all foods as lightly as possible, which will also assist in retaining the functionality of a wide range of micronutrients
- Invest in a reliable toaster and teach all householders how use it

This type of genetic modification project hinders efforts to create a responsible, fair and sustainable food and farming system. We are facing a biodiversity and climate emergency; an obesity epidemic; and an increasing disconnect between people and the origins of the food that they eat. We need a wholesale global shift to sustainable agriculture and diversified local food chains. The focus of this project, using patented technology to create a commodifiable product of extremely limited true value, does not justify the risks outlined in this submission and the trial should not be allowed to proceed.

The proposed trial represents an unacceptable risk to people, wildlife and the wider environment. Further research is needed in a contained environment before any release into the environment. We request, therefore, that the Minister denies consent and prevents the proposed open-air field trial going ahead.

Yours faithfully

Liz O'Neill Director GM Freeze	Clare Oxborrow Senior Sustainability Adviser Friends of the Earth (England, Wales and Northern Ireland)	Dr Helen Wallace Director GeneWatch UK	Claire Robinson Editor GMWatch
Pat Thomas Director Beyond GM	Dr Ricarda Steinbrecher Co-Director EcoNexus	Joanna Lewis Director of Policy and Strategy The Soil Association	Roger Kerr Chief Executive Organic Farmers & Growers CIC
Dr Will Simonson Head of Research The Organic Research Centre	Gabriel Kaye Executive Director Biodynamic Association	Jyoti Fernandes MBE Chair Land Workers' Alliance	Lawrence Woodward OBE Chair Whole Health Agriculture
Helen Woodcock Director The Kindling Trust	Oliver Dowding Managing Director Shepton Farms Ltd	Kate McEvoy and Ben Gabel Directors Real Seeds	Julia Lim Member Banc Hadau Llambed / Lampeter Seed Library
Josiah Meldrum Co-Founder Hodmedod Ltd	Debbie Clarke Co-Operative Member Unicorn Grocery Ltd	Chris Young Coordinator Real Bread Campaign	Jane O'Meara Spokesperson GM Free Somerset
Brian John Co-Founder GM Free Cymru	Jim McNulty Co-Founder Genetic Engineering Network	Gerald Miles Co-Founder Agri Activism UK	Ken Huggins Organiser Resurgence Dorset
Pam Rosling Trustee Sustainable Dorset	Carole Shorney Secretary SE Essex Organic Gardeners	Lee Smith Spokesperson GM Free Dorset	Edward Parker Executive Director The Springhead Trust

References

¹Raffan, S., Sparks, C., Huttly, A. et al. (2021) Wheat with greatly reduced accumulation of free asparagine in the grain, produced by CRISPR/Cas9 editing of asparagine synthetase gene *TaASN2*. Plant Biotechnology Journal doi.org/10.1111/pbi.13573.

²Raffan, S., Sparks, C., Huttly, A. et al. (2021) Wheat with greatly reduced accumulation of free asparagine in the grain, produced by CRISPR/Cas9 editing of asparagine synthetase gene *TaASN2*. Plant Biotechnology Journal doi.org/10.1111/pbi.13573.

³ <u>https://www.rothamsted.ac.uk/news/rothamsted-research-granted-permission-defra-carry-out-field-trial-with-gm-wheat-plants</u>

⁴ UK Research and Innovation (2021) Outcomes of the project: 15-IWYP Realising increased photosynthetic efficiency to increase wheat yields. <u>https://gtr.ukri.org/projects?ref=BB%2FN021045%2F1#/tabOverview</u>

⁵ <u>https://www.rothamsted.ac.uk/news/ge-field-trial-application-submitted-defra</u> <u>https://www.rothamsted.ac.uk/articles/ge-field-trial-low-asparagine-wheat-qa</u> <u>https://www.rothamsted.ac.uk/news/genome-edited-wheat-reduce-cancer-risk-bread-and-toast</u>

⁶ Raffan, S., Sparks, C., Huttly, A. et al. (2021) Wheat with greatly reduced accumulation of free asparagine in the grain, produced by CRISPR/Cas9 editing of asparagine synthetase gene *TaASN2*. Plant Biotechnology Journal doi.org/10.1111/pbi.13573.

⁷ Smits, A.H., Ziebell, F., Joberty, G. et al. (2019) Biological plasticity rescues target activity in CRISPR knock outs. Nature Methods 16: 1087–1093 with background information and interpretation at <u>https://www.gmwatch.org/en/news/latest-news/19280-researchers-assumed-that-crispr-mediated-disruption-of-genes-was-turning-them-off-but-they-were-wrong</u>

⁸Raffan, S., Sparks, C., Huttly, A. et al. (2021) Wheat with greatly reduced accumulation of free asparagine in the grain, produced by CRISPR/Cas9 editing of asparagine synthetase gene *TaASN2*. Plant Biotechnology Journal doi.org/10.1111/pbi.13573.

⁹ Raffan, S., Sparks, C., Huttly, A. et al. (2021) Wheat with greatly reduced accumulation of free asparagine in the grain, produced by CRISPR/Cas9 editing of asparagine synthetase gene *TaASN2*. Plant Biotechnology Journal doi.org/10.1111/pbi.13573.

¹⁰ See, for example:

Agapito-Tenfen, S.Z., Okoli, A.S., Bernstein M.J., Wikmark, O.G., Myhr, A. (2018) Revisiting risk governance of GM plants: The need to consider new and emerging gene-editing techniques. Frontiers in Plant Science 9: 1874. doi:10.3389/fpls.2018.01874

Eckerstorfer, M.F., Dolezel, M., Heissenberger, A., Miklau, M., Reichenbecher, W., Steinbrecher, R.A., Wassmann, F. (2019) An EU perspective on biosafety considerations for plants developed by genome editing and other new genetic modification techniques (nGMs). Frontiers in Bioengineering and Biotechnology 7: 31. doi:10.3389/fbioe.2019.00031

Kawall, K., Cotter, J. & Then, C. (2020) Broadening the GMO risk assessment in the EU for genome editing technologies in agriculture. Environmental Sciences Europe 32: 106. https://doi.org/10.1186/s12302-020-00361-2

Modrzejewski, D., Hartung, F., Sprink, T., Krause, D., Kohl, C., Wilhelm R. (2019) What is the available evidence for the range of applications of genome-editing as a new tool for plant trait modification and the potential occurrence of associated off-target effects: a systematic map. Environmental Evidence 8 (27). doi:10.1186/s13750-019-0171-5.

ENSSER (2021) Scientific critique of Leopoldina and Easac statements on genome edited plants in the EU.

https://ensser.org/wp-content/uploads/2021/04/Greens-EFA-GMO-Study-1.pdf

¹¹ See, for example:

Kapahnke, M., Banning, A., Tikkanen, R. (2016) Random splicing of several exons caused by a single base change in the target exon of CRISPR/Cas9 mediated gene knockout. Cells 5 (4). doi:10.3390/cells5040045

Norris, A.L., Lee, S.S., Greenlees, K.J., Tadesse, D.A., Miller, M.F., Lombardi, H.A. (2020) Template plasmid integration in germline genome-edited cattle. Nature Biotechnology 38: 163-164. doi:10.1038/s41587-019-0394-6

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